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Sequential Partial Migration Across Monarch Generations in Michigan

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Abstract: We collected 434 adult monarchs and surveyed milkweeds for immature monarchs in southwest Michigan, USA in order to test the hypothesis that monarchs are temporally variable, sequential partial migrants rather than partial migrants that may be spatially separated. Adult size, wing wear, female egg counts, fat content and sequestered chemical defenses were measured in monarchs across an entire season from spring migrant arrival, through breeding, until autumn migrant departure. We predicted that a population characterized by starting from all migrants and no residents, through breeding residents, to all migrants and no residents should show life history measures consistent with changes in these proportions. Results show that female monarch spring migrants arrive with chorionated eggs and high wing loads in both intact and fat-extracted adults. Wing loads of both males and females decrease during the summer and increase again immediately before autumn departure, when the fat content of all adults increases markedly. The high fat content of spring arrivals is also characteristic of migrants. Cardenolide content of adults showed a similar pattern of high content in spring arrivals, a decrease in the summer and then an accumulation of cardenolide defenses in adults in late summer just before migratory departure. We conclude that these results are consistent with temporally variable, sequential partial migration in a short-lived insect that contrasts with spatially variable partial migration in longer-lived vertebrates.

Keywords: monarch butterfly, partial migration, voltinism, milkweed hostplant, wing wear, wing load, fat content, cardenolide sequestration

1 Introduction

Populations of many mobile vertebrate species such as birds, mammals and fish with multi-year lifespans show mixtures of migratory and non-migratory, or resident life histories characteristic of partial migration [1-3]. Chapman et al., [2] and Shaw and Levin [3] describe three forms of seasonal partial migrants in which (1) migrants and residents breed together, but overwinter apart, (2) migrants and residents breed apart and overwinter together, or (3) migrants breed together, but not every year, generating a mix of migrants and non-migrants. Short-lived insects that include migration behaviors in their life histories are also thought to reflect these patterns [1-4]. However, the short life-span of most mobile insects is likely to preclude separation into these three categories of “non-breeding partial migration”, “breeding partial migration”, or “skipped breeding partial migration.” Instead, migrant and non-migrant insects may be separated *temporally* rather than *spatially* at breeding or overwintering locations and show *sequential partial migration*. Here, we test this interpretation with a seasonal analysis of monarch butterflies, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae) as they arrive, breed and depart from Michigan, USA near the center of the monarch breeding distribution in North America east of the Rocky Mountains [5]. We predict that spring arriving and autumn departing adults will show life history measures characteristic of migrants and between these defining points the adults will have measures characteristic of breeding residents.

The monarch butterfly is considered an iconic example of insect migration by virtue of its predictable, long-distance annual migration in North America between overwintering resources in Mexico and breeding resources distributed across the USA and southern Canada east of the

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Rocky Mountains [5-19]. From early wing-tagging studies by Nora and Fred Urquhart in Canada [5,7,8] and later studies coordinated by Orley Taylor of Monarch Watch at the University of Kansas (<http://www.monarchwatch.org/index.html>) we know that adult monarchs fly each autumn to 12 locations in oyamel fir forests above 3,000 m elevation in the Sierra Transvolcanica mountains of central Mexico, west of Mexico City [10,11,14,15]. There the butterflies remain for up to 5 months over winter in tightly aggregated clusters until their return migration north in spring at the end of March. We also know that the same butterflies that left the USA and southern Canada in the autumn, and spent the winter in Mexico, return to the southern USA where they mate, lay eggs and die [16,17,20,21]. The offspring of these migrants from Mexico then feed on southern milkweed hostplant species and upon emergence as adults continue the migration north to recolonize the full spatial extent of their milkweed hostplants across North America, east of the Rocky Mountains [20,21].

In addition to their spectacular annual migration in North America, monarch butterflies are well known for their specialized larval feeding on milkweeds in the genus *Asclepias* and their ability to sequester toxic steroids known as cardenolides for use in defense against natural enemies such as birds [22-32]. Each *Asclepias* hostplant species generates a different pattern of sequestered cardenolides in adult monarchs and Malcolm et al. [21] used these chemical “fingerprints” as indicators of migratory strategy in spring migrants. Evidence from cardenolide fingerprints and wing wear patterns [21], as well as isotopic signatures [16,17,33,34], show that spring migration does occur primarily via successive broods in which migrating spring butterflies reach the southern USA from Mexico, mate, lay their eggs on southern *Asclepias* species and die, leaving their offspring to continue the migration north.

While much is known about migration, mating behavior and physiology, hostplant use and the operation of defense against natural enemies in monarch butterflies, we still know little about the dominant phase of their life history when their annual populations increase during the summer or about how monarchs both migrate and lay eggs as they encounter milkweed resources. While monarchs are thought to occur as either migrant or non-migrant populations [5,6,14,60] they are better described as “partial migrants” with a life history characterized by a mix of migrant and non-migrants [1-4]. This mix of

life histories is likely to vary seasonally and may help to explain some of the variation seen among monarch populations. Here, we describe data collected from both migrant and non-migrant monarch butterflies from southwest Michigan starting with their arrival in spring, through summer breeding until their departure in autumn. The data we collected include variation in adult size, wing loading, wing wear, fat content, female egg load and sequestered cardenolides; together these data aid in the interpretation of how monarchs move to exploit seasonally and spatially separated host plant resources. These data allow us to test the prediction of Berthold [61] that “improved conditions” (i.e. good quality breeding resources) increases the proportion of non-migrants in a population of partial migrants [62].

Michigan is in the center of the extensive distribution of the common milkweed, *Asclepias syriaca*, [35,36] which is the most important hostplant for monarchs [21,32,36,37] out of the 108 species of *Asclepias* milkweeds described by Woodson [35] from North America. It is in this northern extent of the range, where *A. syriaca* predominates, that monarchs generate up to three successive generations [38,39], to establish the large numbers of butterflies that fly to Mexico each autumn.

Migrating insects are thought to show a distinct trade-off between flight and reproduction so that migrating individuals are in reproductive diapause and reproductive individuals do not migrate. This trade-off is known as the “oogenesis-flight syndrome” and is argued to occur in monarchs [6,9,40-46]. However, like Thomas and Showers [47] we suspect that this is not the case for migratory monarchs and we have some evidence from prior work that migrating female monarchs lay eggs and that they carry fully developed, or chorionated eggs during migration.

We researched monarch breeding ecology during the summer throughout southwest Michigan in order to determine (1) changes in adult wing size, wing loading, wing wear, fat content, and host plant-derived chemical defense content, (2) variation in female monarch egg counts in migrants and non-migrants as a test of the “oogenesis-flight syndrome”, and (3) the relationship between these measures and the timing and abundance of immature monarchs on milkweed hostplants as a measure of the duration of breeding behavior.

Our goal was to determine whether collected data are consistent with an interpretation of partial migration in which spring arrivals and autumn departures are migrants and summer breeders are non-migrants.

2 Methods

2.1 Immature monarch monitoring and butterfly collection

Adults and eggs of the monarch butterfly, *Danaus plexippus*, were collected from spring arrival in late May until autumn departure in late September 2011 at 8 locations in Allegan, Barry, Kalamazoo and Van Buren counties of southwest Michigan. The sites varied in habitat and included a range of genet sizes of the common milkweed *Asclepias syriaca* as well as occasional plants of the milkweeds, *A. incarnata* and *A. tuberosa*. We collected adults and observed immatures at each of the sites once per week. We also supplemented these data with more extensive “citizen science” data for Michigan accessed with permission from Dr. Karen Oberhauser of the Monarch Larval Monitoring Project, at the University of Minnesota (<http://www.mlmp.org>). At each site, we randomly checked individual milkweed ramets within genets for eggs and larvae while we searched for adult monarchs. The location of collected monarch adults and eggs, and observed larvae was determined with a Garmin (eTrex Legend HCx) GPS unit and recorded in both field notebooks and a GIS database in addition to date, time, and habitat, as well as butterfly sex and behavior. Adult butterflies were placed in glassine envelopes and all samples were stored frozen at -20°C.

2.2 Adult size measures, wing wear and female eggcounts

The right forewing length of each adult butterfly was measured to the nearest 0.5 mm on the dorsal surface from the attachment base to the wing tip. The wings were then removed from frozen butterflies and photographed digitally with the same ruler scale used to measure the wings. The areas of the right fore and hind wings were then measured using ImageJ (version 1.48, <http://imagej.nih.gov/ij>) on thresholded images. Where possible the right fore and hind wings were measured, summed and doubled for total wing area without considering overlap during flight [48,49] – if a wing was damaged we measured the least damaged alternative wing. Wing loading was calculated as N/m^2 following Corbet [49], where wing load = (body mass(kg) * gravitational field ($g = 9.80665 \text{ m/s}^2$))/wing area (m^2). We used both dry weights of intact butterflies and the dry weights of lipid-extracted butterflies to calculate intact and lean wing loads.

As a measure of age and activity, wing wear of all adult butterflies was estimated four times, independently, by two observers using a scale from 1 (newly emerged) to 5 (extremely worn), at 0.5 intervals, taking into account wing fading, scale loss, fraying, tears and other damage to the wings [21]. The average of the four values was used for analysis.

Female monarchs were then dissected in a tray over ice to count the numbers of fully chorionated eggs. The abdomen was opened ventrally with fine scissors and the ovarioles were pulled apart carefully to count the eggs and then replaced in the abdomen. The bursa copulatrix was removed and weighed from all females and extracted for cardenolide content as described below. Bursa cardenolides were analyzed in addition to whole female cardenolides so that male contributions to female cardenolide content could be determined. Adult butterflies and the bursae were then freeze-dried in a LabConco lyophilizer to determine dry weights before the extraction of fat from adults and cardenolides from both adults and bursae.

2.3 Fat and cardenolide analyses

Fat was extracted from freeze-dried butterflies with methods modified from [50-53]. Monarchs were ground with 4 ml petroleum ether in an 18 x 150 mm glass tube using a motorized homogenizer. A further 2 ml of petroleum ether was used to rinse any remaining insect material on the homogenizer into the tube containing the thoroughly ground butterfly material. Each tube was centrifuged for 10 minutes and the supernatant decanted into a preweighed, labeled 12 x 75 mm glass tube and left in a fume hood overnight to evaporate the ether, leaving behind the fat extract. The extracted fat weight was then recorded. After fat extraction, 4 ml of 100% methanol was added to the insect residue in the original tube, which was then vortexed and sonicated in a heated water bath at 55°C for 10 minutes. The extract was centrifuged for 10 minutes and the supernatant was poured into a 13 x 100 mm glass tube. A further 2 ml of methanol was then added to wash the residue from the previous step and pooled to give a 6 ml extract. The methanol extract was dried under nitrogen in a water bath at 55°C. Once dry, the methanol extract was resuspended in 1 ml acetonitrile, vortexed, and filtered through a 0.45 μm Luer-lock syringe filter on a 3 ml plastic syringe and placed into a 1 ml, labeled autosampler vial, ready for HPLC.

The extraction of cardenolides from the dissected bursa followed the same procedure, except that ether extraction of fat was not performed because no fat was

associated with the bursae. Instead, each bursa copulatrix was ground with the homogenizer in 4 ml methanol and the procedure described above was followed.

Cardenolide analyses of butterfly and bursa extracts were performed using the method of Wiegrebe and Wichtl [54] on a Waters gradient HPLC system with WISP autosampler, 600E pump, 996 diode array detector and Millennium 2010™ chromatography software. The reverse-phase elution gradient was acetonitrile: water at 1.2 ml·min⁻¹ at 40°C, with 20% acetonitrile at start, 32% after 35 min., 40% after 45 min., 50% after 55 min., then back to 20% at 61 min., and 20% at 65 min., on a 2 50-4LiChroCART® RP-18 column packed with LiChrospher® 100,5 µm (E. Merck) with a 10 mm guard column. The 20 µl sample injections were separated over 65 minutes with 10 min. equilibration between samples and cardenolides were detected at 218.5 nm and identified by their symmetrical spectra between 205 and 235 nm and a λ_{max} of between 214 and 224 nm. Cardenolide concentration for each peak (µg/g sample DW) was calculated from a calibration curve with the external cardenolide standard digitoxin (Sigma, StLouis, Missouri). Only cardenolide peaks reported by Millennium 2010® software as consistently pure were considered for analysis.

2.4 Data analysis

Statistical analysis was performed with JMP11 software (SAS Institute). Arc Map 10.0 (ESRI) was used to map sample locations. Location data were obtained using a Thales Navigation MobileMapper™ GPS/GIS receiver and a Garmin eTrex Legend HCx GPS unit and imported into ArcView 10.0 (ESRI) for analysis in the Western Michigan University GIS lab.

3 Results

3.1 Immature density

Immature monarchs on ramets of the highly modular common milkweed, *A. syriaca*, showed three peaks during the weeks of June 5, July 17 and August 21 (Figure 1), consistent with the production of up to three generations of monarchs in Michigan as found at a similar latitude in Wisconsin [38,39]. There were 42 days between the first and second egg peak and 35 days between the second and third peak of eggs/ramet, which is consistent with the degree-day accumulation required for these generations to be produced by monarchs in Michigan rather than by successive immigrations of monarchs from further south [20].

3.2 Adult size

In June, July, August, September and one October sample 2011, we collected 434 adult monarchs (265 males and 169 females, Table 1). Wing lengths of adults did not vary significantly through these months (males ANOVA $F_{4,261} = 1.50$, $P = \text{NS}$ and females ANOVA $F_{3,165} = 0.84$, $P = \text{NS}$), and surprisingly we did not find significantly larger males than females in any month, other than August when males were significantly larger than females (Table 1).

In contrast, when we scaled size according to weight with both intact wing loads and lean wing loads (Table 1) we did find significant differences. Both males and females showed significantly different intact and lean wing loads by month (male intact wing loads by month ANOVA $F_{4,260} = 13.33$, $P < 0.0001$ and females ANOVA $F_{3,164} = 38.45$, $P < 0.0001$, with lean wing loads for males by month ANOVA $F_{4,252} = 8.65$, $P < 0.0001$ and females ANOVA $F_{3,157} = 29.08$, $P < 0.0001$). For both intact and lean wing loads these differences were mostly driven by the strong increase in wing loads in September because the butterflies accumulated fat reserves (see below) for the onset of southward migration [51,53]. We also found significantly higher intact and lean wing loads for females in June,

Figure 1. Changes in the density of monarch immatures on ramets of the common milkweed, *A. syriaca*, by date (weeks monitored) at 10 locations in Michigan in 2011 (Monarch Larval Monitoring Project data used with permission, based on citizen science monitoring of 10 sites and an average of 185.8 ramets searched per week). The numbers 1, 2 and 3 represent 3 successive generations of monarchs showing increasing overlap with time.

but significantly higher intact wing loads for males in August (Tables 1b and 1c) and no differences between males and females in July and September. However, the overall relationship between both intact and lean

wing loads and time (days) was described by significant second order polynomial regressions for both males and females (Figures 2 and 3 for intact and lean wing loads, respectively). These relationships show that both spring

Table 1. Anova comparisons of mean adult wing lengths, wing loading and wing wear by sex for each month.

a) Wing Length (mm)

Month	June		July		August		September	
sex	M	F	M	F	M	F	M	F
Mean	52.26	50.96	51.53	51.21	52.28	51.23	52.27	51.83
SE	0.41	0.61	0.35	0.52	0.17	0.22	0.35	0.29
N	31	14	54	24	154	95	26	36
F ratio	3.10		0.26		14.06		0.92	
P	NS		NS		0.0002		NS	

b) Wing Loading – intact weight (N/m²)

Month	June		July		August		September	
sex	M	F	M	F	M	F	M	F
Mean	0.46	0.55	0.48	0.50	0.47	0.42	0.61	0.60
SE	0.02	0.03	0.01	0.02	0.01	0.01	0.02	0.02
N	31	14	53	24	154	94	26	36
F ratio	6.14		1.01		17.87		0.12	
P	0.02		NS		<0.0001		NS	

c) Wing Loading – lean weight (N/m²)

Month	June		July		August		September	
sex	M	F	M	F	M	F	M	F
Mean	0.44	0.49	0.45	0.46	0.43	0.39	0.53	0.52
SE	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01
N	31	14	53	24	147	89	25	34
F ratio	4.84		0.24		15.59		0.31	
P	0.03		NS		0.0001		NS	

d) Wing wear (scale = 1 (new) to 5 (very worn)) (male by month $F_{4,260} = 7.68$, $P < 0.0001$; female by month $F_{3,165} = 17.43$, $P < 0.0001$)

Month	June		July		August		September	
sex	M	F	M	F	M	F	M	F
Mean	3.11	3.06	2.49	2.61	2.31	3.14	1.76	1.71
SE	0.16	0.24	0.15	0.22	0.08	0.11	0.12	0.10
N	31	14	53	24	154	95	26	36
F ratio	0.03		0.19		37.78		0.07	
P	NS		NS		0.0001		NS	

migrant arrivals and autumn departures have higher wing loading than mid-summer butterflies and that this relationship is stronger in females than in males.

3.3 Adult wing wear

Our measures of wing wear on a scale of 1 (fresh) to 5 (very worn) show that both males and females vary significantly through the months of June, July, August and September (males ANOVA $F_{4,260} = 7.68$, $P < 0.0001$; females ANOVA $F_{3,165} = 17.43$, $P < 0.0001$), and males show the same levels of wing wear as females, except in August when females were significantly more worn than males (Table 1d). Wing wear was highest in both males and females in June, with the exception of worn females in August that probably reflect

high levels of reproductive activity (mating and oviposition). Other than the August females, wing wear in our adult butterflies steadily decreased from June through July and August to September, when we found the least worn adults.

3.4 Female eggcounts

The distribution of chorionated eggs varied randomly with time (Figure 4), although there is some evidence that early female arrivals in June had low egg counts. All females collected in September (days 244-273, $N = 36$) contained no chorionated eggs (Figure 4), consistent with the onset of reproductive diapause [41-43]. However, all females sampled at the start of the season in southwest Michigan contained chorionated eggs.

Figure 2. The relationship between wing load (N/m^2) and time (day) for (a) females (Wing load = $-0.074 + 0.0022 \cdot \text{Day} + 0.000084 \cdot (\text{Day} - 224.65)^2$, $r^2 = 0.32$, $F_{2,167} = 38.55$, $P < 0.0001$) and (b) males (Wing load = $0.097 + 0.00166 \cdot \text{Day} + 3.66e-5 \cdot (\text{Day} - 218.56)^2$, $r^2 = 0.16$, $F_{2,264} = 24.05$, $P < 0.0001$).

Figure 3. The relationship between lean wing load (N/m^2) and time (day) for (a) females (Lean wing load = $0.099 + 0.00135 \cdot \text{Day} + 5.57e-5 \cdot (\text{Day} - 224.65)^2$, $r^2 = 0.26$, $F_{2,160} = 27.28$, $P < 0.0001$) and (b) males (Lean wing load = $0.2007 + 0.00105 \cdot \text{Day} + 2.44e-5 \cdot (\text{Day} - 218.56)^2$, $r^2 = 0.11$, $F_{2,256} = 15.03$, $P < 0.0001$).

3.5 Fat content of adults

Fat was measured as the percent of total dry weight in adult butterflies and when plotted against the day of collection (Figure 5) the data were described by a significant second order polynomial regression (fat (% dry weight) = $-13.44 + 0.088 \cdot \text{Day} + 0.0022 \cdot (\text{Day} - 220.93)^2$, $r^2 = 0.19$, $F = 47.35$, $P < 0.0001$). This regression indicates higher fat proportions at the start and end of the adult flight period in southwest Michigan. Fat content was also highest at the end of the flight period in September (days 244-273) consistent with a change from reproductive behavior to nectaring behavior

Figure 4. Number of chorionated eggs in females plotted against time (day)

Figure 5. Fat content of all adult butterflies as % of total dry weight against day. Polynomial regression, fat (% dry weight) = $-13.44 + 0.088 \cdot \text{Day} + 0.0022 \cdot (\text{Day} - 220.93)^2$, $r^2 = 0.19$, $F_{2,417} = 47.35$, $P < 0.0001$.

and the onset of southward migration in September [50,51,52,53].

3.6 Sequestered cardenolide content of adults

The patterns of variation in cardenolide concentrations with day of collection in both male and female monarchs were described by significant polynomial regressions (Figure 6) that show higher cardenolide concentrations at the start and end of the period of monarch presence in July (days 182-212) (female cardenolide concentration ($\mu\text{g/g}$) = $-109.71 + 0.734 \cdot \text{Day} + 0.0166 \cdot (\text{Day} - 224.65)^2$, $r^2 = 0.04$, $F_{2,159} = 3.08$, $P = 0.049$; male cardenolide concentration ($\mu\text{g/g}$) = $-76.047 + 0.557 \cdot \text{Day} + 0.0194 \cdot (\text{Day} - 218.56)^2$, $r^2 = 0.05$, $F_{2,250} = 7.19$, $P = 0.0009$).

Figure 6. Second order polynomial regressions of cardenolide concentrations ($\mu\text{g/g}$ dry weight) plotted against time (day) for (a) female (cardenolide concentration ($\mu\text{g/g}$) = $-109.71 + 0.73 \cdot \text{Day} + 0.017 \cdot (\text{Day} - 224.65)^2$, $r^2 = 0.04$, $F_{2,159} = 3.08$, $P = 0.049$) and (b) male monarchs (cardenolide concentration ($\mu\text{g/g}$) = $-76.05 + 0.56 \cdot \text{Day} + 0.019 \cdot (\text{Day} - 218.56)^2$, $r^2 = 0.05$, $F_{2,250} = 7.19$, $P = 0.0009$).

Although male monarchs are known to contribute important nutrients to females with their spermatophores when mating [55,56], it is not known whether they also contribute cardenolides that might influence the defensive effectiveness of both females and their offspring. Thus we measured the cardenolide content of the bursa copulatrix of all females and plotted these values against time (days) in Figure 7a, and also against each female source of the bursa (Figure 7b). Donated male cardenolides in the bursae showed no relationship with time (Figure 7a). However, there was a positive linear increase in bursa cardenolide concentration with female cardenolide concentration (Figure 7b, bursa cardenolide ($\mu\text{g/g}$) = $59.258 + 0.394 \cdot \text{cardenolide conc.}$, $r^2 = 0.144$, $F = 19.91$, $P < 0.0001$).

Figure 7. a) Variation of bursa copulatrix cardenolide concentrations ($\mu\text{g/g}$) with time and (b) cardenolide concentration of each bursa copulatrix plotted against its female cardenolide concentration (bursa cardenolide ($\mu\text{g/g}$) = $59.26 + 0.39 \cdot \text{cardenolide conc.}$, $r^2 = 0.14$, $F_{1,119} = 19.91$, $P < 0.0001$).

4 Discussion

We interpret our data as being consistent with an interpretation of seasonal or temporally variable, sequential partial migration in monarch butterflies. The short life span of monarchs allows successive generations to vary their life histories from migrant, through non-migrant and then back to migrant characteristics within the “window of opportunity” provided by abiotic conditions and day length cues and constraints. Our data for both intact and lean wing loading show distinctly curved relationships with high loads at the start of the season that drop to low loads in mid-season and then increase again at the end of the season (Figures 2 and 3). That lean wing loads also show this relationship suggests that the difference is not simply a result of fat physiology (Figure 5) required to fuel migration, especially in the autumn [51,52,53].

Our results and those of the Monarch Larval Monitoring Project (Figure 1) show evidence for three generations during the summer and changes in synchrony, with a pronounced initial peak of immatures around June 5, then two flatter generation peaks around the weeks of July 17 and August 21, much as described for monarch generations in Wisconsin [38,39]. This flattening of the peaks is probably generated by continual arrivals of migrants from the south and random, non-migratory movements of individuals throughout the Great Lakes region during the summer. Based on the work of Cockrell et al. [20], the degree-day accumulation between these generation peaks was sufficient for the peaks to be generated in-situ rather than to be dependent upon successive colonization events. This is consistent with a model of monarch migration that uses abiotic cues such as the spring equinox around March 21 to trigger northward movement from Mexico and the summer equinox around June 21 to signal monarchs to cease moving north. These equinoctial cues also coincide with the cool spring temperatures that Guerra and Reppert [18] found triggers northward flight in monarch butterflies, in addition to an antenna-dependent, time-compensated sun compass for overall orientation. These may also be the cues that monarchs use to trigger temporal variation in their partial migration life history across generations along a spectrum from migrant to non-migrant morphology, physiology and behavior.

Our evidence shows very little difference in adult wing size through the season in southwest Michigan which suggests to us that this is a single population of monarchs with plastic life histories consistent with sequential partial migration. We were also surprised that males and females

were very similar in size (table 1), which also suggests selection for wing lengths that favor migration in all three generations of the butterflies in southwest Michigan.

When we scaled size with butterfly weights with measures of intact and lean wing loads (Figures 2 and 3) we found that wing loads were highest at the start and end of the season because weights were higher at both the start and end of the season. This relationship was particularly strong for females (Figure 2a) which may be a reflection of female butterflies migrating in spring carrying loads of chorionated eggs and autumn butterflies carrying loads of sequestered fat. This finding provides evidence to refute the predictions of the “oogenesis-flight” syndrome [44-46] which predicts that migratory female insects should either migrate or lay eggs and that they cannot do both. The distribution of chorionated eggs in our female samples (Figure 4) also show that the first females sampled in June contained chorionated eggs and that they were likely to be both laying eggs and migrating into Michigan. The presence of chorionated eggs in females ceased in September (days 244-273, Figure 4), which is consistent with the onset of reproductive diapause and a shift to nectaring behaviors [41,50,51,53].

We were somewhat surprised that the monarch arrivals to southwest Michigan in June did not show lower wing wear scores consistent with very fresh butterflies (Table 1d). However, the mean scores of 3.11 and 3.06 for males and females, respectively, in June are exactly intermediate in the 1-5 scale suggesting that these butterflies had flown from spring breeding sites further south, but that they were not sufficiently worn to represent overwintered butterflies. As the season progressed, wing wear scores decreased, reflecting the emergence of new generations of monarchs.

Part of the change in wing loading was generated by the increase in fat content of all monarchs sampled, especially late in the season (Figure 5). As Masters et al. [51] and Brower et al. [53] argue this is likely to represent the sequestration of fat to fuel southward migration from sugars ingested by extensive nectaring at flowers.

We also expected the first monarch arrivals to show high cardenolide contents to reflect migrants from the south that had exploited the toxic southern milkweed, *A. viridis* [21,32]. However, our data in Figure 6 show highest cardenolide contents in August (days 213-243). There is an early influx of butterflies with fairly high cardenolide in June (days 152-181), which may reflect the arrival of *A. viridis*-derived monarchs. However, *D. plexippus* can sequester cardenolides more efficiently from *A. syriaca* than any other milkweed species from which cardenolide determinations have been performed [32,36] and Malcolm

[32] argues that this effective sequestration may reflect strong evolutionary selection provided by the abundance and both spatial and temporal distributions of *A. syriaca* for the migratory life history of the eastern North American population of *D. plexippus*.

We conclude that the plasticity of the monarch life history allows them to be partial migrants that vary in the proportions of migrants and non-migrants sequentially through successive generations. This plasticity allows monarchs to use migration to find spatially separated resources and to use predictable cues. This contrasts with longer-lived partial migrants in which migrants and non-migrants will be spatially separated either during breeding or during overwintering. Such an interpretation fits well with speculation about the origins of migration in monarchs in which they are thought to have originated with seasonal wet season-dry season migrations in tropical central America [63]. We (NRV and SM) have observed monarchs in the Dominican Republic where, like monarchs from Puerto Rico [60], they are thought to be non-migratory. However, in August along the coast temperatures become lethally hot for monarchs (above 33°C) and although there are extensive milkweed resources available in the form of actively growing, introduced *Calotropis procera* and evidence of monarch larval feeding, no larvae or adults are present. Instead the adult monarchs move to higher, cooler elevations where they lay eggs on native *Asclepias nivea*. To us, this is a migratory response to abiotic conditions and this response is likely to be characteristic of monarchs everywhere. The plasticity we describe in Michigan reflects the evolutionary shifts to spatially separated resources described by Young [63] for North America, but whether this occurs in Caribbean Islands, South America [4] or North America we think this represents temporally variable, or sequential partial migration.

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