Fueling the fall migration of the monarch butterfly

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Synopsis Monarch butterflies in eastern North America accumulate lipids during their fall migration to central Mexico, and use them as their energy source during a 5 month overwintering period. When and where along their migratory journey the butterflies accumulate these lipids has implications for the importance of fall nectar sources in North America. We analyzed the lipid content of 765 summer breeding and fall migrant monarch butterflies collected at 1 nectaring site in central Virginia over 4 years (1998–2001), and compared them with 16 additional published and unpublished datasets from other sites, dating back to 1941. Virginia migrants store significantly more lipid than summer butterflies, and show significant intraseason and between-year variation. None of the Virginia samples, and none of the historical samples, with one exception, had lipid levels comparable with those found in migrants that had reached Texas and northern Mexico. This evidence suggests that upon reaching Texas, the butterflies undergo a behavioral shift and spend more time nectaring. The one exceptional sample led us to the discovery that monarchs that form roosts along their migratory routes have higher lipid contents than monarchs collected while nectaring at flowers. We propose that for much of their journey monarchs are opportunistic migrants, and the variation within and between samples reflects butterflies’ individual experiences. The stored lipids appear to be of less importance as fuel for the butterflies’ migration than for their survival during their overwintering period, in part because soaring on favorable winds reduces the energetic cost of flying. The conservation of nectar plants in Texas and northern Mexico is crucial to sustaining the monarch’s migratory spectacle, and nectar abundance throughout eastern North America is also important. As generalists in their selection of nectar sources and nectaring habitats, monarchs are unlikely to be affected by small changes in plant communities. Agricultural transformations of natural communities in the eastern United States and Great Plains, however, and especially the extensive planting of genetically modified herbicide-resistant soybeans and corn, may be changing the availability of nectar for monarchs and other pollinators. This new technology is eliminating virtually all forbs in and surrounding agricultural fields, including the monarch’s larval hostplants (milkweeds) and native and nonnative nectar sources. To evaluate whether changes in nectar availability are altering the butterflies’ ability to accumulate energy, we recommend that monarchs’ lipid contents be assayed annually at sites throughout eastern North America.

Introduction Animals that migrate long distances face the challenge of fueling their travels. Many shorebird species alternate periods of heavy feeding, during which they accumulate large stores of lipid, with long-distance flights (Myers and others 1987). Red knots (Caladris canutus) replenish their lipids by feeding at a small number of critical stopover sites that have unusually rich food resources, for example, horseshoe crab eggs in Delaware Bay (Botton and Harrington 2003; Baker and others 2004). Other migrants, including many songbirds, make shorter flights and feed at multiple stopovers sites along their route (Yong and Finch 1997; Yong and Moore 1997).

The stopover ecology of animal migrants has received much less study than their ecology in their breeding and wintering habitats (Hutto 1998). An important concern for the conservation of animals that make long-distance latitudinal migrations is ensuring that fueling areas are adequately buffered from threats including habitat destruction, competition with exotic species, competition with humans for food or habitat, introduced predators, and changes in the quantity and quality of the food resource. The conservation of wintering and breeding habitats of migrant species will be fruitless if animals cannot survive the journeys between them (Myers and others 1987; Yong and Moore 1997; Baker and others 2004).

The migration of the monarch butterfly

In the late summer and autumn, monarch butterflies (Danaus plexippus L.) in eastern North America make an extraordinary long-distance migration. Butterflies weighing ~500 mg fly up to 4000 km from breeding areas in the United States and Canada to wintering areas in the mountains of central Mexico (Brower
from November through March the monarchs aggregate in massive numbers (up to 50 million per hectare, Brower and others 2004) in a dozen or so colonies with a combined area that has varied yearly from 2 to 21 hectares (Garcia-Serrano and Mora Alvarez 1999; Garcia-Serrano and others 2004; Rendon-Salinas and others 2006). They spend much of the time quiescent in dense clusters on tree branches and trunks, but they periodically fly to water sources and reform their clusters after dislodgement by storms. Entire colonies usually move down slope as winter progresses (Calvert and Brower 1986). In March, the surviving butterflies become reproductively active, mate, and fly back to the southern United States (up to 2000 km from the overwintering areas) where females lay eggs on newly emergent milkweeds and begin a new generation. Two or three summer generations repopulate the eastern United States, before a new fall generation enters reproductive diapause and heads to Mexico (Herman 1985; Malcolm and others 1993).

Monarch butterflies obtain sugar from nectar, which they convert to lipid, as their energy source. During the migration, large numbers of monarchs are seen nectaring wherever nectar is abundant: in fields of goldenrod and aster, in fields of clover and alfalfa, and along streams and nonherbicided roadways. Once the butterflies have reached the wintering areas in Mexico they do little feeding, and they maintain themselves by metabolizing lipid reserves (Alonso-Mejia and others 1997). At the end of the winter, the degree to which the migrants refuel on their way north is unknown. Urquhart (1960) assumed that spring migrants rarely feed on nectar, but this assumption was based on little data and an incomplete understanding of the migration (Brower 1995). During the fall migration, therefore, the butterflies must accumulate energy not only for the flight itself, but also for the 5 month wintering period and, possibly, for the spring northward flight (Brower 1985).

Lipids in the life cycle of the monarch butterfly

In common with many migratory animals (Perrins and Birkhead 1983), monarch butterflies use lipids as energy storage molecules (Brown and Chippendale 1974). Lipids provide more energy per weight than sugar and can be stored by animals in an anhydrous form, and are therefore the most efficient energy source for flights of long duration (Weis-Fogh 1961; Griffin 1975). Beall (1948) first addressed the question of the relative importance of glycogen and lipid as energy stores in the monarch butterfly. In Ontario migrant collections, he found highly variable lipid weights but relatively constant lean weights. Since the lean weight includes glycogen but showed little variation, he reasoned that monarchs did not accumulate substantial glycogen reserves to fuel their migration. Cenedella (1971) and Brown and Chippendale (1974) found only trace amounts of glycogen in migrating monarchs, in contrast to large quantities of triacylglycerides stored in the fat body. Thus, although glycogen may be of some energetic importance in short-term exertion, the evidence indicates that its role as a substantial migratory energy reserve for monarch butterflies is minimal.

Lipid stores in relation to life stages and migratory condition have been measured for monarch butterflies from California (Tuskes and Brower 1978; Chaplin and Wells 1982), eastern North America (Beall 1948; Cenedella 1971; Brown and Chippendale 1974; Walford 1980; Brower 1985; Cohen 1985; Brower and Malcolm 1991; Gibo and McCurdy 1993; Alonso-Mejia and others 1997) and Australia (James 1984, 1986). Summary statistics from most of these studies are tabulated in the Appendix of Alonso-Mejia and colleagues (1997). To put the following data in perspective, adult monarch butterflies weigh 300–900 mg, with dry weights of 125–350 mg, lean dry weights of 90–220 mg, and lipid weights of 0–296 mg.

Monarchs accumulate energy as caterpillars that is not all metabolized during a brief pupal stage, and adults eclose with small stores of lipids. Freshly eclosed monarch butterflies from Massachusetts had 5–57 mg of lipid (mean 29 mg; Walford 1980), and similar values have been obtained for freshly eclosed butterflies from Ontario (Beall 1948), Florida (Cohen 1985) and Australia (James 1984). The summer breeding generations feed on nectar and maintain low lipid levels during the weeks in which they are dispersing, mating, and ovipositing. The mean lipid mass of monarchs breeding in western Massachusetts in July and August 1979 was 19 mg (range = 3–80 mg; Walford 1980; Brower 1985).

Fall migrants in eastern North America differ from the summer breeding generations by entering reproductive diapause and converting the sugar obtained by nectaring into large but highly variable lipid stores. By the time they reached Texas and north Mexico in October, butterflies had a mean lipid mass of 126 mg (Walford 1980; Brower 1985), and 2 collections made in the Mexico overwintering areas soon after butterflies arrived in November 1982 and 1993 had mean values of 142 and 133 mg (Alonso-Mejia and others 1997). The maximum lipid level so far recorded for an individual migrant is 296 mg (Texas, October 1993, in Alonso-Mejia and others 1997). The maximum level we have measured in an overwintering
butterfly from Mexico was 270 mg (January 1977). By March, lipid levels of butterflies at the overwintering sites had decreased to 59 mg in 1978 (Walford 1980) and to 56 mg in 1994 (Alonso-Mejia and others 1997). The butterflies that survive the winter reach the southern United States with lower lipid levels than butterflies in the colonies in March (Alonso-Mejia and others 1997), indicating that some of the energy reserves remaining after overwintering are used during the northward migration. Whether they supplement the lipid with sugars obtained by nectaring during the spring remigration is unknown.

Previously hypothesized patterns of fall lipid dynamics

Based on the data available to them, Beall (1948), Brower (1985), and Gibo and McCurdy (1993) proposed different models of the timing of lipid accumulation by eastern migrant monarchs, in relation to butterfly age and geographic location along the migration route to Mexico.

Beall (1948) measured high lipid levels in fall butterflies from Ontario (sample means ranged from 39 to 149 mg, grand mean 78 mg), very low levels in late fall butterflies from Louisiana (from 2 to 20 mg), and intermediate levels in winter butterflies from California (43 mg). In 1948 the Mexican wintering sites were not known, and the relationships among monarchs in different regions of North America were not well understood (Brower 1995). Beall hypothesized that fall butterflies rapidly accumulated high levels of lipids which they depleted during their southward migration. Taking into account the higher lipid levels in California wintering butterflies, Beall hypothesized that some butterflies accumulated additional lipids once in their wintering areas.

Walford (1980) conducted an extensive analysis of the lipids in breeding, migrant, and overwintering monarchs. In contrast to Beall’s Ontario migrants, samples of fall migrants from Massachusetts, New Jersey, Kansas, and Florida had means of 18–29 mg lipid. Also in contrast to Beall’s finding of low lipids in Louisiana fall butterflies, fall generation butterflies collected from central Texas and central Mexico had high lipid levels, with a mean of 126 mg. Integrating Walford’s data with insights gained from the early years of research in the Mexican overwintering sites, Brower (1985) rejected Beall’s model of monarchs building up lipid reserves before heading south, and concluded that they accumulate most of their lipids when they reach Texas, and in north and central Mexico.

Gibo and McCurdy (1993) focused on the differences between Beall’s and Brower’s conclusions. Emphasizing the temporal component of the migration, they grouped the Ontario monarchs they collected in fall 1986 into early phase, middle phase, and late phase samples. Lipid mass increased from early to middle phase migrants, and decreased from middle to late phase migrants. Gibo and McCurdy proposed that early in the migration, recently emerged individuals would have little lipid because they have not had much time to forage. In the middle phase, the proportion of adults that have been foraging for 1 or more weeks would increase, and they would have accumulated large reserves. The late phase migrants would have lower lipid reserves as deteriorating weather conditions and decreased nectar availability force them to consume some of the energy stores they had already accumulated. Gibo and McCurdy proposed that if this pattern were found all along the migration route, the differences between Beall’s and Walford’s samples could result primarily from differences in the migration phases during which the samples were collected.

In summary, the 3 models that have been proposed for the accumulation of lipids in migratory monarchs differ in proposing that monarchs accumulate the bulk of their stored lipids soon after eclosion (Beall 1948), within a few weeks of eclosion (Gibo and McCurdy 1993), or after they have reached Texas and the Gulf coast (Brower 1985).

Conservation implications

Concern has been raised that many insect pollinators may be in decline (Buchmann and Nabhan 1996; Allen-Wardell and others 1998), although this is proving to be difficult to evaluate (Cane and Tepedino 2001; Ghazoul 2005). Variability in the distribution, phenology, and abundance of nectar sources will affect pollinators, both year to year as weather conditions vary, and, over a longer time frame, as a result of climate change, ecological interactions with exotic species, shifting agricultural practices, and other land use modifications (Cane and Tepedino 2001). Nabhan (2001, 2004) has drawn attention to the particular vulnerability of migratory insect, bird, and bat pollinators, which may be dependant upon very localized nectar corridors along their routes.

Understanding where and when the monarch butterfly requires nectar during its fall and spring migrations, and monitoring the nectar resources in these areas, will contribute to maintaining the quality of migrating monarchs, and conservation planning in general. The migration and wintering biology of the eastern population of the monarch butterfly has been labeled an “endangered biological phenomenon” (Brower and Malcolm 1991) due to continual loss
and degradation of winter habitat in Mexico (Brower 1996, 1999; Brower and others 2002; Honey-Roses and Galindo 2004) and alteration of larval milkweed habitat by agricultural practices (Brower 1995, 1999, 2001; Oberhauser and others 2001; K. Oberhauser, personal communication).

In this paper we will examine the fueling behavior of migrating monarch butterflies, and address the significance of our findings for conservation. We will evaluate alternate hypotheses for the timing and geography of migratory feeding, based on 4 years of censuses and butterfly lipid measurements made during the fall migration through central Virginia.

Methods
Censuses
Butterfly censuses were conducted for 4 years, from August through October, at 2 large patches of buddleia (Buddleia davidii) on the Sweet Briar College campus, Amherst County, Virginia (37° 32’ 43” N, 79° 05’ 38” W, elevation 240–270 m). One 100 m long strip of shrubs was in the median of a parking lot. A larger, X-shaped patch of 79 buddleia shrubs was planted on the edge of a large hayfield in spring 1998. The 4 arms of the cross were each 22 m long by 3 m wide and pointed north, south, east, and west.

We censused each patch once daily. Most censuses were done between 1100 and 1500; occasional censuses were done earlier or later, but only when the air temperature was well above the butterflies’ flight threshold. The observer recorded temperature, wind, and cloud conditions, and then walked slowly around the full perimeter of each patch counting all monarchs seen at the flowers or flying in the area. The data presented are the total butterflies counted at the 2 patches each day. By circling the patch we risked double-counting some butterflies, but our method was standardized and we periodically checked the consistency of observers by having them make simultaneous but independent counts. When there were large numbers of monarchs at a patch, the perimeter was circled twice and the higher of the 2 counts was recorded.

In 1998–2000 we censused through October 31, but in 2001 the Buddleia blossoms had senesced by October 22 and we terminated the census on this date. For 1998–2000, 97–99% of the full seasons’ totals had been counted by October 22.

Butterfly collections
On several dates each season we netted all of the butterflies we could. Summer butterflies were collected both from the buddleia and while they were cruising and nectaring on milkweeds (A. syriaca) in the adjacent field; fall butterflies were collected from the buddleia. Each butterfly was put in an individual glassine envelope and at the end of the collecting period the butterflies were brought to the lab. On warm days the butterflies were stored in the field in a cooler with an ice pack or ice. Within a few hours after capture, each butterfly was weighed to the nearest 0.1 mg, killed by freezing, and stored in a freezer until analyzed. Wing condition was rated on a 4 point scale (1 = fresh, bright colors, and few missing scales; 4 = many missing scales, color dull), forewing length was measured with a ruler to the nearest 0.5 mm, and the mating status of females was determined by abdominal palpation (Van Hook 1999). Forewing length was not measured in 1998.

Lipid analyses
Lipid extraction followed Alonso-Mejia and colleagues (1997). The previously frozen butterflies were dried for 16 h at 60°C in a forced air oven. The dry weight of each butterfly was determined, and the butterfly was then placed into a 35 ml centrifuge tube and crushed with a glass rod. Ten milliliters of petroleum ether was added and the butterfly was ground for 2 min with a Janke-Kunkel SDT1810 Ultra-Turrax tissueizer. The capped tube was vortexed for 10 s and placed in a shaker bath at 35°C for 30 min. Every 10 min the tube was removed and vortexed for 10 s. The tube was then centrifuged for 10 min at 1000 rpm (Dynac centrifuge), and the supernatant was pipetted into a preweighed aluminum pan. Twenty additional milliliters of petroleum ether was then added to the centrifuge tube, the extraction procedure was repeated, and the supernatant was added to the aluminum pan. Aluminum pans were placed on a slide warmer at 30°C overnight to evaporate the petroleum ether and the mass of the pan plus lipid was then determined. Lipid weight was obtained by subtracting the pan weight, and lean mass was determined by subtracting the lipid weight from the dry weight of the butterfly.

Gibo and McCurdy (1993) used 2:1 chloroform/methanol, rather than petroleum ether, as the solvent to extract lipids, as have several other monarch butterfly studies (Cenedella 1971; Brown and Chippendale 1974; Tuskes and Brower 1978; James 1984, 1986). We used 2 pooled samples of butterflies to compare the lipid mass extracted with the 2 solvent systems. In each pool, 6 females were dried, ground together, and the powder was divided into 6 samples. Three samples were extracted with 2:1 chloroform/methanol, and 3 with petroleum ether. One set used monarchs reared in the laboratory on Asclepias curassavica, which have low lipid stores. The second set used butterflies collected in Sierra Chincua,
Statistical analyses

Data were analyzed using JMP 4.0 (SAS Institute Inc. 2001). Lipid data were skewed and variances were highly heterogeneous, and therefore groups were compared with nonparametric Kruskal–Wallis (3 or more samples) or Wilcoxon rank-sum (2 samples) tests. Kruskal–Wallis tests were followed by multiple comparison tests as outlined by Siegel and Castellan (1988), using an alpha level of 0.05. Unless indicated otherwise, the remaining data were analyzed with t-tests or ANOVA, log10 transformed when necessary (variances were tested for homogeneity with Levene’s test). Multiple comparisons among means used the Tukey–Kramer honestly significant difference test, using an alpha level of 0.05.

Results

Migration samples

The individual lipid mass of the 765 Virginia butterflies ranged from 0 to 169 mg. To analyze the variation in lipid mass through time, we used the temporal pattern of the migration to assign butterflies to 4 groups: (1) Summer (August): Many of these butterflies showed some wing wear and females were virtually all mated. Butterflies were mating, cruising, and ovipositing on Asclepias syriaca in a field adjacent to the larger buddleia patch, as well as nectaring from the buddleia. (2) Late summer/early migrants (early September): Wing condition was variable: the population included both worn and fresh individuals. Mating pairs were observed at or near the nectar sites, but some females were unmated. This sample includes all migrants collected prior to the first notable migration day (defined below). (3) Middle migrants (late September to mid-October): All butterflies captured between the first and last notable migration days. (4) Late migrants (mid-October to early November): Butterflies captured after the last notable migration day.

The migration through central Virginia

1998–2001

The migration through central Virginia peaked between late September and early October in all 4 years, but the magnitude and pattern of the migration varied (Fig. 1 and Table 1). Most butterflies censused before September 1 were summer breeders. Table 1 compares summary statistics for the migration between September 1 and October 22 each year. We followed Walton and colleagues (2005) in defining “notable” migration days as those having counts greater than the seasonal daily average (total monarchs/number of census days), “waves” as 1 or more successive notable days, and “peak” days as those with >5.5% of the annual season’s total [this value equals the average of the 4 seasons’ mean daily percentages (2.1%) plus the average of the 4 standard deviations of the daily counts (3.4%)].

In 1998 a small generation of local August breeders, identified by their worn wing condition and by palpation of females indicating that they were mated, was separated by almost 4 weeks from the fall migrants, identified by their fresh wing condition. Migrants arrived in 5 waves, the second of which, from September 28 to October 2, brought the largest numbers (44% of the season total).

The 1999 migration was more spread out than in other years. A much larger local summer breeding population overlapped temporally with the migrants.
From August 26 to 30, most butterflies were worn and most females were mated, but on August 31 almost all butterflies were fresh. Daily counts fluctuated through the entire migration season, with 8 waves, the largest of which accounted for just 16% of the season total. Butterflies were present on every census day except one.

In 2000 there were very few local breeders. The size of the migration, measured as total butterflies counted and number of days with zero butterflies, was the lowest of the 4 years. The wave from October 2 to 4 accounted for 46.5% of the season total; if the wave is considered to extend to October 6, it accounted for 68% of the season total.

In 2001, as in 1999, a sizeable summer breeding population overlapped with the migrants. The migration itself was the largest of the 4 years. We counted more than twice as many monarchs as in 1999, the second highest year (Table 1). Two very large waves came through, the first, from September 17 to 25, accounting for 33% of the season total; and the second, from October 1 to 5, accounting for 50%.

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### Lipid analyses of Virginia migrants 1998–2001

The dates of collection and number of butterflies collected for lipid analyses are indicated in Table 2. We collected butterflies in all 4 categories in 1999 and 2001, but in 1998 and 2000 there were almost no summer or early migrants to collect. The strong male bias in most of the collections is representative of the apparent sex ratio of butterflies visiting the buddleia patches.

The results are summarized in Table 3 and Figure 2. Lipid mass values for males and females did not differ (Wilcoxon rank-sum tests, $P > 0.7$ each year; $z = 0.28, P > 0.75$ for all years combined), so the sexes were combined for analyses. Lean weights of males were significantly higher than females (Welch ANOVA for unequal variance, $t = 3.86$, df $= 490$, $P < 0.001$ for all years combined), so the sexes were analyzed separately.

#### Summer versus migrant samples

Migrant butterflies had significantly more lipid than summer butterflies (Fig. 2 and Table 3) in both 1999 and 2001. The mean lipid content of the summer breeders in both years was 17 mg (range $= 0–92$ mg); the mean lipid content of migrants was 44 mg in 1999 and 45 mg in 2001 (range $= 4–169$ mg). The lipid content of summer butterflies was highly skewed: 81% had $<20$ mg and only 4% had $>40$ mg. In contrast, only 32% of the migrants had $<20$ mg of lipid and 37% had $>40$ mg.

Lean weights of summer breeders were lower than migrants for both sexes in both 1999 and 2001; the differences were statistically significant for males in both years and for females in 1999.

#### Early, middle, and late migrants

Butterflies collected at different stages of the migration did not reveal consistent changes in lipid mass (Fig. 2 and Table 3). In 1998 and 1999 the late migrants had significantly less lipid than middle migrants, but in 2000 and 2001 there was no drop in lipid content between the middle and late migrant samples.

In 1999, 2000, and 2001, within the migrant generation there were no significant changes in lean weight over the course of the migration (Table 3), but this conclusion should be interpreted cautiously due to the small sample sizes for some groups. (A laboratory error prevented the calculation of lean weights for most of the 2001 middle migrant butterflies.) In 1998 the lean weight of the 4 late migrant females was significantly

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### Table 1 A comparison of the fall migration of monarch butterflies at Sweet Briar VA, from September 1 to October 22, 1998–2001

<table>
<thead>
<tr>
<th>Year</th>
<th>1998</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Census days</td>
<td>51</td>
<td>46</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Total monarchs</td>
<td>1081</td>
<td>1262</td>
<td>628</td>
<td>2733</td>
</tr>
<tr>
<td>Average monarchs per census day</td>
<td>21.2</td>
<td>27.4</td>
<td>14</td>
<td>58.1</td>
</tr>
<tr>
<td>Number of days with 0 monarchs</td>
<td>7</td>
<td>1</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>Number of notable migration days</td>
<td>13</td>
<td>17</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>Number of migration waves</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Largest wave as % of total</td>
<td>44</td>
<td>16</td>
<td>46.5</td>
<td>50</td>
</tr>
<tr>
<td>Dates of largest wave</td>
<td>9/28 to 10/2</td>
<td>10/1 to 10/3</td>
<td>10/2 to 10/4</td>
<td>10/1 to 10/5</td>
</tr>
<tr>
<td>Number of peak migration days</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

The daily census data from August 1 to October 31 are available online as Supplementary Table 1.

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lower than the middle migrants, but the males did not show such a difference.

Year to year variation

Because the lipid mass varied over the season, the middle migrants were used for between-year comparisons of lipid mass. In 3 of the sampling years (1998, 1999, and 2001) the mean lipid content of butterflies was similar (mean = 43–49 mg; Table 3). The butterflies collected in 2000, however, had significantly less lipid (mean = 24 mg).

For lean weight comparisons among years, the early, middle, and late migrant samples were combined. For males, most years did not differ, but the lean weight of the 2001 butterflies was significantly lower than the 1998 butterflies. No transformation succeeded in making the variances of the males’ lean weights homogeneous; this dataset, therefore, was analyzed with a Kruskal–Wallis test. Females in 2001 also had the lowest lean weight, but the difference among years was not statistically significant (ANOVA, $F_{2,187} = 1.62, P = 0.19$). Wing lengths did not differ significantly in 1999, 2000, and 2001 [wing lengths were not measured in 1998; ANOVA of log_{10}(forewing length), females, $F_{2,123} = 2.36, P = 0.10$; males, $F_{2,267} = 2.01, P = 0.14$].

Lipids and wing condition

For summer butterflies, the individuals showing more wing wear had lower lipid levels than individuals in fresh condition. Of 180 summer butterflies, 18% were assigned a wing condition of 1 (fresh), 46% were condition 2, and 36% were conditions 3–4 (worn). The lipid mass for butterflies with wing conditions 1 (median = 18 mg) and 2 (median = 14 mg) differed significantly from butterflies with wing conditions 3–4 (median = 12 mg; Kruskal–Wallis test, $\chi^2 = 11.46, 2$ df, $P = 0.0032$).

We collected too few migrant butterflies with appreciably worn wings to determine if there is a relationship between lipid content and wing condition for this generation. Of 580 migrants, only 13 had wings of condition 3 and 3 had wings of condition 4. These 16 worn butterflies had low lipid levels, with a mean of only 12 ± 6.1 mg of lipid (median = 10.5 mg); however, most of these were probably late summer breeders rather than migrants. All were collected in 1999 and 2001, which were the years with large local summer populations, and 11 of these were from the late summer/early migrant samples.

Chloroform/methanol and petroleum ether as extraction solvents

The 2:1 chloroform/methanol extracted more lipid than the petroleum ether. For A. curassavica-reared butterflies, 36 ± 0.3 mg of lipid was extracted by chloroform/methanol (mean ± standard error for 3 replicates) and 21 ± 0.2 mg by petroleum ether.
For the Mexican butterflies, 122 ± 0.6 mg was extracted by chloroform/methanol, and 103 ± 0.8 mg by petroleum ether. The differences between the 2 methods (15 and 18 mg) were similar for the low lipid and high lipid samples. If lipid mass is expressed in milligrams per butterfly, the overestimate from the chloroform/methanol method will be constant. Many papers present lipid data as percent of total weight or percent of lean weight; for these, the percent error will be greater for butterflies with low lipid levels.

### Discussion

#### Geographic and temporal patterns of lipid storage

The Virginia data do not support Beall’s (1948, p 92) proposal that “very considerable fat reserves must be at once laid down” by young migrants. Neither, however, do they conform to the model of Brower (1985) that butterflies accumulate little lipid before reaching Texas. Virginia migrants had accumulated considerably more lipids than the summer generation (Table 3), but still much less than Texas migrants and Mexican overwintering monarchs (Brower 1985; Alonso-Mejia and others 1997). For example, 32% of the Virginia migrants had 20 mg of lipid and only 4% had >110 mg; in contrast, 0% of overwintering butterflies in November 1982 and 1993 had 20 mg, and 65–79% had >110 mg (Fig. 3R and S).

Figure 3 collates the available lipid data from 15 separate fall migrant samples and includes summer breeding samples from Massachusetts (Fig. 3C) and Virginia (Fig. 3H) and overwintering samples from Mexico (Fig. 3R and S) for comparison. In addition to the data from this study and from Walford (1980), Figure 3 includes data from Beall (1948), Brown and

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**Table 3** Summary statistics for monarch butterfly lean and lipid mass, Sweet Briar, VA, 1998–2001

<table>
<thead>
<tr>
<th></th>
<th>Lean weight (mg) female</th>
<th>Lean weight (mg) male</th>
<th>Lipid weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean ± SD</td>
<td>1</td>
</tr>
<tr>
<td>Summer versus all migrant butterflies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>19</td>
<td>140 ± 17</td>
<td>a</td>
</tr>
<tr>
<td>Migrants</td>
<td>74</td>
<td>171 ± 28</td>
<td>b</td>
</tr>
<tr>
<td>2001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>28</td>
<td>157 ± 26</td>
<td>a</td>
</tr>
<tr>
<td>Migrants</td>
<td>19</td>
<td>167 ± 25</td>
<td>a</td>
</tr>
<tr>
<td>Migrants only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle migrant</td>
<td>60</td>
<td>180 ± 28</td>
<td>a A</td>
</tr>
<tr>
<td>Late migrant</td>
<td>4</td>
<td>147 ± 13</td>
<td>b</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early migrant</td>
<td>15</td>
<td>170 ± 16</td>
<td>a</td>
</tr>
<tr>
<td>Middle migrant</td>
<td>59</td>
<td>172 ± 30</td>
<td>a A</td>
</tr>
<tr>
<td>Late migrant</td>
<td>0</td>
<td>4</td>
<td>185 ± 33</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle migrant</td>
<td>21</td>
<td>171 ± 19</td>
<td>a A</td>
</tr>
<tr>
<td>Late migrant</td>
<td>12</td>
<td>172 ± 17</td>
<td>a</td>
</tr>
<tr>
<td>2001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early migrant</td>
<td>5</td>
<td>150 ± 31</td>
<td>a</td>
</tr>
<tr>
<td>Middle migrant</td>
<td>12</td>
<td>164 ± 6</td>
<td>a A</td>
</tr>
<tr>
<td>Late migrant</td>
<td>12</td>
<td>174 ± 22</td>
<td>a</td>
</tr>
</tbody>
</table>

1Within a year, rows with different lowercase letters are significantly different (P < 0.05). Lean weight data were analyzed using an unpaired t-test (2 groups) or ANOVA (3 groups). Lipid data were analyzed using the Wilcoxon rank-sum test (2 groups) or Kruskal–Wallis test (3 groups). Among years, years with different uppercase letters are significantly different. For lean weight, the early, middle, and late migrant samples were combined for the comparison. For lipid weight, only the middle migrant samples were compared. Female lean weights were compared with ANOVA. Male lean weights and lipid weights were compared with the Kruskal–Wallis test.

2Lean weights were not calculable for 16 additional butterflies.
The samples are arranged roughly by latitude and they reveal substantial spatial and temporal heterogeneity in lipid accumulation.

Methodological differences in lipid analyses

Differences in lipid extraction protocols (Dobush and others 1985; Christie 1993) mean that caution must be used in comparing Figure 3A (from Beall 1948), 3B (from Gibo and McCurdy 1993), and 3K (from Brown and Chippendale 1974) to the remaining samples, which were analyzed in our laboratory using the method described in this paper. Gibo and McCurdy (1993) and Brown and Chippendale (1974) used 2:1 chloroform–methanol as the solvent rather than petroleum ether, and Beall (1948) used diethyl ether and a different extraction method (a Bailey–Walker fat extraction apparatus). Chloroform–methanol extracts polar phospholipids as well neutral lipids (Christie 1993), and can also extract considerable amounts of nonlipid contaminants (Dobush and others 1985). The polar lipids are not used by monarchs as an energy source (Brown and Chippendale 1974), and therefore petroleum ether is the more appropriate solvent unless further fractionation will be done. We determined that using chloroform/methanol results in a 15–18 mg overestimate of available lipids. This is similar to the values obtained in previous studies that fractionated the total lipids that were extracted from samples of migrant monarchs with 2:1 chloroform/methanol. Cenedella (1971) determined that the butterflies contained 14 mg phospholipid and 86 mg neutral lipid, and Brown and Chippendale (1974) measured 8 mg phospholipid and 129 mg neutral lipid. In constructing Figure 3B and K, we subtracted 10 mg from the values of Gibo and McCurdy (1993) and Brown and Chippendale (1974). (Several datasets used to construct Figure 3 were only available as histograms with differing intervals; we therefore could not adjust Gibo and McCurdy’s data by 13 mg, the average from the 3 experiments.) Beall’s lipid values were not adjusted, because Dobush and colleagues (1985) determined that variation in extraction methods was less problematic than variation in solvents, and that diethyl ether and petroleum ether did not differ in the percent lipid they extracted.
Fig. 3 Lipid mass distribution in 19 samples totaling 2431 monarch butterflies from eastern North America. The samples are arranged roughly by latitude. They indicate variable lipid accumulation during the fall migration, with the exception of consistently high lipid levels in butterflies collected in Texas and northern Mexico. Means are given in parentheses. (No mean was provided for sample B.) Open bars indicate summer-breeding samples, dark gray bars indicate fall migrant samples and light gray bars indicate overwintering samples. Locations and dates of collections (sample sizes): (A) “Point aux Pins” (Rondeau Provincial Park) Ontario, September 7, 1943 (N = 67). (B) Missisauga Ontario, August–October 1986 (N = 234). Ten milligrams have been subtracted from these values; see text for explanation. (C) Amherst, MA, July 21 to August 17, 1979 (N = 230). (D) Amherst, MA, Aug 18 to September 28, 1979 (N = 190). (E) Eastern Point, MA, October 4, 1979 (N = 105). (F) Beach Haven NJ, September 30, 1979 (N = 14). (G) Cape May NJ, September 29, 1979 (N = 36). (H) Sweet Briar, VA, August 1999 and 2001 (N = 184). (I) Sweet Briar, VA, September 1 to October 31, 1998, 1999, 2001 (N = 488). (J) Sweet Briar, VA, September 1 to October 31, 2000 (N = 93). (K) Boone County, MO, October 2–4, 1972 (N = 18). Ten milligrams have been subtracted from these values; see text for explanation. (L) Lawrence, KS, September 23, 1979 (N = 122). (M) Lighthouse Point, Gulf Coast, FL, October 28–29, 1979 (N = 108). N. Austin, TX, October 10, 1979 (N = 42). (O) Windemere, TX, October 11, 1979 (N = 66). (P) Uvalde, TX, October 11, 1982 (N = 59). (Q) San Javier, Queretaro, Mexico, October 31, 1977 (N = 101). (R) Sierra Chincua, Michoacan, Mexico, November 9, 1982 (N = 174). (S) Sierra Chincua, Michoacan, Mexico, November 8, 1993 (N = 100). Sources: Beall (1948) (A); Gibo and McCurdy (1993) (B); Walford (1980) (C–G, L–O, Q); this study (H–J); Brown and Chippendale (1974) (K). Previously unpublished data from our lab (P). Data from our laboratory, used in Alonso-Meja and colleagues (1997) (R and S). Additional details about each collecting site are provided in Supplementary Table 2.
Lipids in migrating monarch butterflies

Variability among samples north of Texas
Brower (1985) based his model of lipid dynamics on 8 migrant samples collected in 1979 in Massachusetts, New Jersey, Kansas, Florida, and Texas, and one 1977 sample from northern Mexico (Walford 1980). Five of these, from western Massachusetts (Fig. 3D), eastern Massachusetts (Fig. 3E), Cape May NJ (Fig. 3G), Kansas (Fig. 3L) and Florida (Fig. 3M) had positively skewed distributions in which few butterflies had >50 mg of lipid. In contrast, the 2 samples from Texas (Fig. 3N and O) had negatively skewed distributions, with most butterflies having >50 mg of lipid.

In 1979, therefore, migrating monarch butterflies apparently had low lipid levels throughout much of the eastern United States but accumulated substantial lipids in Texas. The Virginia sample from 2000 had a similar distribution to the 1979 samples (compare Fig. 3J and E), but other samples had significantly higher lipid levels. Samples from Ontario in 1986 (Fig. 3B), Virginia in 1998, 1999, and 2001 (Fig. 3I), and Missouri (Fig. 3K) had a significantly higher proportion of butterflies with >50 mg of lipid. These 3 samples did not differ from one another ($\chi^2 = 14.6, df = 8, P = 0.068$) but differed significantly from the five 1979 samples listed above ($\chi^2 = 168, df = 4, P < 0.001$). Two samples of butterflies, from Ontario in 1943 (Fig. 3A) and Beach Haven NJ in 1979 (Fig. 3F), had still higher lipid amounts (see below).

In summary, north of Texas there is substantial heterogeneity in lipid profiles, that does not fall easily into a geographic or temporal pattern. Samples collected in the northern part of the monarch’s range, in Ontario and Massachusetts, do not differ consistently from mid-latitude samples from New Jersey, Virginia, Missouri, and Kansas; nor do mid-western samples (Missouri, Kansas) differ from east coast samples. Gibo and McCurdy (1993) proposed that butterflies collected early and late in the season at a particular site would have lower lipids than samples collected in the middle of the migration. This temporal pattern was found in Virginia migrants in 1998 and 1999, but not in 2000 or 2001 (Table 3; Fig. 2). Among the remaining fall samples, most were collected entirely or primarily during the middle of the migration at the collection sites (see Supplementary Table 2). The samples from eastern Massachusetts (Fig. 3E), Missouri (Fig. 3K), and Florida (Fig. 3M) were collected later in the migration. Two of these had low lipid levels, but they were not different from other samples collected in the same year during the middle of the migration, and the Missouri sample had higher lipid levels than many of the middle migration samples. Thus, although Gibo and McCurdy (1993) are probably correct that many late season butterflies are unable to accumulate lipids because they encounter deteriorating weather and/or dwindling nectaring opportunities, their hypothesis does not explain the heterogeneity in lipid levels among the full array of samples.

The September 7, 1943, Ontario sample analyzed by Beall (Fig. 3A) is markedly different from all other samples north of Mexico, with >75% of the butterflies having >110 mg lipid, and 0% having <20 mg. Beall (1948) chose this sample for intensive analysis specifically because he noted that it had the fattest butterflies of the season. He also analyzed additional pooled samples of migrant butterflies on 8 different dates in 1940, 1941, and 1943. These had average lipid masses ranging between 39 and 136 mg per butterfly. The highest value was on September 7, 1943, the same date as the group he analyzed individually (Fig. 3A). The pooled samples show within-season and between-year heterogeneity. Butterflies collected on August 31, 1943, were also fat, averaging 101 mg, but butterflies collected on September 15, 1943, averaged only 67 mg lipid. The 1940 and 1941 butterflies had less lipid than 1943 (1940: 39, 72, and 48 mg; 1941: 58 and 72 mg). Even taking the variability within his samples into consideration, Beall’s Ontario samples are consistently heavier than the majority of other samples from north of Texas. If the explanation for the difference was that Beall’s extraction method pulled out other molecules in addition to neutral lipids, the lean weights of his samples would be low, but they are, in fact, similar to other migrant samples (Alonso-Mejia and others 1997 and this study). We cannot rule out a long-term change in nectar abundance in Eastern North America as a possible explanation (see the nectar conservation section below).

Nectaring versus roosting butterfly samples
In addition to Beall’s 1943 Ontario sample (Fig. 3A), the Beach Haven NJ sample (Fig. 3F) also stands out for having more lipids than the majority of 1979 samples. The Beach Haven sample was significantly different from the Cape May NJ sample (Fig. 3G) collected 1 day earlier, ~90 km to the southwest (Wilcoxon rank-sum test, $P < 0.01$). The Ontario and Beach Haven samples were both collected from temporary roosts (small groups of monarchs that cluster together on branches of trees or shrubs), rather than at flowers (Beall 1941; L. P. Brower 1979, unpublished field notes). Knowing that nectaring butterflies in the overwintering areas have significantly less lipid than butterflies clustering in the colonies (Brower and Malcolm 1991; Alonso-Mejia and others 1997) led us to ask whether nectaring and roosting butterflies might differ in their lipid contents during the migration, as well. Combing our data and other published records, we
identified 6 paired sets in which it was possible to compare nectaring and roosting migrant butterflies (Table 4). For 4 of these comparisons we compared lipid weights directly, but 2 of the comparisons use butterflies collected for an ecological chemistry study in 1970 that were not analyzed for lipids (Brower and others 1972). For these comparisons we examined dry weights, which are strongly correlated with the weight of the lipid [for \( N = 1521 \) butterflies collected in 1979–1980, dry weight = 1.066 * (weight of fat) + 163, \( r^2 = 0.848 \); Walford 1980]. In all 6 comparisons, the roosting butterflies are heavier than the nectaring butterflies, and in 4 of the comparisons the differences are statistically significant.

Why would roosting butterflies along the migratory routes to Mexico have higher lipid levels than nectaring butterflies? One hypothesis is that once they have accumulated substantial energy reserves, nectaring butterflies shift to a roosting mode and wait for favorable winds to continue the southward migration. Low lipid butterflies, on the other hand, would continue nectaring as long as flowers were available and, if they joined in the migration, would be forced to stop after shorter flights, or nectar more heavily on arrival at the next stopover point. This would be consistent with the roosting monarchs in Mexico which have high lipid contents compared with those collected while nectaring.

### Table 4 Lipid weights (A) or dry weights (B) of nectaring versus roosting monarch butterflies

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Collection location, date (N)</th>
<th>Weight in mg (mean ± SD)</th>
<th>Significance</th>
<th>Source2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nectaring</td>
<td>Roosting</td>
<td>Nectaring</td>
<td>Roosting</td>
</tr>
<tr>
<td>(A) Samples analyzed for lipids</td>
<td>Lipid weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ontario Missisauga, Ontario, August–October 1986 (73)</td>
<td>“Pt. aux Pins,” September 7, 1943 (67 females)</td>
<td>79 (median)</td>
<td>141 ± 49</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Missouri, 1972 Boone County, October 2–4 (18)</td>
<td>Franklin County, September 24 (30)</td>
<td>55 ± 26</td>
<td>129</td>
<td>Not tested</td>
</tr>
<tr>
<td>New Jersey, 1979 Cape May, September 29 (36)</td>
<td>Beach Haven, September 30 (14)</td>
<td>21 ± 17</td>
<td>61 ± 30</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Texas, 1979 Windemere, October 11 (66)</td>
<td>Austin, October 10 (42)</td>
<td>100 ± 39</td>
<td>112 ± 42</td>
<td>n.s.</td>
</tr>
<tr>
<td>(B) Other samples</td>
<td>Dry weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maryland, 1970 Baltimore MD and Delmarva Peninsula MD, September 7–26 (19)</td>
<td>Catonsville MD, September 8–10 (38)</td>
<td>206 ± 40</td>
<td>277 ± 60</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>MA and MD, 1970 Hockanum MA, September 19–26 (122)</td>
<td>Catonsville MD, September 8–10 (38)</td>
<td>191 ± 23</td>
<td>277 ± 60</td>
<td>( P &lt; 0.01 )</td>
</tr>
</tbody>
</table>

1Statistical tests:
Ontario: Individual values were not available for Missisauga; the frequency distribution in Gibo and McCurdy (1993) Figure 1 was compared with Beall’s data with the Kolmogorov–Smirnov 2 sample test (Siegel and Castellan 1988), \( D_{73,67} = 0.77, P < 0.05 \).
Missouri: Individual nectaring data were not available.
New Jersey, Maryland, and Massachusetts: Wilcoxon rank-sum test.
Texas: Individual data were not available; the frequency distributions were compared with the Kolmogorov–Smirnov 2 sample test, \( D_{42,66} = 0.13, P > 0.10 \).

2Sources:
a: Gibo and McCurdy (1993). Butterflies were collected foraging in open fields. Frequency distributions for early, middle, and late migration samples were presented; we used the middle migrant sample, which had the highest median lipid mass.
b: Beall (1948) Table 3. Butterflies were collected from roosts on the north shore of Lake Erie. Beall (1941, 1948) called his collection area Pt. aux Pins; this is part of Rondeau Provincial Park (Peter Sorrill, Natural Heritage Information Center, Ontario Ministry of Natural Resources, personal communication).
c: Brown and Chippendale (1974). Boone County butterflies were nectaring on Aster pilosus. Franklin County butterflies were roosting in hickory and oak trees. The roosting mean was taken from their Table 2. Values for the individual nectaring butterflies were read from their Figure 1.
d: Walford (1980). Cape May NJ butterflies were nectaring on Solidago. Beach Haven NJ butterflies were “sitting on bushes.” Windemere TX butterflies were nectaring on Liatris punctata. Austin butterflies were roosting.
e: Data from our lab, from samples used in Brower and colleagues (1972), Hockanum, Massachusetts sample was collected on flowers by L. P. Brower. The Maryland samples were collected by A. P. Platt: 38 were collected on September 8 and 10 from a roost on the University of Maryland Baltimore County campus, Catonsville MD; 19 were collected on flowers in Baltimore and on the Delmarva peninsula.
Texas and northern Mexico

Three Texas samples (Fig. 3N–P) and a northern Mexico sample (Fig. 3Q), plus an October 1993 Texas sample with a mean lipid mass of 120 mg (reported in Alonso-Mejía and others 1997), all have high lipid profiles. Although the 1982 Texas sample had significantly lower lipid levels than the 1979 Texas samples ($\chi^2 = 37.3$, df = 8, $P < 0.001$), it nonetheless had higher lipid levels than all of the migrant samples collected further north, with the exception of Beall’s Ontario 1943 sample (Fig. 3A). The San Javier, Mexico sample (Fig. 3Q) had the highest lipid level of any sample, and was collected at a roost (W. H. Calvert, personal communication). Collected in 4 different years, these Texas and northern Mexico samples provide consistent evidence that as butterflies approach the overwintering region they alter their behavior and increase their rates and/or duration of nectaring in order to build up large lipid reserves. They also lead to the conclusion that the overriding factor favoring extensive migratory lipid deposition is the energy requirement not of the journey, but of overwintering.

Monarch butterflies are opportunistic migrants

Beall (1948) emphasized the significance of the large differences among samples taken within a few days of one another along Lake Erie, and the high variation within samples. He concluded that the differences among samples represented variation “in the circumstances of their arriving” and that “the variation within a collection, per individual, is great, as if the different butterflies had had extremely various adventures” (p 92). In agreement with this conclusion, the datasets in this paper support a model both of opportunistic fueling by individual butterflies along their route, and a major change in strategy as they near the overwintering sites. The butterflies collected at any location are a heterogeneous group that are different ages, have traveled different distances across varied landscapes, and have encountered diverse nectar resources. Their lipid levels reflect the particular weather and feeding opportunities they have encountered.

Flight strategies

During migration, monarchs travel by cruising flight (“a leisurely flight interrupted by gliding,” Urquhart 1960), gliding and soaring. Using binoculars in open habitats in Ontario, Gibo and Pallett (1979) observed equal proportions of 358 migrating monarchs soaring, combining soaring and powered flight, and using powered flight alone. In a second study (Gibo 1986), 60.5% of 575 butterflies were soaring. The proportion soaring was higher when winds were favorable (from the E, N, and NW).

The energetic costs of soaring are several orders of magnitude lower than powered flight (Gibo and Pallett 1979). A soaring monarch butterfly probably expends energy at its basal metabolic rate, dependent only on its thoracic temperature (Gibo and Pallett 1979; Masters and others 1988). Gibo and Pallett (1979) estimated that a soaring monarch could theoretically travel as much as 13 800 km on an initial lipid mass of 140 mg. This is obviously unrealistic, but it illustrates that monarchs are able to travel long distances between fueling stops while burning very little lipid. Whenever a monarch encounters winds that are favorable for soaring, in direction and speed, it should take flight. Under other wind conditions, however, when a flying monarch would expend more energy in cruising flight, and might risk being carried long distances off its course by crosswinds, its decision about whether to fly, nectar or roost should be affected by the predictability and abundance of nectar along its route, and by its own energy budget. On a given day, a butterfly without much stored lipid might interrupt its flight in order to nectar, while a butterfly with a larger lipid store would have more options, to nectar, roost, or use powered flight to continue its migration.

Without an understanding of the decision rules individual butterflies use for initiating and terminating their nectaring bouts, migratory flights, and roosting behavior, we do not know whether low lipid levels reflect limited prior foraging opportunities, or favorable wind conditions such that traveling preempted foraging. Adverse weather conditions may have contributed to the low lipid levels of the 2000 Virginia and the 1979 samples, if they limited opportunities for monarchs to accumulate lipid by decreasing nectar abundance and/or foraging time. In 1979, the temperatures in the northeast and central regions of the United States were below average in August and September, and temperatures in the east north central region were below average in August but not September (National Climatic Data Center, http://www.ncdc.noaa.gov). Similarly the 2000 migration through Virginia, which produced low census counts (Table 1) as well as low lipid levels, coincided with colder than normal summer temperatures in the northeast region (Table 5), and an October rainfall for VA that was much below average. A very different hypothesis for the low lipid profiles is that these butterflies may have encountered weather conditions especially favorable for soaring, with light to moderate winds out of the north and little precipitation. If butterflies have a high probability of locating nectar sources
all along their route, then when they encounter good conditions for soaring they may all take advantage of them, in preference to feeding.

Migration rates

Ground speeds of migrating monarchs depend both on the flight technique and on the wind conditions. When head winds are strong, monarchs can make little or no forward progress, and they do not take flight (Urquhart 1960; Gibo and Pallett 1979; Davis and Garland 2004). In calm air, gliding speeds close to the ground have been estimated as 9–13 km/h (Gibo and Pallett 1979), and cruising flight speeds as 17.6 km/h (Urquhart 1960). With the benefit of tail winds individual butterflies achieved ground speeds in excess of 50 km/h over short distances (Gibo and Pallett 1979).

Estimates of the average daily travel distances of fall migrants can be derived from information in the Monarch Watch tag recovery database (http://www.monarchwatch.org). Thirty-eight butterflies tagged in 12 states were recaptured in Texas 3–71 days later. Straight-line distances between tagging and recapture sites ranged from 314 to 4293 km. Their average travel distances varied from 18 to 245 km per day (median = 45 km), with half of the butterflies traveling between 28 and 63 km. The actual distances traveled by individual butterflies are longer, since their flight paths are not straight, and are strongly affected by wind conditions (Schmidt-Koenig 1985; Gibo 1986). Without tracks of individual butterflies, however, the actual distances and trajectories cannot be determined. Over 3 seasons, Garland and Davis (2002) recaptured 6 monarchs in Kiptopeke State Park, Virginia, 1–9 days after they had been tagged 226 or 333 km away. The average distance traveled per day ranged between 28 and 226 km. Captures of occasional monarch butterflies in England are evidence that major storms can passively transport individuals great distances (Brower 1995), but the number that will be transported closer to the overwintering destination in Mexico is probably exceedingly low.

The rough estimates of ground speeds and daily travel distances suggest that monarchs can spend much of their migration time either nectaring or roosting. In most years, therefore, butterflies can probably afford to be selective about the weather conditions during which they travel.

Fuel loading

The net aerodynamic and energetic consequences of transporting a larger or smaller fuel load are unknown. A large flight tunnel experiment with red knots, for example, determined that for these birds the metabolic cost of carrying large fuel loads was lower than predicted by aerodynamic models (Kvist and others 2001). For an early fall monarch butterfly with a wet weight of 500 mg (an approximate average value for migrants in Minnesota; Herman 1988; Borland and others 2004), adding 50–200 mg of lipid will increase its wet weight by 10–40%, distributed almost entirely in its abdomen. During powered flight, an insect with high lipid stores may use energy at a higher rate and may have reduced maneuverability (Dudley 2000); on the other hand, a heavier fuel load may increase its flight velocity and improve its ability to overcome head winds (Pennycuick 1975; Dudley 2000). During soaring, increasing wing loading may increase a butterfly’s airspeed, but may also increase its rate of descent through areas of subsiding air and give it less time to locate areas of lift (Gibo and Pallett 1979). The heterogeneity in the lipid levels stored by monarchs along their migration route suggests that their transportation costs may be insignificant; it is possible, however, that costs of high

<table>
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<tr>
<th>Temperature</th>
<th>June–August (Northeast)</th>
<th>June–August (VA)</th>
<th>September (Northeast)</th>
<th>September (VA)</th>
<th>October (Northeast)</th>
<th>October (VA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>N</td>
<td>+, N</td>
<td>–</td>
<td>+</td>
<td>N</td>
<td>–</td>
</tr>
<tr>
<td>1999</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>2000</td>
<td>N</td>
<td>N</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>2001</td>
<td>N</td>
<td>N</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

N = normal.

+ +, – – = somewhat above or below normal.

*Categories and data are from the National Climatic Data Center (1999a, 1999b, 2001, 2002). For the Northeast region, if multiple symbols are shown, individual states had different conditions.
fuel loading may be a factor that has selected against maximal lipid storage until butterflies reach Texas.

**The geography of nectaring**

Where and when are nectar resources important for migrating monarch butterflies? Within Texas, Calvert and Wagner (1999) proposed that the migrating fall monarchs may be concentrated in 2 flyways, 1 in central Texas, more than 300 miles wide, and the second along the coast, extending from Louisiana to Mexico. The data in Figure 3 confirm that extensive nectaring and lipid storage take place in these areas. Within these flyways, and in northern Mexico, it is possible that specific locations will be found to be of key importance. Along most of the migration route, however, and in contrast to the requirements of many migratory shorebirds (Tsipoura and Burger 1999; Baker and others 2004), specific stopover sites do not seem to be used for refueling by a large proportion of the migrants. Along their migration routes, monarchs display neither feeding nor habitat specialization. They nectar on dozens of flower species (Robertson 1928, in Tooker and others 2002) in almost any open habitat.

The monarchs’ spring remigration, from the Mexican overwintering sites to breeding areas in the southern United States, is the most poorly studied phase of their annual cycle. How much lipid reserve is necessary to fuel the return flight? Do butterflies have opportunities to refuel, or are they dependent on the reserves left from the overwintering period? Do favorable winds allow them to travel much of the distance by soaring, or are they more dependent on energetically expensive powered flight?

**Conservation of nectar sources**

Monarch butterflies are specialists as caterpillars, feeding only on milkweed plants in the genus *Asclepias*. They are also extreme specialists as adults in the habitat they select for overwintering. The eastern population overwinters in central Mexico only in high elevation oyamel fir and pine forests within an area of 100 km by 100 km (Brower and others 2002); the environmental conditions these forests provide are not found elsewhere in Mexico (Oberhauser and Peterson 2004). Both of these specializations are creating conservation risks for the butterflies, as milkweeds are eliminated by herbicides from vast parts of their range, and as Mexican oyamel forests are degraded and lost to illegal logging (Brower and others 2002; Honey-Roses and Galindo 2004; Ramirez and others 2005).

During the migration, in contrast, the adult monarch population will not be sensitive to vegetation changes that affect small areas, or to the increase or loss of any individual plant species. Does this mean that nectar availability for monarchs during the fall and spring migrations is not of conservation concern? Plant communities of North America have undergone, and are undergoing, massive shifts through time, from both anthropogenic and nonanthropogenic agents. We propose that the large scale changes in the distribution and abundance of nectar sources may well be approaching a sufficient magnitude to affect the monarchs’ ability to accumulate the lipids they require to survive the overwintering period, but that the data currently do not exist that would allow this to be ascertained.

**Use of herbicides and herbicide-resistant genetically modified crops**

The migration of the monarch butterfly has likely evolved as its hostplants, milkweeds in the genus *Asclepias*, spread and diversified throughout North America during the late Cenozoic era (Woodson 1954; Brower 1995). Until European settlers moved into the Great Plains, fall migrants had nectar available from the highly diverse forbs in the tallgrass and shortgrass prairies, as well as varied and abundant nectar sources in the forested northeast and midwest. Brower (1995) summarized the literature supporting the hypothesis that the principal summer breeding area for monarchs was originally in the Great Plains where at least 21 milkweed species occur. With the advance of industrial agriculture in the twentieth century, over 98% of the prairies and much of the eastern deciduous forest were converted to agricultural fields. Within this vast disturbed area, stretching from the western plains to the Atlantic coast, 1 particularly weedy milkweed species, *A. syriaca*, greatly increased in abundance and in its importance for monarchs. Using cardenolide fingerprinting, Malcolm and colleagues (1993) determined that >90% of adult monarchs collected at Mexican overwintering sites had fed as larvae on this milkweed. Isotope analyses by Wassenaar and Hobson (1998), of butterflies collected in Mexican overwintering areas in 1997, determined that at least half of them had bred within the agricultural midwest. Together, these 2 studies established the current importance of *A. syriaca*, especially in the midwest, for the production of monarch butterflies that migrate each fall to Mexico.

Between 1960 and 1985, the use of herbicides to kill weeds in agricultural fields increased 4-fold and the number of different herbicidal chemicals increased by >50-fold (Lever 1990; Aspelin 1994). Because herbicides kill milkweeds as well as numerous species of herbaceous plants that serve as adult nectar sources throughout the monarch’s annual cycle, Brower (1995, 1999, 2001) predicted that the size of the monarch’s fall
migration would eventually dwindle, a warning echoed in several subsequent studies that substantiated the importance of midwestern agricultural fields as monarch breeding habitats (Hartlzer and Buhler 2000; Oberhauser and others 2001; Jesse and Obrycki 2003). Evidence for the broad scale elimination of A. syriaca from agricultural areas is now emerging. Oberhauser and colleagues (2001) surveyed cornfields and soybean fields in Iowa, Maryland, Minnesota, Ontario, and Wisconsin during the summer of 2000, and documented extensive breeding of monarchs on A. syriaca. By the summer of 2004, the use of herbicides on genetically modified herbicide-resistant soybean seedlings had eliminated virtually all broadleaf plants in these fields, including A. syriaca (K. Oberhauser, personal communication).

The potential threat to biodiversity from herbicides sprayed on herbicide-resistant crops has been discussed by numerous authors (Freemark and Boutin 1995; Buchmann and Nabhan 1996; Allen-Wardell and others 1998; Brower 1999, 2001; Watkinson and others 2000). As repeated annual applications are eliminating native forbs over millions of acres of cropland, potential cascading negative effects include the elimination of foodplants for untold numbers of herbivorous arthropods, and of nectar and pollen sources for native pollinators. Long-term censuses and other data are needed to determine the magnitude of the effects (Watkinson and others 2000; Cane and Tepedino 2001; Marlin and Laberge 2001; Freckleton and others 2003, 2004; Ghazoul 2005).

Exotic plant species as nectar resources

Because the monarch is a nectar generalist (Robertson 1928, in Tooker and others 2002), it may benefit from the spread of certain exotic plant species. Agricultural crops that provide butterflies with nectar include red clover (Trifolium pratense), alfalfa (Medicago sativa), and vetch (Vicia spp.) (Pivnick and McNeil 1985). Invasive alien species that flower in the late summer or fall, are attractive to butterflies, and have become widespread in eastern North America include purple loosestrife, Lythrum salicaria (Swearingen 2005), spotted knapweed, Centaurea biebersteinii (Carpinelli 2005; NABA 2006), and Canada thistle, Cirsium arvense (Roddy 2005). In areas where such invaders are poorly controlled, they may be significant nectar sources for migrating monarchs; successfully bringing such invasions under control, on the other hand, may diminish nectar availability, depending on the characteristics of the plants that replace them.

At the local level, management of other exotic plant species may have unintended effects on nectar availability for migrant monarch butterflies. At Cape May NJ, the US Army Corps of Engineers is carrying out an ecosystem restoration project that includes herbicide control of invasive Phragmites australis. Large numbers of monarchs migrate through Cape May each fall (Walton and others 2005). Aerial application of glyphosate, a broad-spectrum herbicide that kills broad-leaf herbs as well as the target grass, coincided with the middle of the butterfly migration in 2004 and 2005, despite vociferous concerns raised by conservationists (New Jersey Audubon Society 2004; US Army Corps of Engineers 2006).

Prairie management

Historically, the tallgrass prairies of the midwestern United States provided abundant nectar for pollinators, including monarch butterflies (Brower 1995). Towne and colleagues (2005) compared the effects of bison grazing, cattle grazing and no grazing on tallgrass prairie vegetation. In a 10 year experimental study, perennial forb cover increased in grazed pastures compared to the ungrazed controls, and at a greater rate when the herbivores were bison than cattle. Among the plant species that increased in cover at the highest rate were 2 butterfly nectar sources, Missouri goldenrod (Solidago missouriensis) and heath aster (Symphyotrichum ericoides). Other good nectar sources also increased, including Vernonia baldwinii (western ironweed). The implication of Towne and colleagues (2005) for monarch butterflies and other pollinators is that land management decisions within the Great Plains will have a significant effect on the abundance and diversity of nectar resources. As agriculture and urbanization continue to obliterate the native vegetation over most of the landscape, the few natural remnants will increase in importance for resident and migratory pollinators.

Urbanization

We do not have data comparing the abundance and diversity of nectar plants in urban and suburban landscapes to other habitats. One opportunity to increase plant biodiversity within human-dominated landscapes is along the 4 million miles of roadsides (Harper-Lore and Wilson 2000). The Federal Highway Administration guidelines on landscaping practices encourage the use of regional native plants, and reduced use of herbicides (Federal Highway Administration Roadside Vegetation Management, http://www.fhwa.dot.gov/environment/vegmgt/index.htm). The popularity of butterfly gardens and native plant landscaping may enhance pollinator populations locally, but will never be of sufficient magnitude to compensate for the losses of native nectar sources from rural habitats.
Lipids in migrating monarch butterflies

Stopover ecology

Hutto (1998) lamented that migratory birds had received far less study during their migrations than during their breeding or wintering seasons. Addressing this gap, recent studies have examined the stopover ecology of migratory shorebirds and songbirds (Yong and Finch 1997; Yong and others 1998; Piersma and Jakema 2002; Prop and others 2003). In addition, satellite tracking and radiotelemetry have been revealing the migratory flight strategies of individual birds (Bowlin and others 2005; Alerstam and others 2006).

The energetics and stopover ecology of monarch butterflies similarly merit further research (Davis and Garland 2004). Although technological constraints on radiotracking and telemetry have limited their usefulness for all but the largest insects (Ando and others 2002; Cooke and others 2004; Lorch and others 2005; Wikelski and others 2006), much can be learned with other techniques. The monarch butterfly is the subject of citizen-participation projects that monitor the timing and numbers of migrants (Monarch Watch, http://www.monarchwatch.org; Journey North, http://www.learner.org/north) and breeding generations (Monarch Larval Monitoring Program, http://www.mlmp.org), and the incidence of a protozoan parasite (Project Monarch Health, http://www.monarchparasites.org/). Davis and Garland (2004) proposed recruiting this volunteer corps to conduct mark-recapture studies at stopover sites to measure the duration of stopovers. Similarly, we propose that annual monitoring of the lipid stores of migrants in selected stopover sites across eastern North America would enhance our understanding both of the butterflies’ basic energetics, and of the influences of local and regional factors on the migration. Quantitative lipid determinations for large numbers of butterflies is time and labor intensive, but dry weights are highly correlated with lipid weight (Walford 1980), and monitoring dry weights would not require substantial laboratory or financial resources. Such monitoring would also provide regional indices of the abundance of fall nectar sources.

Monarch butterflies continue to reveal novel facets of their biology to curious scientists and naturalists. We hope that the questions raised by this analysis of their patterns of lipid accumulation at one site in Virginia, and in other data spanning 60 years and 14 sites, will spark the imagination of additional scientists with expertise in such fields as satellite tracking, flight aerodynamics and energetics, and conservation physiology.

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