MIGRATION OF THE NORTH AMERICAN MONARCH *DANAUS PLEXIPPUS* TO CUBA

By

CRISTINA DOCKX

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2002

To Nature’s beauty and magic

ACKNOWLEDGMENTS

I would like to thank my advisor Lincoln Brower for introducing me to the monarch butterfly and to the students from his laboratory (who became very good friends as did the monarch butterfly, "the monarch of the team"); and for letting me work in what at that time seemed a "crazy" project; the migration of the monarch butterfly to Cuba. I would like to thank the members of my committee: Richard Kiltie, Thomas Walker, Jackie Miller and Jon Reiskind, all of whom contributed to the success of my research. I extend special thanks to Richard Kiltie, who has been a supportive, patient, and critical member. Thomas Walker provided me with constructive criticism. Jackie Miller was always interested in my work, and offered me her direct knowledge of monarchs in Cuba. Jon Reiskind always had a smile and a hand when I needed it.

I thank my family and friends in the United States, Cuba, Belgium, and Colombia, for being a part of this project. Sandy Lapis and Richard engaged in the big enterprise of smoothing my grammar and making my ideas flow. This is a tenacious endeavor since English is not my native language. I thank them. I thank my friends, Magola Molina (my mom), Tonya VanHook, Amy Knight and Tom Wunderli (my husband), who gave me logistic support: my husband with his wonderful dinners, Tonya for helping me survive the first years of graduate school, Amy for listening and helping me with the development of this project, and Magola for being "my assistant" in the field and the laboratory.

The Tinker Foundation and Sigma Xi provided financial support for the first year of field work. The National Science Foundation grant (Lincoln P. Brower, principal investigator) financed the chemical analyses of the butterflies. Leonard Wassenaar and Keith Hobson (Canadian scientists) finance the majority of the isotopic analyses, I thank them. Lincoln Brower (my advisor) and Richard Wunderli helped me with personal money to pay for the isotopic analyses. I am grateful to them.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

MIGRATION OF THE NORTH AMERICAN MONARCH *Danaus plexippus* TO CUBA

By

Cristina Dockx

August 2002

Chairman: Lincoln P. Brower
Major Department: Zoology

Since the discovery of the Mexican overwintering colonies by Urquhart and his collaborators in the 1970s, it was thought that migrant monarchs from the eastern portion of the North American continent only migrate to Mexico. However, a new migratory route to Cuba was unveiled, as were phenotypic differences between Mexican and Cuban migrants. These migrants do not return to the United States as the Mexican migrants do. Cuba is a new migratory destination of monarchs of the North American continent, and also constitutes a unique migratory strategy. Individuals that follow this migratory route form a distinctive group in terms of their natal ground, phenotype characteristics related to their migration, their reproductive strategy and they do not remigrate back. Three localities in the western Cuba portion were sampled: San Antonio (South of la Havana), Guanahacabibes (the most western portion of the island), and Zapata Swamp (southeast of Havana).

The natal grounds of Cuban migrant monarchs were analyzed through isotopic analyses of hydrogen (δD) and carbon (δ13C). The butterfly isotopic values show that monarchs from the North American continent that migrate to Cuba do not conform to a panmictic model composition, as Mexican monarchs do. Migrants from Guanahacabibes came only from the midwestern United States) and their surroundings. In contrast, migrant monarchs from San Antonio came from southeast Canada and along the east of the United States.

My research shows that Cuban migrant monarchs not only differ in their migratory destination; they differ in their reproductive stage, mating strategy, and some phenotypic traits. Most of these Cuban migrants are reproductively active (90%). My data also suggest that migratory routes and final destinations of the migrant monarchs are linked to phenotypic traits that could be functionally important in their migration. These phenotypic traits are wing size and shape, butterfly lipid, and lean weight. Monarchs that migrate to Cuba maximize their survival and reproductive opportunities migrating to this Caribbean island, opportunities that would be slim or nonexistent if they had migrated to Mexico.

Preliminary results suggest that Cuban migrant monarchs, in contrast to Mexican monarchs, hybridize with the resident Cuban populations and do not return to the United States.

CHAPTER 1
INTRODUCTION AND BACKGROUND

The monarch butterfly *Danaus plexippus plexippus* (Lepidoptera, Nymphalidae) from eastern North America is known for its annual migration to the Oyamel Forest located on the Transverse Neovolcanic Belt in Central Mexico (review in Brower 1995). Most (95%) of the migratory butterflies are hatched in their known breeding range in the United States, Midwest and surrounding areas (Malcolm et al. 1993;Wassenaar and Hobson 1998), where the host plants of the monarch larvae (*Asclepias* spp.) are abundant, especially *Asclepias syriaca* (northeastern region). The other 5% come from the extremes of their breeding range, southern Canada and the southern United States (Wassenaar and Hobson 1998). *Asclepias* spp. (family Asclepiadaceae) have cardenolide compounds that protect the plant from herbivores. The monarch larva has coevolved with these plants, and is able to feed on the plant without being poisoned. The cardenolides are passed from the larvae to the adult, giving it protection against vertebrate predators (review in Brower 1984). After the adults emerge, they move southward as *Asclepias* spp. decrease in abundance at the end of the summer and before the cooler fall temperatures affect them. During their travel they feed on nectar which is transformed into lipids than are then stored for use during the overwintering period (Cenedella 1971).

The migrant monarchs arrive in central Mexico from late October through November in the high altitudes of the Oyamel Forest, where they find cool ambient temperatures (Brower and Calvert 1985; Calvert et al. 1989). These cool temperatures

decrease the monarchs' use of their lipid reserves during the approximately five months that they stay in Mexico (Chaplin and Wells 1982). These migrant monarchs are reproductively dormant, living largely, if not completely, on their lipid reserves (Alonso et al. 1997). The lipid reserves remaining at the end of the overwintering period (between February and March) are used for reproduction and remigration of the butterflies to the southern United States (Malcolm et al. 1993). These remigrants arrive in the southern United States in time to exploit the newly emerging milkweed plants (Herman 1985; Brower and Malcolm 1991).

Between three and five successive generations of monarchs (Brower 1996) move northward to recolonize the summer breeding ranges in the United States and Canada. This northward recolonization is followed by a midsummer nonmigratory phase. The migration cycle begins again at the end of the summer, when migrant monarchs emerge from eggs laid by the summer breeders and the summer nonmigratory monarchs.

Monarch Butterflies in the Caribbean

In contrast to the numerous studies of the migrant monarchs in Mexico (Brower 1985,1995; Malcolm and Zalucki 1993), studies of alternative monarch migratory routes through Florida and Cuba (as well as studies of resident populations of the Caribbean) are few. Migrant monarchs arriving at Englewood in West Florida in late October and November, were reported in the 1930s (Hodges 1937 in Williams et al. 1942). Arrival and roosting of fall migrants have been observed in the Florida Panhandle at St. Marks Natural Wildlife Refuge (Van Hook pers. comm.) and in Central Florida (Brower 1995; Urquhart 1960; Urquhart and Urquhart 1976). Further south, flying monarchs were observed in the Everglades, Florida Keys and, Cape Sable (Urquhart 1960), and a tagged migrant monarch was found in the Keys, probably Key West (Urquhart and Urquhart

1976). The most recent work on the Florida monarchs by Knight (1998) showed a permanent resident population of monarchs in South Florida, that receives an influx of fall migrant monarchs from late October through December.

The movement of North American monarchs *D.p. plexippus,* beyond the Florida peninsula into the Caribbean has long been suspected. Williams at al. (1942) said "... the southern limit of the movement of *plexippus* must be somewhere in Mexico, Cuba and the Bahamas…." *Danausp lexippus. plexippus* refers to the North American monarch subspecies, and *Danaus plexippus megalippe* (Hubner) refers to the monarch from the Caribbean and northern South America. Williams et al. (1942) suggested that these two subspecies hybridize: "it (*D.p. plexippus*) is found in Cuba, Jamaica, Haiti and some of the Virgin Islands along with the intermediates" Here "intermediate" refers to individuals that exhibit mixed phenotypes of *D.p. plexippus* and *D.p.megalippe.*

The tagging program of North American monarchs developed by Urquhart (1987) reported the first confirmed records of the arrival of *D.p. plexippus* butterflies in the Caribbean: one in Cuba, two in Hispaniola, one in Jamaica, one in Puerto Rico, one in the lesser Antilles, and one as far south as Trinidad (only a few miles from the South American continent). Urquhart (1987) also collected four tagged monarchs in the coastal areas of the Yucatan Peninsula. He attributed the presence of migrant monarchs in these regions to strong west and northwest winds blowing the butterflies off their usual southwesterly course, and he calls them "aberrant monarchs." He hypothesized that the monarchs that are blown off follow three different routes:

- Some fly through the Florida Peninsula, then continue flying to Cuba, Yucatan, and on to Guatemala. He suggested that the monarchs overwinter in the mountains of Guatemala and/or Honduras; then during the spring these individual monarchs travel northeastward from Guatemala and Yucatan to Cuba and then to Florida (Urquhart and Urquhart 1976).

- Other migrating monarchs arrive in Bermuda carried by eastward cold fronts or hurricanes (Urquhart 1987).

- A group of the monarchs that were carried away to Bermuda resumes a southerly orientation arriving in the Bahamas, then in the Antilles, continuing on to Central or South America; and finally overwintering in the mountains of Guatemala, Colombia and Venezuela (Urquhart 1987).

Knight (1998) indirectly supported Urquharts data on the fall movement of migrant monarchs to the Caribbean, but not their migration back to the United States through Florida in the spring. She found that migrant monarchs appear in the Miami area from October through December, but the other nine months they are absent from this area (including the spring months). She collected monarchs during 1994 and 1995 around Miami and analyzed their cardenolide "fingerprints" through Thin Layer Chromatography (TLC) to determine if these butterflies were resident or migrant. Cardenolides are incorporated into the monarch larva's body when it feeds on its host plants, *Asclepias* spp., and these cardenolides are retained through metamorphosis and finally passed on to the adult (Brower 1984). Many species of *Asclepias* have a unique cardenolide "fingerprint." By matching the fingerprint of the monarch adult with the plant species and its distribution, it is possible to determine the butterfly's natal ground: Knight determined whether each butterfly was a Miami area resident or if it was a migrant coming from the northeast. Her evidence supported the hypothesis that her study area has a permanent monarch resident population all year around, and that it receives an influx of migrant monarchs from the end of October to early December. However, she did not find any migrant monarchs from January to September: only resident ones.

If Urquhart and Urquhart's (1976) hypothesis is correct, Knight would have found migrant monarchs during March through April, when they would have returned from the overwintering areas in Guatemala. But that was not the case.

This Study

The research presented here explores the possibility of an annual migration of North American monarchs to Cuba and the incorporation of individual monarchs and their genes into the Cuban monarch population during the fall. This implies that the North American monarchs that arrive in Cuba and other Caribbean islands are lost from the North American gene pool population, because they will not return to the United States in the spring. However, according to the hypothesis presented here, they may play a major role in shaping the population structure of Caribbean monarchs through a periodic introgression of genes into their populations.

The term migration in this paper refers to the definition of Dingle (1996), meaning persistent and straightened-out movement of the animal that depends on temporary inhibition of a site-staying response. Specific patterns of energy allocation and specific departing and arriving times are other characteristics of this animal movement.

This dissertation is subdivided into four chapters. Chapters 2, 3 and 4 each discuss methods and materials used in the research. Chapter 2 deals with the following questions:

- Do North American monarchs arrive in Cuba?
- Do they arrive on a regular basis or are they sometimes *blown off* course to Cuba, as Urquhart (1987) proposed?
- Can this movement to Cuba be considered a migration?
- Where are the natal grounds of Cuban migrant monarchs?

To answer the last question ratios of stable-hydrogen (δD) and carbon ($\delta^{13}C$) isotopes and Thin Layer Chromatography (TLC) were used. With these isotopic values it is also possible to establish if migrant North American monarchs are coming from throughout their breeding range (panmictic model), or if instead some geographical regions are better represented than others (non panmictic model).

Chapter 3 explores the questions: (1) Why do some migrant monarchs migrate to Cuba instead of Mexico?, and (2) Are there some significant differences between Mexican and Cuban migrant monarchs in their phenotypes (such us wing length, wing shape and condition, lipid mass, and lean mass) that can explain why some North American monarchs go to Cuba rather than Mexico?

Chapter 4 addresses the question: What happens to these migrant monarchs when they arrive in Cuba? Two scenarios have been analyzed: Urquhart and Urquhart (1976) stated that some "aberrant" migrant monarchs deviate from their usual southwest course ending up in the Florida Peninsula. Later they fly to Cuba, then to Guatemala and/or Honduras, where they overwinter, and in the spring return to the North American continent. An alternative scenario has been proposed: some migrant monarchs coming from North America and Canada, pass through the Florida Peninsula, then arrive in Cuba (and/or other close areas), where some of them hybridize with the resident monarch population, and others continue to other areas of the Caribbean. These migrant monarchs do not return to the North American continent. The entrance of North American and Canadian monarch genes into the Caribbean could partially explain the distinctive phenotypes of resident Cuban monarchs at the end of the year, when migrants potentially can hybridize with them.

Chapter 5 is a summary and conclusion of this work, as well as suggestions for further research. Suggestions for future work include an extension of the work that was done in western Cuba to the East of the island, and to other areas of the insular Caribbean and continental Caribbean (as Yucatan peninsula), to see if migrant monarchs reach these areas as well. Other suggestions for further research include the exploration of genetic differences of Mexican and Cuban monarchs, as these groups differ in their natal grounds, reproductive stage, mating patterns, and phenotypic traits.

CHAPTER 2
DO NORTH AMERICAN MONARCHS MIGRATE TO CUBA?

Introduction

The monarch butterfly, *Danaus plexippus* (Lepidoptera, Nymphalidae), is a cosmopolitan species present throughout the Americas. In southern South America (Bolivia, southern Brazil, and Argentina), however, it is replaced by *Danaus erippus* (Cramer). *Danaus plexippus* has two subspecies, *Danaus plexippus plexippus* (Linnaeus) in North America, and *Danaus plexippus megalippe* (Hubner) in the Caribbean and northern South America (some specialists recognize more than two). The subspecies *D.p. plexippus* in the North American continent has two populations divided by the Rocky Mountains. The monarchs east of the Rockies undergo an extraordinary fall migration of 4000 kilometers from their natal ground, mainly the Midwest of the United States (Brower 1995; Wassenaar and Hobson 1998), to the volcanic mountains of central Mexico. These migrants arrive between the end of October and beginning of November at the overwintering colonies in Mexico and return to the southern United Sates in late March. A fall migration of a lesser scale occurs west of the Rocky Mountains, mainly to overwintering sites along the California coastline, from San Francisco to Tijuana, Mexico (Brower 1995).

In the late 1800s monarchs were introduced into Australia (Kitching and Scheemeyer 1993), where they, too, exhibit migratory behavior (Ackery and Vane-Wright 1984). The monarch sister species, *Danaus erippus,* also exhibits migratory behavior; however, information of its migratory routes is scarce and is based on

observations only (Urquhart 1987). Malcolm Burr (in Williams 1942, p 157), talking about *Danaus erippus*, described a " … big flight of this butterfly at the mouth of the river Plate, near Buenos Aires, at the beginning of April 1891…." April corresponds to fall in the Southern Hemisphere. Wetmore (1926, p 22) says about *D. erippus*, "… It was interesting also to observe the migrational movement of a form of the monarch butterfly (*Anosia erippus*) that wintered in numbers in the Chaco, and in the Spring flew southward to spread over the pampas…." *Anosia erippus* is an early reference to *D. erippus*. Other genera of the subfamily Danainae in the New World, such as *Anetia* (Ivie et al. 1990; Wang and Emmel 1990; Scheermeyer 1993), do not migrate but do form clusters and have altitudinal movement with the seasons, both rainy and dry.

In contrast, monarchs in the Neotropics, such as *D.p. megalippe,* do not migrate, but instead they breed all year round, and are localized in open grasslands where their host plant *Asclepias spp.* Are present, especially *Asclepias curassavica* (Williams et al., 1942; Alayo and Hernandez 1981; Salazar pers. comm; pers. obs.). These differences in behavior between the monarchs in the temperate and Neotropical zones are correlated with phenotypic differences in size, color and wing pattern-differences that resulted in the recognition of them as two different subspecies (Clark 1941; Williams et al., 1942; Brown and Heineman 1972: Alayo and Hernandez 1981; Schwartz 1989). But these phenotypic differences between the two subspecies are not so clear in the Caribbean islands close to the continental United States, perhaps because of the arrival of *D.p. plexippus* in this area. Brown and Heineman (1972) state, "The islands of Cuba, Jamaica, and Hispaniola undoubtedly harbor indigenous sedentary subspecies that have not yet been recognized because of the confusing mixtures present on the islands." They

continue: “Much of this mixing is undoubtedly the result of the southward movement of *D.p. plexippus* deep into the northern part of the tropics, probably during the peak of the *Wisconsin glacial stage*.”

One record exists from Urquhart’s (1976) tagged monarchs, collected in Cuba (Luis de Armas pers. comm.), that shows that this movement currently occurs, and was not restricted to the Wisconsin glacial stage as Brown and Heineman (1972) proposed. In November during the 1970s a girl was at Havana beach with her parents and saw a tagged monarch butterfly. Her parents took the butterfly to the scientists at the Museum of Natural History in La Havana, who sent a picture of the butterfly (not the specimen) to Urquhart (Luis de Armas pers.comm), who later reported that this monarch was tagged in the vicinity of Lake Ontario (Urquhart 1976). Until the work presented in this dissertation, this was the only confirmed report of monarchs from the North American continent reaching Cuba. Observations also have been made of southward flying monarchs in the insular Caribbean from September through November (Williams 1941; Sein 1929). Alayo and Hernandez (1985) hypothesized that these south-flying monarchs came from Canada and North America aided by the cold fronts that start to occur in September.

The central question that this dissertation addresses is: Do Canadian and North American monarchs currently migrate to Cuba? To answer this question, 166 monarch butterflies were collected in Cuba beginning November 1995 through 1997, and 15 more monarchs collected by Luis Roberto Hernandez in 1993 were analyzed. The natal grounds of these butterflies were determined by analyzing their cardenolide fingerprint through Thin Layer Chromatography (TLC) and the isotopic values of carbon ($\delta^{13}C$) and

hydrogen (deuterium, δD). Because Cuba has a permanent resident population of monarchs, it was essential to determine their natal ground; that is, to determine whether they were born in Cuba or in the United States-Canada. All the butterflies collected were analyzed with TLC, but the isotopic analyses were performed on butterflies collected only in November 1993, March 1995, and November 1997.

TLC is a technique developed by Brower et al. (1982) that produces a cardenolide spot pattern that is unique to the milkweed species on which the monarch larva feed. By matching the cardenolide spot pattern of individual monarch to a particular milkweed species, and knowing the general geographical distribution of the plant, it is possible to determine the origin of the butterfly. In this way, one can distinguish between Cuban resident monarchs and migrants coming from Canada and the northern United States. If a monarch has a fingerprint of an *Asclepias spp*. that is only present in the United States, it can be classified as a migrant with high degree of certainty. The majority (95%) of the migrant monarchs that overwinter in Mexico originate in the Midwest and surrounding areas (Wassenaar and Hobson 1998) where *Asclepias syrica* is very common (Woodson 1954). In the absence of any other information, it is assumed that that the majority of Cuban migrant monarchs also come from these areas, which would result in their having an *A. syriaca* TLC fingerprint.

The isotopic technique gives a more precise determination of the natal ground of the monarch than TLC, because one can combine the latitude (hydrogen) and longitude (carbon) data and determine on a broad scale the geographical area from which the butterfly emerged (Wassenaar and Hobson 1998). The isotopic values for hydrogen (δD) and carbon ($\delta^{13}C$) of the keratin of monarch wing membranes is highly correlated (r =

0.99) with the isotopic composition of its larva food source (Hobson and Wassenaar 1999). The isotopic composition of the milkweed is, in turn, controlled by continental isotopic patterns. The values for these two isotopes are distinctive within the North American continent: enriched values for the hydrogen isotope (deuterium) occur toward lower latitudes, in contrast to the carbon isotope ($\delta^{13}C$) that is depleted toward the southwest areas (Wassenaar and Hobson 1998). The isotopic values for the monarchs collected in Cuba were comp7red with the extensive data bank for the North American continent gathered by Wassenaar and Hobson (1998), and analyses made of seven Cuban plant samples for $\delta^{13}C$ values, and of three samples for δ D. These plants were collected in March 1995 and July 2000.

The value of using these two techniques together is that in cases where the isotopic values (hydrogen, δD and carbon, $\delta^{13}C$) or TLC fingerprints were unavailable, the other technique could fill this information gap (with the exception of TLC plant fingerprints found in the southern United States and Cuba). Another advantage is that their results can be used to see the extent to which these two techniques agree, since this is the first time that the same samples of butterflies were simultaneously analyzed using the two techniques.

Methods and Materials

Monarch Collection and Sampling Sites

Monarchs were collected in Cuba at three different locations: San Antonio de los Banos (31 km southwest of La Havana), Zapata Swamp, and Guanahacabibes Peninsula (the westernmost portion of Cuba). Collecting localities are shown in Figure 2-1 and Table 2-1 summarizes sites, dates, samples size and collectors.

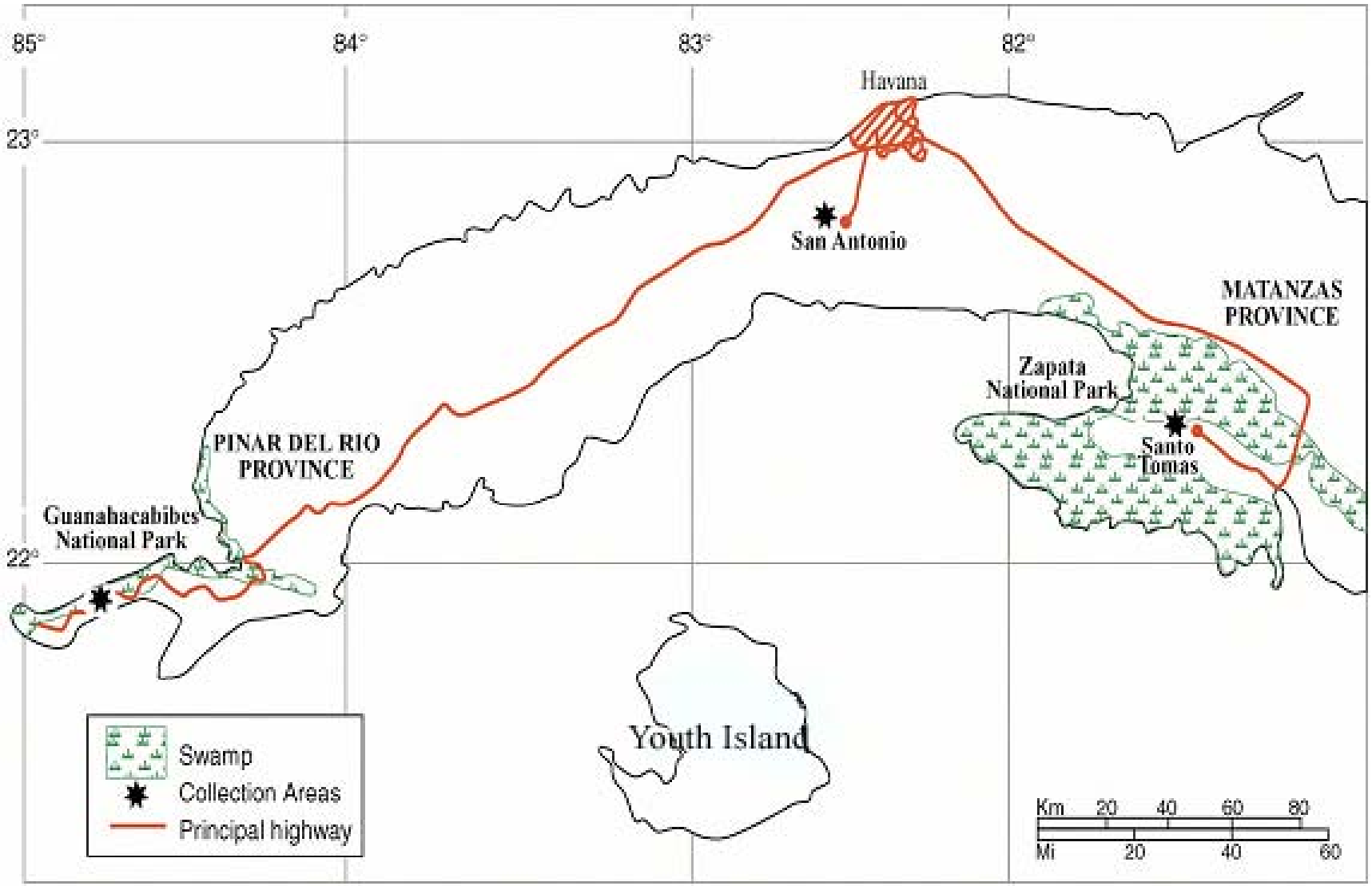


Figure 2-1. Western portion of Cuba showing the three collecting localities: San Antonio, Zapata swamp and Guanahacabibes.

Table 2-1. Sites, dates, number of individuals collected (N) and collectors from 1993 through 1997.

Sites	Date		N	Collectors
Guanahacabibes Peninsula	10-25 Nov	1993	15	Hernandez
San Antonio	6-9 Mar	1995	10	Knight and Dockx
San Antonio	6-11 Nov	1995	30	Dockx
Zapata Swamp	14-15 Nov	1995	2	Dockx
Guanahacabibes Peninsula	18- 24 Nov	1995	3	Dockx
San Antonio	15-23 Nov	1996	78	Dockx
Guanahacabibes Peninsula	23-28 Nov	1996	1	Dockx
San Antonio	11-26 Nov	1997	42	Dockx
Total			181	

The only certain siting of a North American monarch arriving in Cuba occurred in Havana Beach (Urquhart 1976; Luis de Armas, pers. comm.), and for that reason the first search for the monarchs (March 1995) was concentrated in areas close to La Havana. During this time, east (coast) and south locations by La Havana were visited where Cuban scientists reported having seen monarchs in the past, but in none of these locations were monarchs found. All these areas had been fumigated against mosquitos around tourist locations, in some, hotels have been built, and others were not suitable for monarchs. The 15 mounted monarchs collected by Hernandez (Guanahacabibes, Nov 1993), were donated to me during my visit to Cuba in March 1995.

In my second visit (Nov 1995), two other areas were included: Guanahacabibes and Zapata Swamp. Guanahacabibes was included because of the results of TLC on Hernandez's monarchs collected in this area in 1993. Zapata Swamp was chosen because of its importance for the migration of temperate birds (Garrido 1988; Gonzalez et al. 1992), and its possible importance for migrant monarchs (Figure 2-1).

These three collecting localities, as with all of Cuba, are subject to seasonal changes in temperature and precipitation during the dry period that extends from November to April (called the "winter") and the rainy season, May to October. These three places are very similar in terms of their temperature and precipitation (Table 2-2).

Table 2-2. Elevation mean, temperature and precipitation in the dry period (November-April) and wet period (May-October) in the three areas of study (Atlas de Cuba 1978).

Sites	Temperature (C^o):		Elevation (m):	Precipitation (mm):	
	Dry	Wet period		Dry	Wet period
Guanahacabibes Peninsula	22.5	27.5	0-50	300	1100
San Antonio	21.5	27.5	75	300	1300
Zapata Swamp	21.5	27.5	0-50	300	1200

The collections were done during November in 1995, 1996, and 1997, because migrant monarchs arrive in South Florida at this time (Knight 1998) and some may continue to Cuba. After the butterflies were collected, they were placed in glassine envelopes, and transferred to a standard refrigerator to keep them alive and cold during the fieldwork in Cuba, where the butterflies stayed approximately 20 days. Afterwards, butterfly samples from March and November of 1995 were brought to the University of Florida, where they were stored after their arrival in a standard laboratory freezer. In a March of 1995 sample, 2 of the 10 butterflies and 8 of 30 November 1995 samples were dead on their arrival at the University of Florida. The dead butterflies were always maintained in a standard freezer and during transport they were transferred to a cooler that was maintained full of ice to ensure the quality of the samples. In no case was there any evidence of butterfly samples being corrupted, as would be indicated by fungi and/or mold.

Butterflies captured in November of 1996 and 1997 were transported to UNAM (Autonomous National University of Mexico in Mexico City), where they were killed (some had already died) by exposing them to –70C ^{o} for 20 minutes. In the sample of November 1996, 50 of 79 butterflies were dead and 3 of the 42 November 1997 butterflies were dead at the time of their arrival in Mexico. The heads of the butterflies that were alive were immediately removed, placed in microtubes, individually labeled, and stored in a -70 C ^{o} freezer. The alloenzymes analysis requires the organisms to be alive at the time that the sample is taken. These head samples will be used in the future for alloenzymes analyses. Finally, all the butterflies were transferred on ice to the University of Florida, where they were stored in a standard freezer before being analyzed.

San Antonio de los Banos

This collecting field, approximately 33 km (23 miles) southwest of La Havana (Figure 2-1), is 180m by 200 m of pasture in a dairy farm owned by the state, located 1 km from San Antonio and Guira (22^{o} 8'N, 82^{o}4'W). Dirt roads surround two sides of the field and the other two sides are dairy farm constructions.

This field has a large patch of *Asclepias curassavica*, where most of the monarchs were captured, and where all the larvae instars were observed. *A. curassavica* is the only *Asclepias* on which monarch larvae were observed. None of the larvae or eggs were removed from their plants.

Zapata Swamp

This swamp is south-east southeast of La Havana and is a national park that annually receives birds in their migration to the Neotropics. The general vegetation of the area is herbaceous swamp plants, such as *Cladium jamaicense* (Cyperaceae),

Chrysobalanus icaco (Chrysobalanaceae) and lianas (Gonzalez et al., 1982). The sampling area was grassland around a small village called Santo Tomas (22 3'N, 81 5'W) where *Sarcostemma clausum (Jacq.)* (Asclepiadaceae) was present (Figure 2-1).

Guanahacabibes Peninsula

This peninsula is the westernmost portion of Cuba, and its northern region is a national park, declared a Biosphere Reserve by UNESCO (Figure 2-1). Mangroves, swamp vegetation and forest cover the park. The forest is managed in a sustainable way by the government. Monarchs were collected here by Hernandez in November of 1993 by the road along the northern portion of the peninsula, in grasslands close to forest and/or swamp vegetation (21 8'N, 85 0'W). During November 1995 and 1996, monarchs were observed and collected only around the lighthouse in the westernmost portion of the peninsula. During November 1995 and 1996, two plants in two years of *A. curassavica* were seen without eggs or larvae of monarch butterflies, suggesting that this area does not support a resident population. This last observation was confirmed by Alfredo, the Guanahacabibes Park manager, during research conducted for this dissertation in November 1996.

Measurements and Chemical Analysis

Before any laboratory analysis was performed, a picture of the butterfly was taken under standardized conditions of light, background, and distance to the specimen. Then, the right forewing length was measured; and the sex and wing condition also recorded.

Next, the right forewing and hindwing of each butterfly were saved for isotopic determination of carbon ($\delta^{13}C$) and deuterium (δD), analyses that later were made at the National Hydrology Research Center, Saskatoon, Canada. A detailed description of isotopic analysis methodology can be found in Hobson and Wassenaar (1999) and

Wassenaar and Hobson (1998). The other two wings, the thorax and the abdomen (not the head in the majority of the cases) were used for three chemical analyses: fat quantification, cardenolide quantification (spectrophotometry), and Thin-Layer Chromatography (TLC).

The butterflies were then dried at 60° C for 16 hours in a forced draft oven and later weighed on a Mettler AK 160 balance. The dried specimens were later ground in a centrifuge tube in 20 ml petroleum ether with a Janke& Kunkel SDT Ultra Turrax tissuemizer and the lipids were extracted following the methodology used by Alonso (1996). The defatted butterfly material was dried and weighed to determine the lean mass. The remaining butterfly material was extracted in ethanol for determination of cardenolide concentration in ug/0.1g dry butterfly material using a Perkin-Elmer Lambda IIs dual beam spectrophotometer. The cardenolide determination was done following the methodology used by Brower et al. (1982) and Malcolm et al., (1989).

Seven ml of the cardenolide-ethanol mixture that resulted from spectrophotometry analysis were cleaned of contaminants (Malcolm et al. 1989), and used for TLC. The clean extract was dissolved in chloroform and spotted, along with digitoxin and digitoxigenin cardenolide standards, on Silicagel plates (Malcolm et al. 1989). Then each TLC plate was developed, and chromatograms of monarchs were visualized as blue spots by spraying with a saturated solution of TNDP (2,2'-4',4'-tetranitrodiphenyl) in benzene, and the developed TLC plate was immediately photographed. Finally, the slide photograph was scanned and transferred to a CD disk or computer file.

Cardenolide Fingerprint Determination

The match of the TLC pattern of each butterfly to milkweed was done by visualizing spot mobilities (relative to digitoxin) from projected 35-mm color slides of

TLC plates and slide scan computer images. These TLC spot patterns were matched to a specific milkweed species through comparisons with published studies (Malcolm et al. 1993; Moranz 1996; Knight 1998).

Potential problems arise using TLC in Cuban monarch populations, because of an overlap of *Asclepias* species present in Cuba and the southern United States. In Cuba the following species are reported: *Asclepias nivea* (L.), and *Asclepias curassavica* (Leon and Alain, 1957; Roig and Mesa 1988). Other *Asclepias* species collected in Cuba for this work were *Asclepias fruticosa,* as well as *Sarcostemma clausum* and *Calotropis procera* (occurs in the United Sates as well). *Calotropis gigantea* has been introduced in Cuba (W.D. Stevens pers. comm). *Asclepias curassavica*, one of the species of Asclepias that appears simultaneously in Cuba and the southern United States, has been expanding its natural range because it has been used as an ornamentals and recently used in butterfly gardens. Thus, *Asclepias curassavica* has been used in gardens outside of its natural range, as far north as the northeastern United States and possibly southeast Canada. Hence, if a monarchs captured in Cuba had any of the TLC patterns of the *Asclepias* that are present in both Cuba and United States, they could not be classified as migrant or resident. They were classified using only their isotopic values, which were only available for butterflies collected in March 1995, November 1993 and November 1997.

If isotopic values were not available, then the butterflies were not classified as migrants or residents, and they were not included in the analysis. This was the case for nearly all the butterflies for November 1995 (33 of 35 monarchs) and 1996 (76 of 79 monarchs). But if a monarch butterfly had a TLC fingerprint of an *Asclepias spp*. present only in the northeast of the North American continent such us *Asclepias syriaca*) the

butterfly was classified as migrant. Such was the case for butterflies collected in November 1995 and 1996 (where isotopic values were not available) and for some (N=4) butterflies collected in November 1993 and 1997.

Results

TLC Cardenolide Fingerprints

From 168 butterflies sampled in Cuba, nine (5%) have no visible patterns, 95 (56%) display *A. curassavica* patterns that can not be classified as migrant or residents because they were not analyzed with the isotopic technique, 29 (17%) show *A. curassavica* pattern and were identified as Cuban residents by the isotopic technique, 18 (11%) individuals show *A. syriaca* pattern, and 17 (10%) samples were lost at some point during the chemical analysis (Table 2-3 and Figure. 2-2).

The presence of the *Asclepias syriaca* TLC fingerprint in butterflies collected in the four sampled years (November 1993 and 1995-1997), and in all three sampled areas (San Antonio, Zapata Swamp and Guanahacabibes Peninsula) shows that monarchs arrive in Cuba from the northeast of the North American continent. All butterflies with the *A. syriaca* fingerprint match the expected values of hydrogen isotope (deuterium) and the carbon ($\delta^{13}C$) values for monarchs coming from the northeast (Table 2-3). In contrast, no *Asclepias syriaca* pattern (or any TLC fingerprint from an *Asclepias* species of the northeast) was found in the sample of 10 individuals in March 1995, indicate the absence of migrant monarchs (Table 2-3).

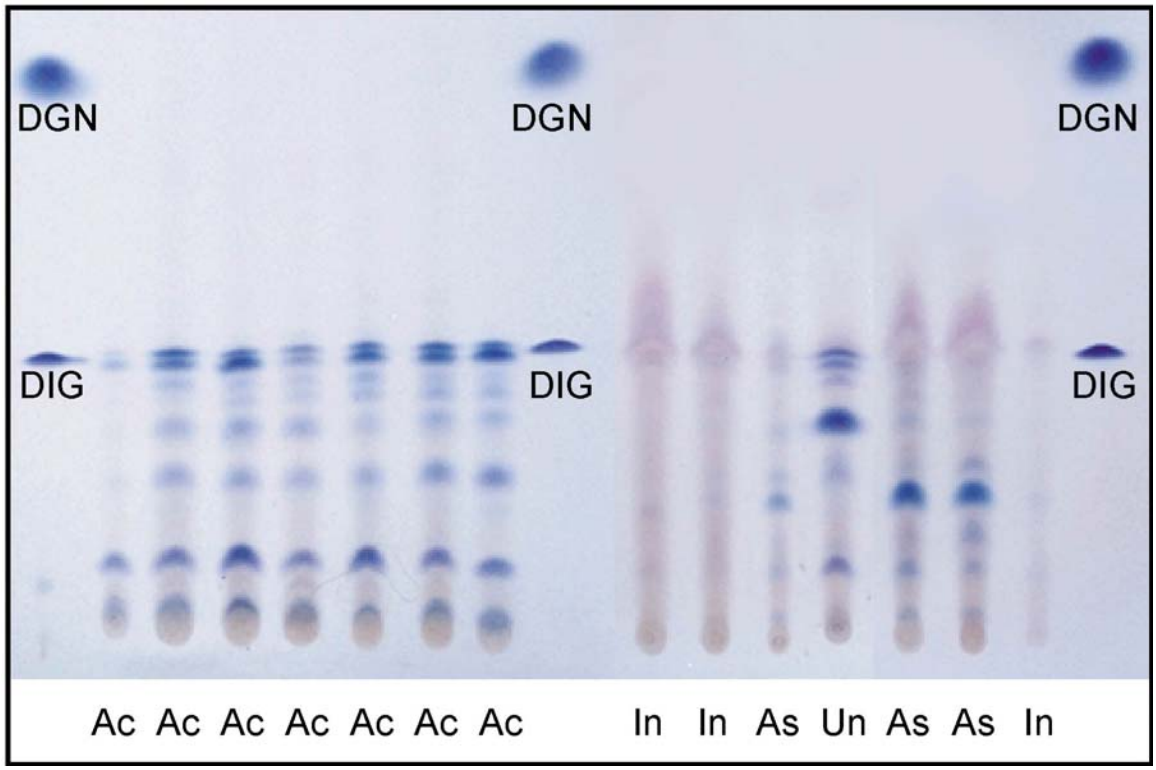


Figure 2-2. Thin-layer chromatograms of 14 monarchs captured in Cuba with digitoxin (Dig) and digitoxigenin standards. The first seven sample columns came from monarchs collected in San Antonio, in March 1995 (starting from the left), showing an *Asclepias curassavica* (*Ac*) cardenolide fingerprint. The next seven sample columns represented monarchs collected in Guanahacabibes, in November 1993. Three columns have invisible (In) patterns, three have an *Asclepias syriaca* (*As*) cardenolide fingerprint, and one has an undetermined fingerprint (Un). This plate is composed of different channels coming from different plates. Each channel represents a butterfly sample. This image was assembled using Adobe Photoshop 5.5, as in Moranz and Brower (1998).

Table 2-3. Summary of TLC fingerprint results of 168 monarchs collected in Cuba in Guanahacabibes, San Antonio and Zapata Swamp, during 1993 and 1995-97. The column labeled as "lost" accounts for butterflies that were lost at any point of the chemical analyses.

Monarch samples				Asclepias	Cardenolide	Patterns	
Site	Date	N	Invisible	A.curassavica (from ?)	A.curassavica (from Cuba)	A.syriaca	lost
Guanahacabibes	Nov'93	15	2	1	1	11	0
	Nov'95	2	0	1	0	1	0
	Nov'96	1	0	0	0	0	1
San Antonio	Marc'95	10	0	0	10	0	0
	Nov '95	18	0	18	0	0	0
	Nov '96	78	4	55	0	3	16
	Nov '97	42	3	19	18	2	0
Zapata Swamp	Nov '95	2	0	1	0	1	0
Total		168	9	95	29	18	17

Isotopic Analysis

The isotopic results for monarchs collected in November 1993 and 1997 show that of the total 57 butterflies, 36 (63%) are migrants from the United States and southern Canada, 19 (33%) are Cuban residents, and two (5%) defy classification because their hydrogen (δD) was missing (Table 2-4). As with the isotopic results, TLC indicates the absence of any migrant monarchs in the March 1995 sample. The isotopic values for this March 1995 sample agree 100 percent with the TLC fingerprints. These two techniques agree as well for monarchs with the *A. syriaca* TLC fingerprint. That is, monarchs that have the *A. syriaca* TLC fingerprint have isotopic values for hydrogen (δD) and carbon ($\delta^{13}C$), typical of the region were this *Asclepias* occurs. The values of the hydrogen isotope (deuterium) and the carbon ($\delta^{13}C$) of the breeding range of eastern North American and Cuba are shown in Figure 2-4.

However, monarchs with the *Asclepias curassavica* TLC fingerprint from November 1997 samples produce contradictory results. The contradiction comes from 14 of 42 monarchs having the *A.curassavica* fingerprint but isotopic values of geographical areas where this species is uncommon (e.g., southeast Canada and the northeastern United States). This may be explained by the recent use of *A. curassavica* in butterfly gardens outside of their natural range, such us southeastern Canada and the northeastern United States. Other possible explanation is that could be another *Asclepias spp*. that has a very similar pattern to *Asclepias curassavica.*

Monarchs that migrate to Cuba come from Southern Canada and throughout all of eastern North America: regions 1, 2, 3 and 4 (Table 2-4 and Figure 2-3). Twenty (36%) of the butterflies collected in November came from the periphery of the Midwest (region 2) and six (10.4%) came from the Midwest itself (region 3). Six (10.6%) came from the extreme ranges of their breeding range, southeast Canada (3) and the Southeast of United States (3) (Table 2-4).

The natal grounds for the two Cuban migrant populations, Guanahacabibes and San Antonio, appear to be different. Twelve of the fourteen North American monarchs that were collected in Guanahacabibes Peninsula had hatched in the Midwest and surrounding area (regions 2 and 3), in contrast to the 22 of 42 monarchs collected in San Antonio that came from the entire United States (regions 1, 2, 3, and 4) and southeast Canada (region 1) (Table 2-4 and Figure 2-3).

The migrant monarchs in San Antonio and Guanahacabibes are male biased. There is, however, a difference in the magnitude of this bias as well as the number of migrants as opposed to resident monarchs in the two locations, Guanahacabibes and San

Antonio. The sex ratio in migrant monarchs in San Antonio is 13 to 9 and in Guanahacabibes is 13 to 1. Only one of 14 Guanahacabibes individuals (collected in November 1993) was a Cuban resident. In contrast, of 42 total individuals collected in November 1997 in San Antonio, 18 (43%) were resident monarchs. The remaining 24 individuals (57%) were migrants.

Signs of the deterioration of San Antonio breeding grounds are already visible. During March 1995 Knight and Dockx visited eastern (coastal) and southern locations near La Havana where Cuban scientists had seen the monarchs, but in none of these locations were monarchs found. All of these areas had been developed for tourism or become so dry that they were not suitable for monarchs.

Monarch Migration to Cuba?

The arrival of monarchs in Cuba can be considered migratory according to the Kennedy (1985) and Dingle (1996) definition: persistent and straightened-out movement of individuals that depends on temporary inhibition for site-staying response. Specific patterns of energy allocation and specific departing and arriving times are other characteristics of this animal movement. Monarchs migrated to Cuba in a persistent manner during the four sampled years, and probably also to other areas of the Caribbean.

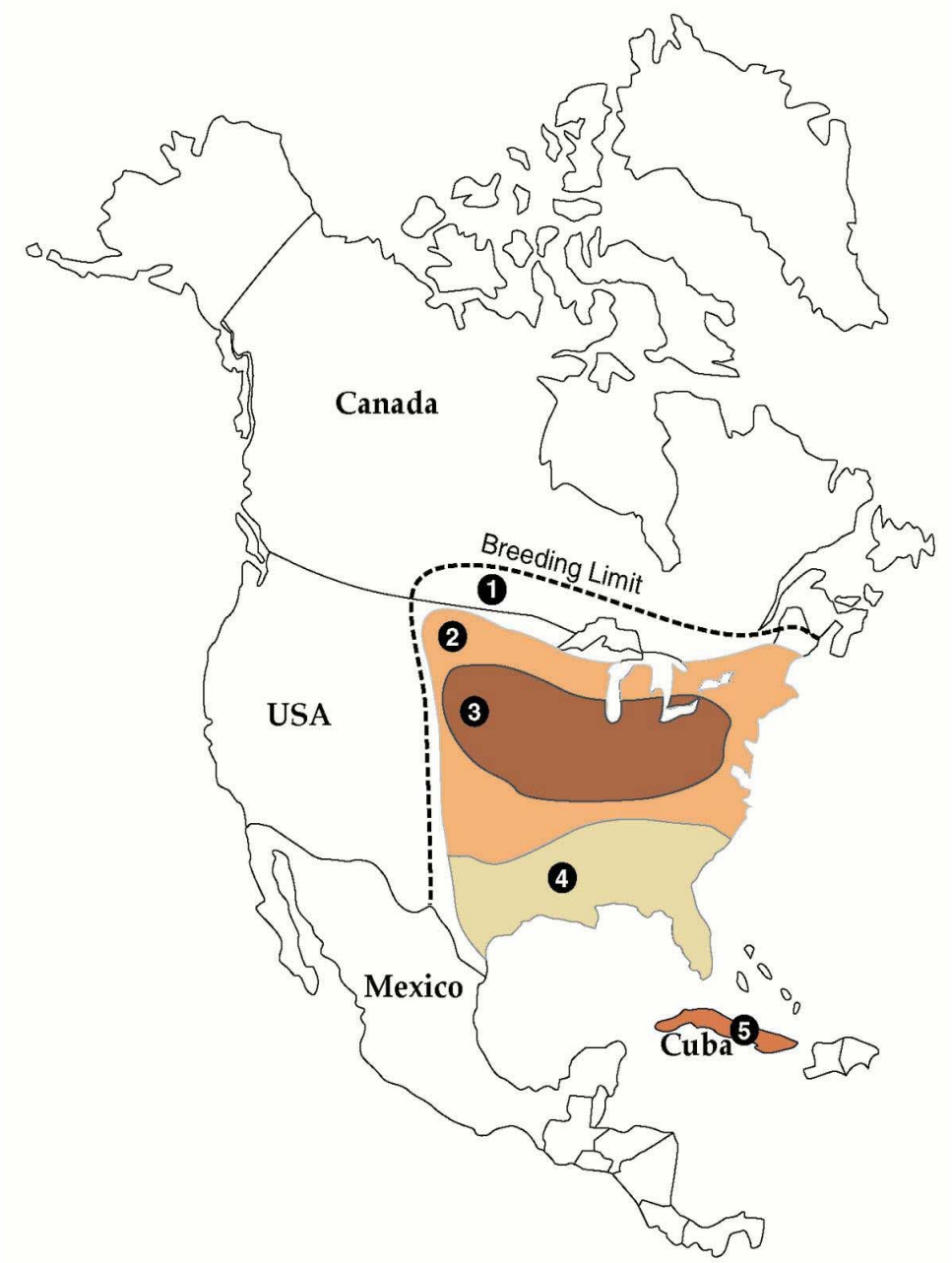


Figure 2-3. Natal regions for the monarchs collected in Cuba during November 1993 and March 1995 (N=67). These natal grounds are determined from the values of hydrogen (δD) and carbon ($\delta^{13}C$) obtained from the migrant monarchs. The dashed line represents the approximate breeding range of monarchs east of the Rockies. The values for hydrogen (δD) and carbon ($\delta^{13}C$) for monarchs of the North American continent comes from Wassenaar and Hobson (1998). Isotopic contours for δD of field reared monarchs were interpolated at arbitrary 10 per mil contours using Surfer (Golden Software) and imported into a basemap using Mapviewer (Golden Software) (Hobson and Wassenaar 1999).

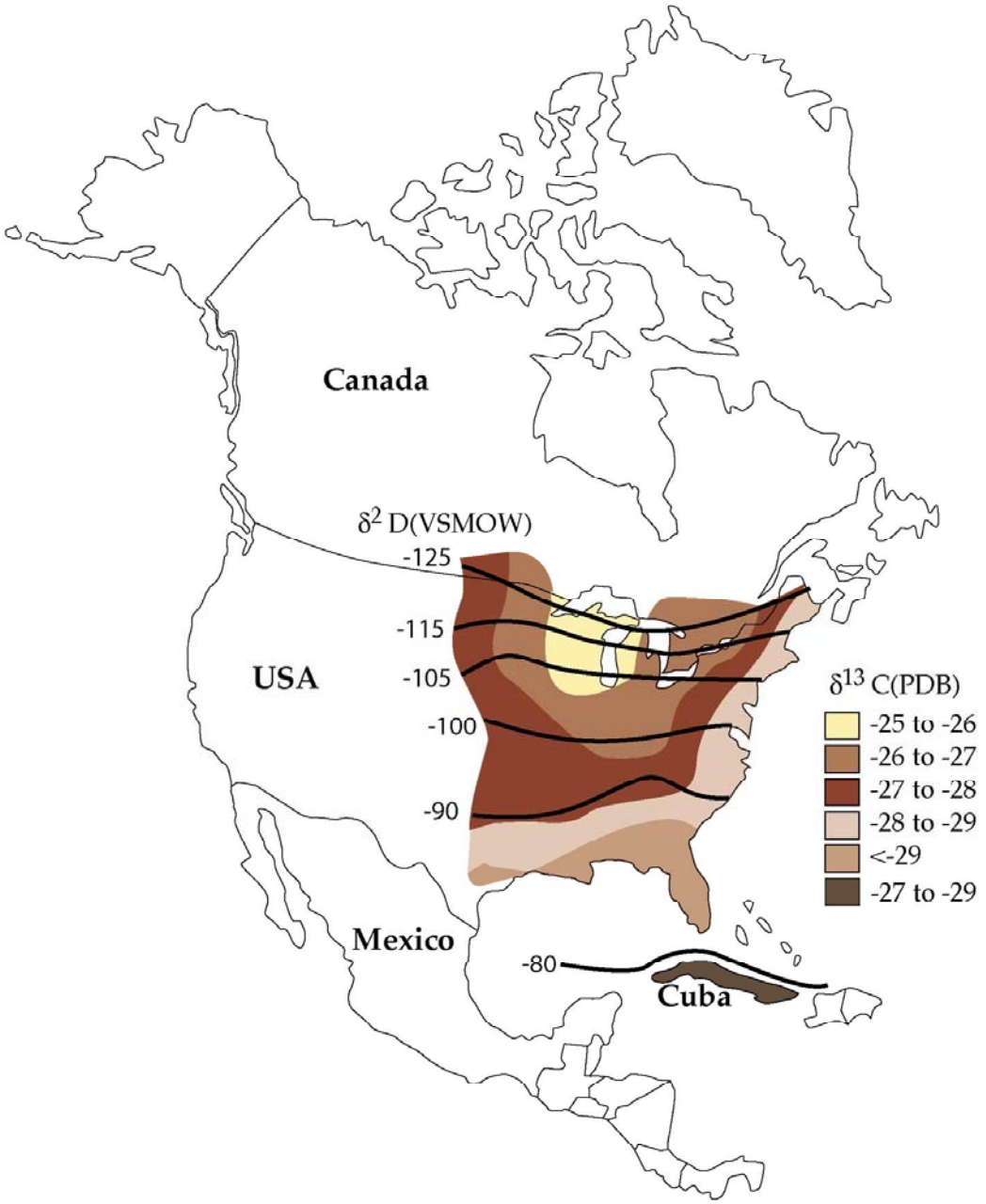


Figure 2-4. Geographic patterns of hydrogen (δD) and carbon ($\delta^{13}C$) values in monarch wings from natal sites across the breeding range of eastern North America and in monarch wings from Cuban resident populations. The values for hydrogen (δD) and carbon ($\delta^{13}C$) for the North American continent come from Wassenaar and Hobson (1998), and for Cuba come from seven *Asclepias* plants collected on this island. Values for δD values are expressed in per mil notation relative to the –132‰ Vienna Standard Mean Ocean Water standard, VSMOW. Values for δC^{13} are expressed in parts per thousands (‰) deviation from the Pee Dee belemnite (PDB) standard.

Table 2-4. Summary of isotopic and TLC results of 57 monarchs collected in Cuba in November 1993, and 1997, plus 10 monarchs collected in March 1995, the regions are marked as Figure 2-3. Sample size (N) and percentages per year were included in the right side of the table. The number in parentheses in the TLC column shows the number of individuals with that specific TLC fingerprint.

Site	Collection date	Region	N	Percent (total)	Percent (per year)	TLC
Guanahacabibes	Nov '93	2	9	14.5	60	A.syriaca (7) Unknown (2)
		3	3	4.8	20	A.syriaca (2) Unknown (1)
		Northeast	1	1.6	6.7	A.syriaca (2)
		Cuba	1	1.6	6.7	Unknown (1)
		Unknown	1	1.6	6.7	A.curassavica (1)
		Total	15		100	
San Antonio	Nov '96	Northeast	3	4.8	100	A.syriaca (3)
Zapata Swamp	Nov '95	Northeast	1	1.6	100	A.syriaca (1)
Guanahacabibes	Nov '95	Northeast	1	1.6	100	A.syriaca (1)
San Antonio	Nov '97	1	3	4.8	7.1	A. curassavica (1) A. syriaca (1) Unknown (1)
		2	12	19.3	28.6	A. curassavica (12)
		3	3	4.8	7.1	A. curassavica (2) Unknown (1)
		4	3	4.8	7.1	A. curassavica (3)
		Cuba	18	29.0	42.9	A. curassavica (18)
		Northeast	1	1.6	2.4	A. syriaca (1)
		Undetermined	2	3.2	4.8	A. curassavica (2)
	Total	42			100	
	Total	62	100			
San Antonio	March 95	Cuba	10	100		A. curassavica

Discussion

Migration of North American Monarchs to Cuba and Their Influence on the Cuban Population of Resident Monarchs

TLC cardenolides fingerprints from November 1993', 1995', 1996', and 1997' and stable isotopic values from November 1993'and 1997' show that monarchs coming from Canada and the United States migrated to Cuba during November in the four sampled years. The migrant monarchs outnumbered the resident ones during this month for November '1993 and '1997. In San Antonio (November '1997) and Guanahacabibes (November '1993), migrant monarchs constituted 63.1% of all butterflies (N=57) collected during these two years, the resident monarch population 33.3%, and the remaining 3.5% were butterflies that could not be determined by TLC or isotopic analysis (Table 2-4). This difference is very conspicuous in Guanahacabibes, where only one resident monarch was found (of 15). This is in contrast with only one previously definitive record of a monarch reaching Cuba having come from the northeast of the North American continent, which had been tagged around Lake Ontario (Urquhart 1987). Information about the total numbers of migrants versus residents collected in November 1995 and 1996 is not available because isotopic analyses were not performed on these butterflies.

Since this migration occurs on a regular basis and the numbers of migrants outnumber the resident population, migrants can be an important force in shaping the resident Cuban monarch population. It is suspected that this same migration from the North American continent occurs as well to the Caribbean islands and to the Yucatan Peninsula; however the impact on resident monarch populations probably becomes diluted the further the migrants are from their source, the United Sates and Canada.

Migrant Monarchs and Their Natal Grounds

Migrants that overwinter in Mexico have natal grounds very similar to the migrants collected in Guanahacabibes. Wassenaar and Hobson (1998) studied 597 monarchs at 13 wintering roost sites in the Oyamel Forest in central Mexico and found that 95% of these butterflies came from regions 2 and 3 in Figure 2-3. The same pattern was found in the Guanahacabibes Peninsula. All the migrant butterflies collected in this most western portion of Cuba, came from the Midwest (region 3) and surrounding area (region 2) (Table 2-4). The isotopic values from the 13 monarch overwintering colonies in Mexico support a generally panmictic model (Wassenaar and Hobson 1998), which is also true for migrant monarchs in Guanahacabibes, but not for those in San Antonio.

The monarchs collected in San Antonio came from regions 2 and 3 and from the extremes of their breeding range as well: southern Canada (region 1) and the southeastertn United States (region 4) (Figure 2-3). Butterflies that have an *A. syriaca* TLC pattern are know to come from the northeast North American continent where this *Asclepias* species grows. However, because their isotopic values are missing they cannot be assigned to any region (Table 2-4). Five percent of migrant monarchs that overwinter in Mexico came from the extremes of their breeding range (Wassenaar and Hobson 1998): southeast Canada and the northeastern United States (region 1) and the southeastern United States (region 4). However, 27% of the migrant monarchs in San Antonio originated from regions 1 and 4 (Figure 2-2). Because migrant monarchs from regions 1 and 4 (Figure 2-3) seem to migrate only to San Antonio (not a single one to Guanahacabibes), the loss of their breeding ground around San Antonio may result in the disappearance of their genes from this area. This may also have an effect on the population dynamics of the Cuban resident

CHAPTER 3
WHY DO MONARCHS MIGRATE TO CUBA?

Millions of eastern North American monarchs migrate each year during the fall from their breeding ranges to the overwintering colonies in the Oyamel Forest in the transvolcanic mountains in central Mexico (Alonso 1996; Brower et al. 1991). The breeding ranges for many (95%) of these migrant monarchs are the U.S midwest and surrounding areas (Wassenaar and Hobson 1999). After hatching, the migrants start their travel south, during which they accumulate their lipid reserves, which are critical to their survival during their overwintering months in the Oyamel Forest, where they will stay in a semi-dormant state (Alonso 1996). These migrant monarchs arrive from late October through November at the Mexican overwintering colonies, and there they remain inactive until March, when they start to mate and then migrate back to the southern United States. During this remigration, the descendants of the Mexican migrants feed on freshly emerging milkweed plants (Herman 1985; Brower and Malcolm 1991; Malcolm et al. 1993).

Not all eastern North American monarchs migrate to Mexico, however. Some move eastward toward the Atlantic coast of the United States (Urquhart 1987), arriving in South Florida (Knight 1998), and Cuba. The current research shows that monarchs coming from Canada and the United States migrated to Cuba during November in the four sampled years (’1993, ’1995, ’1996, and ’1997). The majority (95%) of the Mexican migrant monarchs came from the U.S. midwest and surrounding areas and only 5% of these migrants came from the extreme ranges; that is, southeastern portion of

Canada and the United States (Wassenaar and Hobson 1998). In contrast, 16.6% of the migrants collected in Cuba whose origin was determined came from the extremes of their breeding range (Chapter 2).

In addition to the differing natal grounds ranges of Mexican versus Cuban migrant monarchs, there could be phenotypical differences that can explain why North American and Canadian monarchs migrate to Cuba instead of Mexico. Potentially important phenotypical traits for migration include wing length, wing shape, wing condition, lipid mass, and lean mass. The phenotypic traits of the monarchs are compared in this paper to determine if Mexican migrants and migrants moving through an alternative migratory route (Southeast of the United Sates and Cuba), Cuban migrant monarchs, the resident Cuban monarchs, and both males and females in the resident and migrant groups are homogeneous in terms of their particular phenotypic traits.

Studies with the hemipteran genera *Oncopeltus* and *Dysdercu*s show a positive association of body size and migratory capacity (Dingle and Arora 1973; Dingle et al. 1980). Because wing length and lean mass are good estimates of body size among Lepidoptera (Miller 1990; Alonso 1996), these two measurements were taken in order to distinguish, by comparison of body sizes, between and within resident and migrant monarchs in Cuba. Wing length and lean mass of the migrants in Mexico (Alfonso 1996 and Vanhook 1996), Georgia (Brower without publishing), Florida peninsula (Knight 1996 and without publishing) were compared, to explore differences between different migrant monarch populations. The study of wing length, and subsequently of body size differences has long been used in the scientific literature. Beall and Williams (1945) reported that the mean wing length of monarchs throughout South Americas, where the

monarch does not exhibit migratory behavior, ranges from a mean of 4.6 cm in Ecuador to a mean of 4.8 cm in Brazil. In contrast, the wing length of migrant monarchs in Mexico is 5.2 cm (Alonso 1996). The difference in wing length between migratory and nonmigratory populations is a generalized pattern in Lepidoptera (Arango 1996).

Wing size by itself (not as an indicator of body size) is also important for the migratory butterfly. It has been shown that for wings longer than 1 cm, gliding distance increases with wing length (Kingsolver and Koehl 1985). This enables the migrant monarch to fly farther since migratory monarchs rely heavily on gliding during migration (Gibo 1981). Thus, because of the importance of gliding, wing size can be an important phenotypic trait for migrant monarchs.

The significance of wing shape in flight performance in animals such as birds and bats is known (Norberg 1981; Rayner 1987). It is possible that wing shape also varies between migrant and resident monarchs. Migrant monarchs fly extensively, an average of 4,000 km for those migrating to Mexico and approximately the same for migrants reaching Cuba. Resident monarchs do not migrate, but rather move between *Asclepias* patches and nectaring flowers. As a result, one expects that the resident monarchs will have shorter and broader wings that favor slow, agile flight between vegetation patches, and that migrant monarchs will have more elongated wings to reduce turbulence and drag.

Because of the differences in flight between migrant and resident monarchs, one would also expect resident monarchs to exhibit greater wing wear because they spend the majority of their time flying close to the vegetation, looking for plants on which to oviposit or patrolling for females. In contrast, migrant monarchs are usually in

reproductive diapause, and they do not exhibit these behaviors, perhaps resulting in wings in better condition.

Lipids are also critical to migrant monarchs. They are used as major source of energy in their migration and to survive the overwintering period. Mexican migrant monarchs depend almost exclusively upon these lipids to survive during their stay in Mexico (Alfonso et al. 1997). Autumn migrant monarchs build their lipid reserves during their journey as they migrate southward. It is possible that Cuban migrants differ in their lipid content from Mexican migrant monarchs. Because lipids are so critical, a difference in the lipid content of Cuban migrants, as compared to Mexican migrants, could explain their different migratory routes, destinations, and behaviors.

Methods and Materials

Monarch Collection and Sampling Sites

Monarchs were collected in three locations in the western part of Cuba: in San Antonio de los Banos (31 km or 19 miles southwest of la Havana, Havana Province),in the Zapata Swamp (Matanzas Province), and in the Guanahacabibes Peninsula (the westernmost portion of Cuba, Pinar del Rio Province). Monarchs were collected in November of 1995, ’1996 and ‘1997. Fifteen other mounted monarchs collected by Hernandez in Guanahacabibes in November 1993 were also included in this work. A complete description of localities, maps, and collection methods is found in Chapter 2.

Measurements and Chemical Analysis

Before any laboratory analysis was performed on a butterfly, the butterfly was photographed under standardized conditions of light, with a circular flash attached to the camera lens, at a speed of 16 hundredths of a second, on a gray background, and at a distance 20 cm from the specimen. The camera was a Leica 35 mm with Provia SLR

daylight slide film. After the butterfly was photographed, the right forewing length was measured from the white spot at the wing base on the underside of the wing to its apex. Sex and wing condition were also recorded. Wing condition was rated from 1 (very fresh, virtually no scales missing) to 5 (very worn, many scales missing, and in some cases wing sections absent) in increments of 0.5.

Next, the right forewing and hindwing of each butterfly were saved for isotopic determination of carbon ($\delta^{13}C$) and deuterium (δD), analyses that were made at the National Hydrology Research Center, Saskatoon, Canada (Chapter 2). The other two wings, as well as the thorax and abdomen (not the head in the majority of the cases) were used for fat quantification.

Then the butterflies were dried at 60^{o} C for 16 hours, weighed, and ground with a tissuemizer, and the lipids were extracted following the methodology used by Alonso (1996). The defatted butterfly material was dried and weighed to determine the lean mass.

Statistical Analyses

Wing length, wing condition, lipid mass, and lean weight were analyzed using Wilcoxon's nonparametric test (two-tailed) because wing condition and lipid mass did not have normal distributions and they could not be transformed to have a normal distribution. The chosen significance value for the Wilcoxon test (Z) was 0.05. This nonparametric test was performed to determine if there were mean differences of four variables: (1) females versus males for the migrant and resident group, (2) migrant versus resident monarchs, (3) migrants from Guanahacabibes versus San Antonio, and (4)

migrant males versus resident males. Butterflies were classified as migrant or resident based on the combination of TLC fingerprint and isotopic results (Chapter 2).

Shape Analysis

A method based on the Fourier series, elliptical Fourier analysis, was used to quantify shape of the wing outline. The study of shape differences of structures between populations and/or species using Fourier coefficients has been carried out by Ferson et al. (1985) and Rohlf and Archie (1984). Rohlf and Archie (1984) studied wing differences among 127 species of mosquitoes using elliptical Fourier analysis as well as other methods, and they concluded that elliptical Fourier analysis appears the most promising. Ferson et al. (1985) also used elliptical Fourier analysis to study shape between two groups of mussels, *Mytilus edilus,* that differ with respect to the presence/absence of alleles at two loci. Elliptical Fourier analysis showed an association between genotype and phenotype for the two populations of mussels.

Fourier series are mathematical expressions of sine and cosine curves that can describe a two dimensional outline (Christopher and Waters 1974) and three dimensional data (Ferson et al. 1985). The results of the Fourier series are harmonics and their coefficients. Individual harmonics can be visualized as ellipses that can be added together to represent the specific outline (Figure 3-4). There are several methods of calculating the coefficients of these harmonics. Some of these methods are called centered polar, raw polar and elliptical Fourier. In the first case the centered polar data set is obtained as equally spaced radii from a centroid of the outline; the raw polar data set is obtained as radii originating from a landmark of the outline. All the methods, given enough harmonics, can encode the outline; however, there are different criteria in the selection of landmarks or centroids, and some outlines may not have a single centroid or landmark,

leaving elliptical Fourier as the most generalized approach. Since elliptical Fourier does not require a center or landmarks, it does not require the points to be spaced equally and can fit an arbitrary contour (Rohlf and Archie 1984).

Fourier data are sensitive to information that is not needed for this particular study, such as location, size, and orientation of the object. Luckily, Kuhl and Giardina (1982) were able to remove this information (normalization), and as a result elliptical Fourier analysis can quantify shape per se. The program used to perform elliptical Fourier analysis, EfaWIN (http://life.bio.sunysb.edu/morph), incorporated Kuhl and Giardina's normalization, and as a result the analysis was insensitive to differences in size and location of the wing in the image.

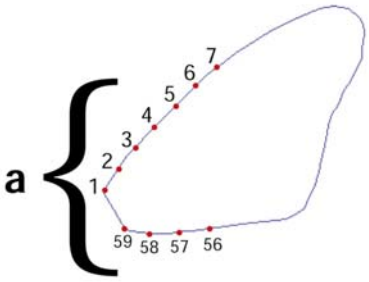
An outline reconstructed by Fourier analysis is the sum of harmonically-related ellipses that describe a closed curve. The closed curve in this case is the right forewing of each butterfly. These harmonics represent independent contributors to the wing outline. The amplitude of each harmonic can be obtained as the sum of the squares of the four harmonics' coefficients. Figure 3-1 (section 3), shows harmonics (in this case nine) with their four coefficients (a, b, c and d).

In the present work, elliptical Fourier analysis was used to determine if migrant and resident monarchs, and if Guanahacabibes and San Antonio migrants in Cuba differ in their wing shape. The Fourier analysis was performed on the right forewing of each butterfly; however if the right forewing of the butterfly was damaged, the analysis was done on the left forewing; and if neither of the two forewings was in good condition, then the analysis was not performed. The first step

Figure 3-1. Input and output data sets of the shape analyses. The butterfly picture was scannned (1). The outline of the right forewing (2a) is digitized with WINDIG. At the right of the butterfly outline we can see some of the digitized points (in red) as well as the average distance between them. The output of this WINDIG was an average of 50 x and y coordinates for each digitized wing (2b). WINDIG These digitized points were the input for EfaWIN, elliptical Fourier program, and the data output of this program were the harmonics and their coefficients (3), that is coefficients that were used in a multivariate analysis, Principal Component Analysis, to study shape differences between different monarch populations.

1.

2.



	X	Y
1.	195	122
2.	202	132
3.	210	140
4.	223	151
5.	236	161
6.	252	170
7.	268	180
•	•	•
•	•	•
•	•	•
•	•	•
56.	224	102
57.	218	103
58.	213	104
59.	208	107

b

3. Coefficients for harmonics

	A	B	C	D
1.	1	0	0	-0.596
2.	-0.0099	0.0671	-0.0906	-0.0324
3.	0.0838	-0.0017	-0.0148	-0.0085
4.	-0.0139	0.0283	0.0316	-0.0001
5.	0.0216	0.0031	0.0007	-0.0287
6.	-0.0058	0.0085	-0.0013	-0.0023
7.	0.0010	-0.0028	0.0016	-0.0030
8.	-0.0054	0.0060	0.0082	0.0013
9.	0.0001	-0.0030	0.0004	-0.0036

was to scan the right forewing of each butterfly slide (Figure 3-1, section 1). The butterfly picture was scanned by Harmon's Photo Shop (Gainesville, Florida). Each original, color-scanned picture was 3190 by 2127 pixels, with a resolution of 960 pixels/cm, and a mean luminosity of 65.41. The original color image was then converted to a gray scale with a resolution of 399 by 269 pixels to make the image more manageable on the computer screen and to more easily load it into the next program, WinDIG. Then, the points in the outline of the right forewing were extracted from the image using WinDIG 2.5 (http://life.bio.sunysb.edu/morph). The extraction of the points in the outline of the wing was performed manually, starting at the upper left of the right forewing (point 1 in Figure 3-1, section 2a). The typical average distance between each digitized point is shown in Figure 3-1 (section 2a); however, this distance was less in the upper tip, because more points were needed to reproduce this part of the wing. For each digitized datum, an x- and y-coordinate was produced, and an average of 50 x and y coordinates were produced for each digitized wing (Figure 3-1, section 2b). These x- and y-coordinates were analyzed by Fourier analysis, EfaWIN (http://life.bio.sunysb.edu/morph). The Windows shell for EFA (WINDOWS) was designed by Mike Isaev and runs Rohlf and Ferson's 2D elliptic Fourier analysis program. The result of the Fourier analysis is a group of harmonics with their four coefficients (a, b, c and d) (Figure 3-1, section 3).

Individual Fourier coefficients do not have biological meaning by themselves (Bookstein 1982); however, they can uncover relevant biological information about shape in the context of multivariate analysis (Ferson et al 1985). A multivariate analysis, Principal Component Analysis (PCA), was performed in the current work to explore

possible wing shape differences between: (1) migrant and resident monarchs, and (2) migrant monarchs from Guanahacabibes and San Antonio.

The PCA analysis was done on the correlation matrix, and the number of PCA axes retained was based on the "broken stick expectation" (Jackson 1991). The PCA analysis was done using Statistical Analysis System, SAS 6.12 (SAS Institute Inc., 1989-1996, Cary, NC, USA.). Because the PCA analyses revealed that there were significant differences in wing shape, three angles of the right forewing were measured: α, β, and λ (Figure 3-2 and 3.3). If the right forewing was missing critical parts, the angle measurement was taken from the left forewing, if neither of the two wings was in good shape the analysis was not performed. This is the reason why in the tables of the result section the sample size varies.

Since angles can be only measured between two lines, lines were traced along each contour of the wing, however this was not possible for angle λ, since the forewing tip does not have a good landmark. A circle section was drawn in the tip following its margin, and the angle λ was formed between a hypothetical tangent to this circle and the line labeled X, in Figure 3-3. The derivation of the formula used to calculate λ is as follows:

The equation of the circle of radius r and center at the origin (0, 0) is:

$$x^2 + y^2 = r^2$$

(1) The formula for the derivative of y with respect to x for y > 0 is:

$$\frac{dy}{dx} = \frac{-x}{\sqrt{r^2 - x^2}}$$

Figure 3-2. Triangulation of the right forewing and the two measured angles: α and β

Figure 3-3. Circle section at the right forewing tip, and the angle λ formed between a hypothetical tangent to this circle and the line labeled, X. The yellow dot represents the center of the circle and r shows its radius. X and r are perpendicular to each other and X is always smaller than r.

(2) Therefore, the angle λ that the tangent line to the circle at (x, y) for x, y > 0 makes with the segment joining the points (x, y) and (0, y) is:

$$\lambda = \arctan\left(\frac{x}{\sqrt{r^2 - x^2}}\right)$$

Results

Wing Length

Mean wing length for migrant monarchs collected in the three locations ranged from 4.8 cm to 5.1 cm (Table 3-1). Migrant monarchs collected in Guanahacabibes have larger wings and lower mean wing length variation (coefficient of variation, C.V.) than the migrant monarchs captured in San Antonio. The mean wing length for Cuban resident monarchs captured in San Antonio in November is 4.7 and shows a greater wing length variation (C.V.) than the wing length of migrants in Guanahacabibes and San Antonio (Table 3-1).

Table 3-1. Mean right forewing length for migrant and resident monarchs collected in November 1995, 1996 and 1997 from San Antonio, Guanahacabibes and Zapata Swamp. Males and females were included.

Migrant/ Resident	Location	Date	N	Wing length (cm)		
				Mean (S.D.)	Range	C.V.
Migrant	Guanahacabibes	Nov '93 and '95	14	5.1 (0.146)	4.9-5.3	2.8
	San Antonio	Nov '95, '96, and '97	25	4.8 (0.298)	4.2-5.4	6.2
	Zapata Swamp	Nov '95	1	4.9 (-)	-	-
Resident	Guanahacabibes	Nov '93	1	5.2 (-)	-	-
	San Antonio	March '95	10	4.5 (0.244)	4.1-4.9	5.4
	San Antonio	Nov '97	18	4. 7 (0.348)	3.9-5.1	7.3
Total			69	4.8 (0.34)	3.9-5.4	7.04

Wing length differs significantly between migrant females (N=10) and males (N=29), in that females have smaller wings than males (Z=-2.42, p=0.016; Table 3-2). A

comparison of the 12 females *versus* 7 males from the Cuban resident monarchs captured in November of ’1993, ’1995, ’1996 and ’1997 revealed that males and females do not differ significantly in their wing length (Z=1.95, p=0.05; Table 3-2). However, the mean difference between males and females as well as the standard deviation (S.D.) between the two groups is similar. But this similarity is the result of combining migrants from Guanahacabibes (largest wing length, Table 3-1) and San Antonio (wing length similar to the Cuban resident monarchs from November, Table 3-1). A comparison of migrant males and females from Guanahacabibes and San Antonio with San Antonio resident males and females, could not be made since only one female was captured in Guanahacabibes.

Table 3-2. Wing length, wing condition, lipid mass and dry lean mass compared between migrant and resident males and females collected in November 1993, ’95, ’96 and ’97 in Guanahacabibes and San Antonio. Assignment to migrant or resident was determined by isotopic analyses and TLC combined.

	N	Wing (cm) Mean (S.D.)	Wing cond. Mean (S.D.)	Lipid (mg) Mean (S.D.)	Lean (mg) Mean (S.D.)
Migrant					
Females	10	4.7 (0.3)	3.2 (0.7)	22.7 (19.8)	88.9 (26.4)
Males	29	5.0 (0.2)	2.6 (0.6)	16.9 (18.0)	107.8 (26.2)
Z value		-2.42	2.28	1.029	-1.913
P-value		0.016	0.02	0.3	0.05
Resident					
Females	12	4.6 (0.4)	3.08 (0.8)	13.8 (9.3)	91.8 (27.1)
Males	7	5.0 (0.2)	2.5 (0.4)	13.4 (4.1)	102.4 (25.1)
Z-value		1.95	-1.46	0.55	0.63
P-value		0.05	0.14	0.58	0.53

There was a significant difference in wing length between resident (N=29) and migrant (N=40) monarchs collected in the three localities in Cuba during March 1995 (only residents) and November 1993, ’1995, ’1996, and ’1997 (Z=-3.19, p=0.001;Table

3-3). Migrant monarchs have larger wings (larger mean length) and the same standard deviation (S.D.) as resident monarchs.

Table 3.3. Wing length, wing condition, lipid mass and lean mass compared between migrant and resident monarchs that were collected in the three locations in Cuba during November in 1993, ‘ 95,’ 96 and ‘ 97. Ten Cuban resident monarchs collected in San Antonio during March 1995 were also included. The independent variable was migratory vs. resident.

(mg)	N	Wing (cm) Mean (S.D.)	Wing condition Mean (S.D.)	Lipid (mg) Mean (S.D.)	Lean weight Mean (S.D.)
Migrant	40	4.9 (0.3)	2.7 (0.7)	18.9 (18.5)	104.12 (27.8)
Resident	29	4.7 (0.3)	2.9 (0.8)	11.8 (7.02)	94.5 (24.1)
Z value		-3.19	1.16	-0.67	-1.60
P-value		0.001	0.24	0.5	0.10

Migrant male monarchs collected in Guanacabibes during November ’1993 have significantly larger wings (Z=2.51, p=0.011; Table 3-4) and wings in better condition (Z=-2.2, p=0.03; Table 3-4) than migrant males collected in San Antonio during November ’1995, ’1996, and ’1997. This comparison was made only for males since only one migrant female was captured in Guanahacabibes.

Table 3-4. Wing length, wing condition, lipid mass and lean mass compared between migrant males collected in Guanahacabibes and San Antonio during November in 1993, ’95, ’96 and ’97. The independent variable was the locality.

Location of collection	N	Wing (cm) Mean (S.D.)	Wing cond. Mean (S.D.)	Lipid (mg) Mean (S.D.)	Lean (mg) Mean (S.D.)
Guanahacabibes	14	5.1 (0.1)	2.3 (0.3)	23.0 (23.8)	113.0 (14.7)
San Antonio	15	4.9 (0.3)	2.8 (0.7)	11.2 (3.9)	103.0 (23.3)
Z value		2.51	-2.2	0.06	1.09
P-value		0.011	0.03	0.948	0.275

However, when compared, migrant and resident monarchs collected in San Antonio in November 1993, ’1996 and ’1997 do not differ in their wing length (Z=0.675,

p=0.499; Table 3-5). In fact, they are very similar in their wing size, that is, they have similar mean length and virtually the same standard deviation. Only males were included in the analyses.

Table 3.5. Wing length, wing condition, lipid mass and lean mass compared between migrant males collected in San Antonio (SA) during November in 1995, '96 and '97 and resident monarchs collected in November 1997.

Migrant/ Resident	N	Wing (cm) Mean (S.D.)	Wing condition Mean (S.D.)	Lipid (mg) Mean (S.D.)	Lean weight (mg) Mean (S.D.)
SA migrant	15	4.9 (0.3)	2.8 (0.7)	11.17 (6.5)	103.0 (27.9)
Resident	7	5.0 (0.3)	2.5 (0.7)	13.46 (3.8)	102.4 (23.3)
Z value		0.675	-1.089	1.445	-0.07
P-value		0.499	0.275	0.148	0.94

Wing Condition

Mean wing condition for migrant monarchs collected in Guanahacabibes, San Antonio and Zapata Swamp ranges from 2.3 to 3.0. Migrant monarchs collected in Guanahacabibes have better wing condition (lower mean) than migrants from San Antonio and Zapata Swamp, and they have a lower wing condition variation than migrants collected in San Antonio. Mean wing condition for Cuban resident monarchs collected in San Antonio was 3.0 (Table 3-6).

A comparison of the 10 migrant female *versus* 29 migrant male monarchs, revealed that males and females differ significantly in their wing condition (Z=2.28, p=0.02; Table 3-2). Wing condition does not differ between resident males (N=7) and females (12) (Z=-1.46, p=0.14; Table 3-2).

Table 3-6. Mean wing condition ranked from excellent (1.0) to poor (5.0) in increments of 0.5 for migrant and resident monarchs collected in November 1995, 1996 and 1997 from San Antonio, Guanahacabibes and Zapata Swamp.

Migrant/ Resident	Location	Date	N	Wing condition Mean (S.D.)	Range	C.V.
Migrant	Guanahacabibes	Nov '93 and '95	14	2.3 (0.317)	1.5-2.5	13.64
	San Antonio	Nov '95, '96, and '97	25	3.0 (0.789)	1.0-4.5	26.7
	Zapata Swamp	Nov '95	1	2.5 (-)	-	-
Resident	Guanahacabibes	Nov '93	1	2.5 (-)	-	-
	San Antonio	March '95	10	3.0 (0.926)	1.0-4.0	30.37
	San Antonio	Nov '97	18	2.9 (0.758)	2.0-4.5	26.25
Total			69	2.8 (0.753)	1.0-4.5	26.78

There also is not a significant difference in wing condition between resident (N=29) and migrant (N=40) monarchs collected in the three localities in Cuba during March '1995 (only residents) and November 1993, '1995, '1996, and '1997 (Z=1.16, p=0.24;Table 3-3). However, migrant male monarchs collected in Guanacabibes during November '1993 have significantly lower (better) wing condition than males collected in San Antonio during November 1995, 1996, and 1997 (Z=-2.2, p=0.02; Table 3-4). This comparison was made only for males since only one migrant female was captured in Guanahacabibes. Additionally, migrant and resident male monarchs collected in San Antonio in November 1993, '1996 and '1997 do not differ in their wing condition (Z=-1.089, p=0.275; Table 3-5).

Lipid Mass

Mean lipid mass for migrant monarchs collected in Guanahacabibes, San Antonio and Zapata Swamp ranges from 0.5 mg to 69.9 mg. Migrant monarchs from Guanahacabibes have a greater lipid mass variation than migrant and resident monarchs from San Antonio (C.V.). Mean lipid mass for Cuban resident monarchs collected in San

Antonio in November is 14.2 mg, with a lower lipid mass variation (C.V) than migrants from Guanahacabibes and San Antonio (Table 3-7).

Table 3-7. Mean lipid mass for migrant and resident monarchs collected in November 1995, 1996 and 1997 from San Antonio, Guanahacabibes and Zapata Swamp.

Migrant/ Resident	Location	Date	N	Lipid (mg) Mean (S.D.)	Range	C.V.
Migrant	Guanahacabibes	Nov '93 and '95	4	23.0 (23.74)	2.3-69.9	103.28
	San Antonio	Nov '95, '96, and '97	25	15.8 (14.66)	0.5-69.2	92.93
	Zapata Swamp	Nov '95	1	39.8 (-)	-	-
Resident	Guanahacabibes	Nov '93	1	3.4 (-)	-	-
	San Antonio	March '95	10	8.2 (3.82)	4.8-16.3	46.82
	San Antonio	Nov '97	18	14.2 (7.43)	4.7-30.4	52.25
Total			69	15.9 (15.162)	0.5-69.9	95.37

A comparison of the 10 migrant female *versus* 29 migrant male monarchs, reveals that males and females do not differ significantly in their lipid mass ($Z=1.029$, $p=0.30$; Table 3-2). Lipid mass for resident females (12) and males (7) also does not differ ($Z=0.55$, $p=0.58$; Table 3-2).

There also is not a significant difference in lipid mass between resident (N=29) and migrant (40) monarchs collected in the three localities in Cuba during March 1995 (only residents) and November 1993, 1995, 1996, and 1997 ($Z=-0.67$, $p=0.5$;Table 3-3); and there is no significant difference in the lipid mass between migrant male monarchs collected in Guanacabibes during November 1993 and 1995 and males collected in San Antonio during November '1995, '1996, and '1997 ($Z=-0.06$, $p=0.94$; Table 3-4). However, migrant monarchs from Guanahacabibes have a higher (double) mean lipid mass and a six times more variable (S.D.) than migrants from San Antonio. This comparison was made only for males since only one migrant female was captured in Guanahacabibes. Migrant and resident male monarchs collected in San Antonio in

November 1993, ’1996 and ’1997 did not differ in their lipid mass (Z=1.445, p=0.148; Table 3-5)

Lean Mass

Mean lean mass for migrant monarchs collected in Guanahacabibes, San Antonio and Zapata Swamp range from 97.4 mg to 148.0 mg. Migrant monarchs from Guanahacabibes had lower lean mass variation (C.V.) than migratory and resident monarchs from San Antonio. Mean lean mass for Cuban resident monarchs collected in San Antonio was 96.6 mg (Table 3-8).

Table 3-8. Mean lean mass for migrant and resident monarchs collected in November 1995, 1996 and 1997 from San Antonio, Guanahacabibes and Zapata Swamp.

Migrant/ Resident	Location	Date	N	Lean Mass (mg) Mean (S.D.)	Range	C.V.
Migrant	Guanahacabibes	Nov ’93 and ’95	14	113.0 (14.73)	77.6-133.9	13.04
	San Antonio	Nov ’95, ’96, and ’97	25	97.4 (31.05)	41.4-175.9	31.89
	Zapata Swamp	Nov ’95	1	148.0 (-)	-	-
Resident	Guanahacabibes	Nov ’93	1	79.9 (-)	-	-
	San Antonio	March ’95	10	92.2 (20.56)	63.7-119.1	22.28
	San Antonio	Nov ’97	18	96.6 (26.7)	43.7-135.0	27.64
Total			69	100.1 (26.57)	41.4-175.9	26.54

A comparison of the 10 migrant females *versus* 29 migrant males reveals that they do not differ significantly in their lean mass (Z=-1.913; p=0.05 Table 3-2). Mean lean mass does not differ significantly for resident females (N=12) and males (N=7) (Z=0.63, p=0.53; Table 3-2).

There also is no significant difference in lean mass between migrant (N=40) and resident (N=29) monarchs collected in the three localities in Cuba during March 1995 (only residents) and November 1993, 1995, 1996, and 1997 (Z=-1.6, p=0.1; Table 3-3). No significant difference in lean mass was found between migrant male monarchs

collected in Guanahacabibes during November 1993 and males collected in San Antonio during November 1995, 1996, and 1997 ($Z=1.09$, $p=0.275$; Table 3-4). However, migrant monarchs from Guanahacabibes had a higher mean for lean mass and lower variation (S.D) than migrants from San Antonio. This comparison was made only for males since only one migrant female was captured in Guanahacabibes. Migrant and resident male monarchs collected in San Antonio in November 1993, 1996 and 1997, do not differ in their lean mass ($Z=-0.07$, $p=0.94$; Table 3-5).

Wing Shape

Wing shape was encoded by nine harmonics and four coefficients (a, b, c, and d) for each harmonic (Figure 3-4), which resulted in 36 harmonic coefficients (Figure 3-1c). These 36 Fourier coefficients were the input data set for the principal components analysis, PCA.

The "broken stick expectation", g_n (null hypothesis), accounts for the proportion of variance expected for each component by chance alone. Because observed variance for the first three principal components was higher than the variance expected by chance (g_n), only these three components were retained (Table 3-9).

The first principal (Z1) component accounts for 37% of the total variance, the second (Z2) for 14.0% and the third (Z3) for 9% of the total variation. These three components together explain the 60.0% of variation of the data. When the values for these three principal components were plotted for the 48 individual butterflies, well structured data was obtained based on: (1) natal ground of the butterfly, that is, if the butterfly is migrant or a Cuban resident, and (2) the area where the butterfly was collected, Guanahacabibes or San Antonio (Figure 3-5). Butterflies with low values for the first principal component (left group of data, Figure 3-5) are all monarchs collected in

San Antonio (letter S, Figure 3-5). This group of butterflies consists of migrants (number 1, Figure 3-5) as well as residents (number 2, Figure 3-5), and females (N=16) and males (N-8) combined. In contrast, butterflies with larger values for the first principal component (right group of data, Figure 3-5) are all migrants (except one), most are males (21 of 24); and the majority were collected in Guanahacabibes (14 of 24). All migrants from Guanahacabibes belong to this second group.

Table 3-9. Eigenvalues of the correlation matrix and variance for the first four principal components for 48 migrant and resident monarchs collected in Cuba. "Broken stick" expectation for the proportion of variance accounted for each of these four components is listed.

Component	Eigenvalue	Observed variance	Broken stick value (g_n)
Prin1	13.21	0.37	0.11
Prin 2	5.04	0.14	0.08
Prin 3	3.33	0.09	0.07
Prin 4	2.06	0.06	0.06

Wing outlines for individual butterflies in the two groups with the largest values for these three PCA axes were reconstructed to visualize wing shape differences between the two groups (Figure 3-6). The wing outlines of butterflies at the right side of Figure 3-5 are labeled with an R in Figure 3-6, and wing outlines at the left side were labeled L. Butterflies in the group at the right in Figure 3-5 appeared to be different in the angle where the wing joins the body (angle α, Figure 3-2), from the butterflies at the left side. Butterflies in this group also (at the right in Figure 3-5), have a more acute inflection at the opposite side where the wing joins the body, resulting in a narrower and more prominent wing tip. In contrast, butterflies at left side have a less acute inflection, resulting in a more rounded wing tip. This can bee seen by comparing the wing outlines 3L and 3R, in Figure 3-6.

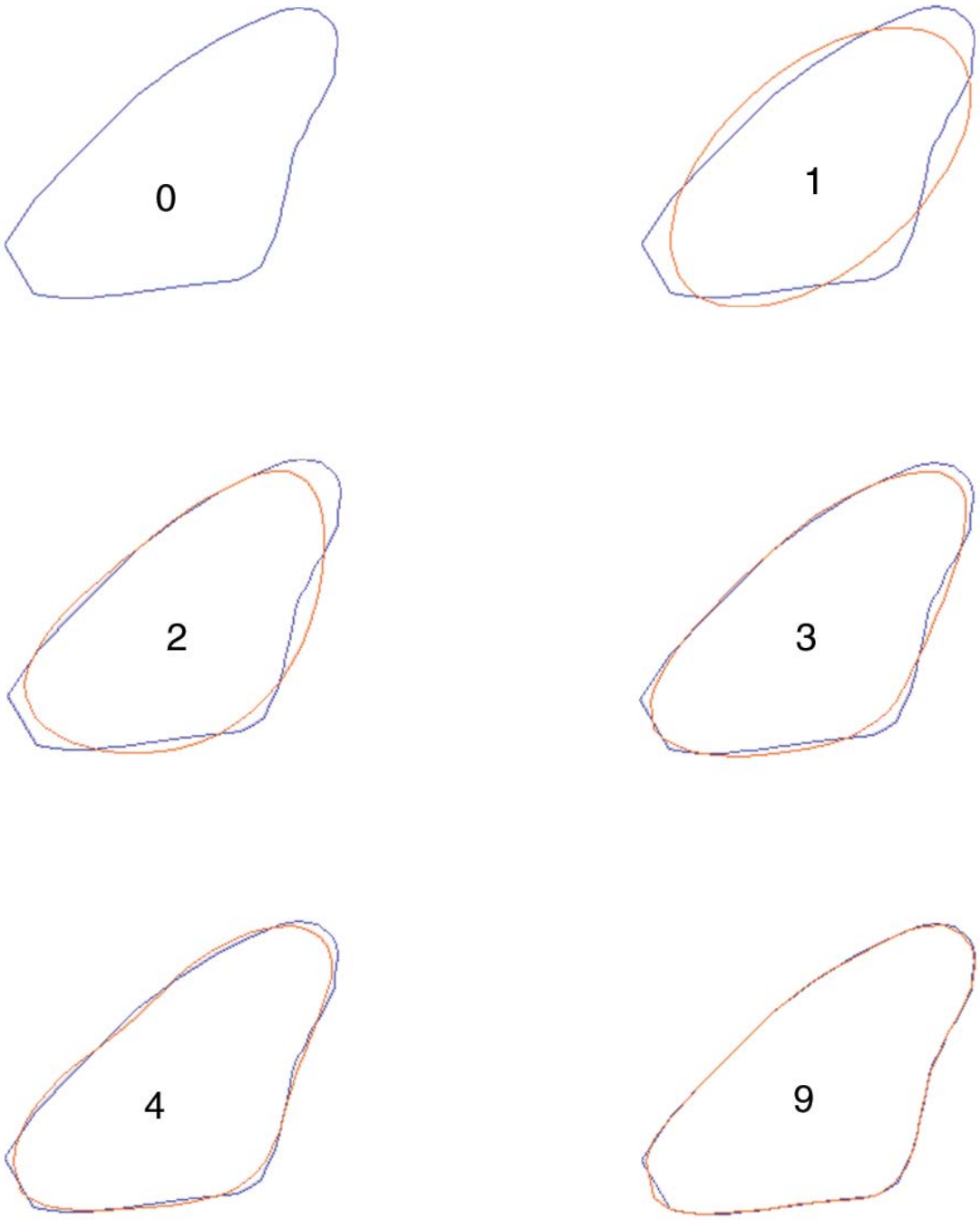


Figure 3-4. Elliptical Fourier harmonics for a right forewing of a butterfly based on x- and y-coordinates of the wing. The numbers inside each wing outline represent the harmonic and the red outline the cumulative contributions of the harmonics in the reconstructions of the wing outline. The number 0 represents the original wing outline, that in each case is shown in blue color.

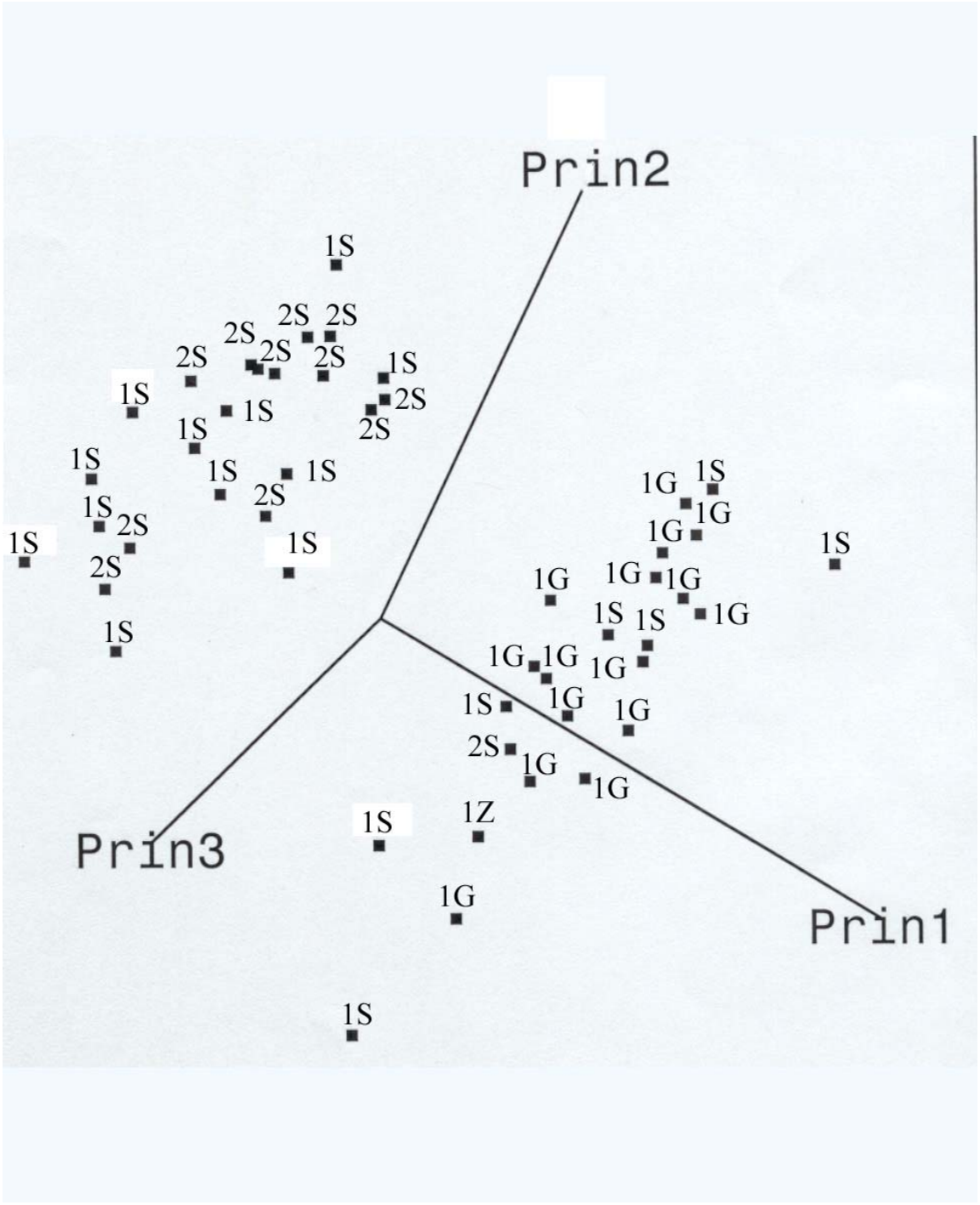


Figure 3-5. Plot for the first three dimensions of the principal components analyses of Elliptical Fourier coefficients for migrants (number 1) and resident (number 2) monarchs. The letter represent the locality were the monarchs were collected: S, for San Antonio, G, for Guanahacabibes and Z, for Zapata Swamp.

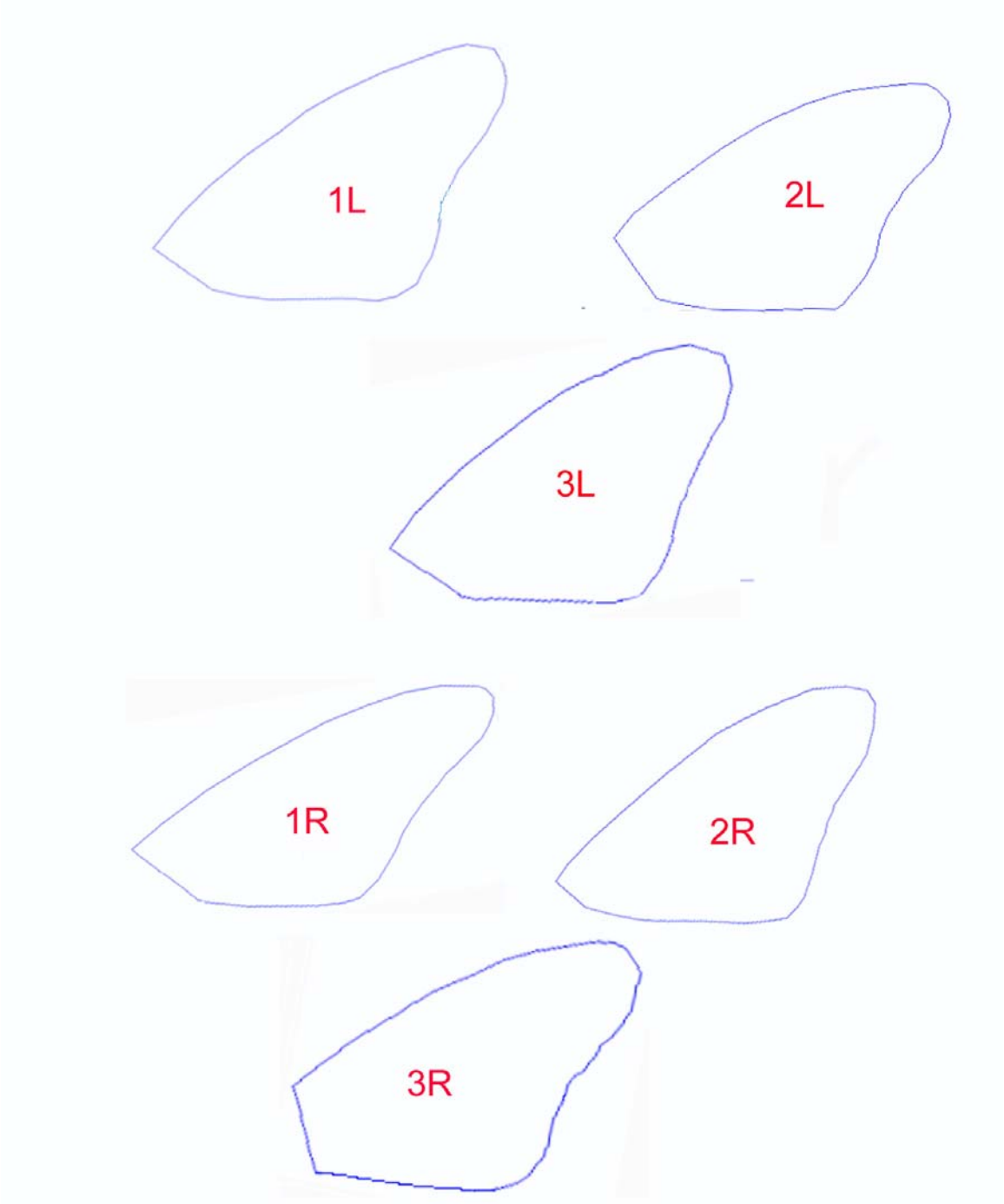


Figure 3-6. Wing outlines for individuals with the largest values for the three principal components. The number inside the outline refers to the principal component and the letter L (for left in Figure 3-3) represents individuals with low values for the first principal component. R (for right in Figure 3-3) represents λ as well. These three angles, α, β, and λ, are shown in Figure 3-2 and Figure 3-3.

The next section offers a quantification of these angular differences between groups individuals with larger values for the principal component. The wing outlines do not show wing size wing differences between butterflies.

Angle Measurements

Migrant monarchs collected in Guanahacabibes have wings with the larger values for angle α and lower standard deviation (S.D) than the migrant monarchs captured in San Antonio. The mean for angle α for the Cuban resident monarchs captured in November is lower than the mean for residents captured in March (Table 3-10).

Table 3-10. Angle's mean –α, β, and λ- for migrant and resident monarchs collected in November 1995, 1996 and 1997 from San Antonio, and Guanahacabibes. Males and females were included.

Migrant/ Resident	Location	Date	N	Angles (Mean and S.D)		
				Angle α	Angle β	Angle λ
Migrant	Guanahacabibes	Nov 93 and 95	14	42.12 (1.64)	113.03 (3.0)	77.19 (10.9)
	San Antonio	Nov 95, 96, and 97	21	39.06 (3.0)	113.42 (3.44)	66.02 (9.88)
Resident	Guanahacabibes	Nov 93	1	40.5 (-)	112.5 (-)	83.33 (-)
	San Antonio	March 95	10	41.74 (1.93)	112.03 (1.71)	63.74 (11.47)
	San Antonio	Nov 95, 96 and 97	17	38. 22 (2.37)	114.61 (3.36)	55.18 (12.61)
Total			63	39.96 (2.85)	113.42 (3.13)	65.51 (13.52)

Angle α differs significantly for migrant females (N=9) and males (N=26), where females have narrow angles than males (Z=-3.06, p=0.002; Table 3-11). A comparison of

Studies of the 11 females *versus* 7 males from the Cuban resident monarchs captured in November of '1993, '1995, '1996 and '1997 revealed that males and females do not differ significantly in their angle α (Z=0.09, p=0.93; Table 3-11). A comparison of migrant males and females from Guanahacabibes and San Antonio with San Antonio resident males and females, could not be made since only one female was captured in Guanahacabibes.

Table 3-11. Angle comparison –α, β, and λ- between migrant and resident males and females collected in November 1993, '95, '96 and '97 in Guanahacabibes and San Antonio. Assignment to migrant or resident was determined by isotopic analyses and TLC combined.

	N	Angle α Mean (S.D.)	Angle β Mean (S.D.)	Angle λ Mean (S.D.)
Migrant				
Females	9	37.73 (3.25)	114.47 (1.82)	64.74 (11.68)
Males	26	41.16 (2.29)	112.85 (3.53)	72.48 (11.05)
Z value		-3.06	1.21	-1.63
P-value		0.002	0.22	0.1
Resident				
Females	11	38.1 (2.77)	114.93 (4.1)	55.5 (14.11)
Males	7	38.74 (1.63)	113.81 (1.41)	58.83 (14.74)
Z-value		0.09	0.36	0.36
P-value		0.93	0.72	0.72

There was not a significant difference in angle α and angle β between resident (N=28) and migrant (N=35) monarchs collected in the three localities in Cuba during March 1995 (only residents) and November 1993, '1995, '1996, and '1997 (Table 3-12). In contrast, these two groups differ significantly in angle λ ($Z=-3.03$, $p=0.002$; Table 3-12). However, apparently there is a discrepancy between migrant monarchs having a lager value for angle λ and having a more elongated forewing tip. This contradiction can be explained by the orientation of the tip forewing. This tip for the right forewing in migrant monarchs is more tilted to the right, creating a more conspicuous inflection of the tip (Figure 3.7) than for resident monarchs.

Table 3.12. Angle comparison –α, β, and λ- between migrant and resident monarchs collected in the three locations in Cuba during November in 1993, ‘ 95,’ 96 and ‘ 97. Ten Cuban resident monarchs collected in San Antonio during March ’95 were also included. The independent variable was migratory vs. resident.

	N	Angle α Mean (S.D.)	Angle β Mean (S.D.)	Angle λ Mean (S.D.)
Migrant	35	40.28 (2.94)	113.26 (3.23)	70.49 (11.56)
Resident	28	39.56 (2.74)	113.61 (3.05)	59.28 (13.38)
Z value		-1.13	-0.31	-3.03
P-value		0.26	0.75	0.002

Angle α and angle λ in migrant male monarchs collected in Guanacabibes during November ’1993 are significantly larger than in migrant males collected in San Antonio during November ’1995, ’1996, and ’1997 (Table 3-13). This comparison was made only for males since only one migrant female was captured in Guanahacabibes.

Table 3.13. Angle comparison –α, β, and λ- compared between migrant males collected in Guanahacabibes and San Antonio during November in 1993, ’95,’96 and ’97. The independent variable was the locality.

Location of collection	N	Angle α Mean (S.D.)	Angle β Mean (S.D.)	Angle λ Mean (S.D.)
Guanahacabibes	14	42.12 (1.64)	113.03 (3.0)	77.19 (10.9)
San Antonio	12	40.05 (2.5)	112.63 (4.19)	66.99 (8.71)
Z value		-2.06	-0.08	-2.63
P-value		0.04	0.94	0.008

However, migrant and resident monarchs collected in San Antonio in November 1993, ’1996 and ’1997 do not differ in their three angles (Table 3-14).

Table 3.14. Angle comparison –α, β, and λ- compared between migrant males collected in San Antonio (SA) during November in 1995, '96 and '97 and resident monarchs collected in November 1997. The independent variable was the locality.

Migrant/ Resident	N	Angle α Mean (S.D.)	Angle β Mean (S.D.)	Angle λ Mean (S.D.)
SA migrant	12	40.05 (2.5)	112.63 (4.19)	66.99 (8.71)
Resident	7	38.74 (1.63)	113.81 (1.4)	58.83 (14.74)
Z value		-1.23	0.72	-1.27
P-value		0.22	0.47	0.2

Summary

When all the phenotypic traits discussed are taken into account, it is apparent that Cuban migrant monarchs are not a homogenous group; San Antonio migrants are more similar to the residents of this area than to migrants from Guanahacabibes.

Migrant monarchs from Guanahacabibes have larger wings, in better condition, than do the San Antonio migrant monarchs. The migrants collected in Guanahacabibes also have larger lean and lipid mass (but higher S.D.) than those from San Antonio (Table 3-4). The wing shape differs between these two groups as well, with Guanahacabibes monarchs having a more elongated wing tip and slimmer wings than migrants from San Antonio. The more elongated wing tip and slimmer wings of Guanahacabibes migrant monarchs result from having a larger angle α and angle λ than San Antonio migrants (Table 3-13).

Migrant males have larger wings, and wings in better condition, than females (Table 3-2); however, they do not differ in their lean and lipid mass. Migrant males and females differ in their wing shape as well, in that males have a more broader wing (significant larger angle α, Table 3-11). When migrants collected in San Antonio were compared with residents from San Antonio, no significant differences were found between these two groups in terms of lean and lipid mass and wing condition (Table 3-5).

Discussion

Why Does Migration of North American Monarchs to Cuba Occur?

The migration of North American monarchs to the Caribbean has long been suspected and there have been various explanations as to why and when this migration to Cuba has occurred. Brown and Heineman (1976) hypothesized that the presence of *D.p. plexippus* (monarch subspecies present in the North American continent) in the northern part of the tropics was a result of their movement during the peak of the *Wisconsin glacial stage*. We can also hypothesize that migrant monarchs arrive in Cuba and possibly other areas of the Caribbean as a result of hurricanes or storms at the end of the year. A third explanation proposed by Urquhart (1976) states that some monarchs are blown off course, from their usual southwest direction to the Florida Peninsula, flying later to Cuba, then to Guatemala and/or Honduras, where they overwinter, and in the spring returning to the North American continent. A fourth explanation proposed in this dissertation, is that migratory routes and final destinations of the migrant monarchs are linked to phenotypic traits that could be functionally important in their migration. If this is true, then it implies that the migrant monarchs that arrive in Cuba have to significantly differ in their phenotypic characteristics from the migrants in Mexico, those phenotypic characteristics being wing size and shape, and butterfly lipid and lean weight. Monarchs that migrate to Cuba (and Miami) increase their survival and reproduction chances by migrating to this Caribbean island. These particular migrant monarchs fly from North America and Canada to Cuba (and/or other close areas), where some of them hybridize with the resident monarch populations, and others continue on to other areas of the Caribbean. None of these migrant monarchs will return to the North American continent.

According to Brown and Heineman (1972) this movement occurred and was restricted to the peak of the *Wisconsin glacial stage,* between 70,000 and 10,000 years ago (Wicander and Monroe 1993); however Urquhart's single record (1987) and the work presented in this dissertation show that the movement of the monarchs is taking place currently as well, and that it was not restricted to the peak of the Wisconsin glacial stage, if it in fact ever occurred at that time.

A second explanation is that the arrival of monarchs from the North American continent into the Caribbean has been explained through storm influence, because the England scenario has been used as model, and from a lack of knowledge of the scale (in numbers and regularity) of the migration of monarchs to Cuba and possibly to other areas of the insular and continental Caribbean. Monarchs have been reported to arrive during certain years in England (Bowles 1996; Nelson 1996; Skinner and Parsons 1998). Monarchs arrived in southwest England during October and November of 1995 and 1999, and one individual in 1997 (Roger Bristow, pers. comm.). Southwest England was reached by the remnants of hurricane Iris in 1995 (www.nhc.noaa.gov/tracks/1995atl.gif) and Floyd in 1999 (Davey 1999). This suggests an assisted migration to England by extreme weather conditions.

The arrival of migrant monarchs to Cuba as a result of strong winds associate to weather phenomena affecting the east of the North American continent and the Caribbean, requires at least two basic assumptions: (1) that the general movement of storm-depressions and hurricanes affecting eastern North America and the Greater Antilles has to have a general southerly direction, and (2) that in the years when migrant monarchs were found in Cuba (1993, 1995, 1996, and 1997) and/or surrounding area, the

areas were affected by storms or hurricanes. However, these two general assumptions are not met.

Many of the weather phenomena that affect the Caribbean and the eastern United States originate around Cape Verde in Western Africa, and travel west until reaching the insular Caribbean. At this point some of the tropical depressions and hurricanes enter the Gulf of Mexico and the others, the majority, follow the United States coastline in a general northeasterly direction.

If the arrival of migrant monarchs in Cuba is linked to the influence of storms affecting the Caribbean region, then a necessary assumption to explain this movement is that storms favor a southeast movement of the monarchs from their breeding ranges in the northeast of the North American continent; however, in the northern hemisphere the storms usually travel first in a northwesterly direction and in the higher latitudes turn toward the northeast, and do not move in a southeast direction. This storm trajectory does not favor the southerly direction that a butterfly in southern Canada and the eastern United States needed in order to arrive in Cuba. In addition, monarch butterflies generally do not migrate when the wind is coming from the south, southwest or west (Gibo and Pallet 1978). It has been reported that on one occasion in September when the wind was blowing from the south, several hundred butterflies were resting in a large field and only one attempted a long flight (Gibo and Pallet 1978). Similar observations have been reported by Lugger (1890), Urquhart (1960) and Kanz (1977). When the wind came from the northwest or east they resumed migration.

The second general assumption of depressions and/or hurricanes affecting the greater Antilles or surrounding area during the years when migrant monarchs arrived in

Cuba proved not to be true for 1993 and 1995. During these two years there were not any storms around Cuba during the Atlantic hurricane season (www.nhc.noaa.gov/tracks/1993atl.gif) and www.nhc.noaa.gov/tracks/1995atl.gif).

A third explanation for migrant monarchs in the Caribbean was proposed by Urquhart (1987), as an "aberrant migration route of the eastern population." meaning a deviation from the southwest route to the overwintering colonies in Mexico. However, this paper proposes that the presence of migrant monarchs in the insular Caribbean and South Florida is the result of another migratory route that increase the probabilities of their survival and reproduction, that in other conditions would be very unlikely.

Phenotype and Migratory Behavior

Different migratory routes and the final destination of migrant monarchs can be explained by differences in phenotypic characteristics that are functionally related to migration. Two groups of migrant monarchs were used to test the phenotype hypothesis: Mexican migrant monarchs vs. migrant monarchs that move through the southeast of the United States and Cuba. In this southeast migration route the following samples were included: a Georgia sample (Brower et al. unpublished), four Floridian samples (Knight without publishing and Knight 1998), and Cuban samples. Georgia (undetermined location), Florida (North, Central and South; Figure 3-7; Table 3. 15), and Cuban (west portion) migrant monarchs are considered a single group, since it appears they follow the same migratory route, that is, some of the migrant monarchs that pass through Georgia, arrive in Florida and continue to Cuba. According to this hypothesis Mexican vs. Georgia-Florida-Cuban migrant monarchs could potentially differ in phenotype traits that

Figure 3-7. Localities along the southeast migratory route.

can be directly linked to the migratory behavior, such as wing length, wing condition, lipid content and lean mass. Samples from four different migratory monarchs were collected (Knight unpublished) at different points along their migration through the Florida peninsula, as well as Georgia. Georgia monarchs were roosting at the time of their collection by Lincoln Brower in January of 1988 (not specify location was given). Migrant monarchs in Florida were collected on the Panhandle area (San Blas Peninsula and St. Marks), Sanibel Island northwest of Fort Myers, and in Miami (Figure 3.7). Monarchs from St. Marks were roosting at the time of their collection by Tonya Vanhook at the end of October of 1994; however they do not overwinter there. Sanibel Island migrant monarchs were nectaring when Amy Knight collected them in November 1995. Miami migrant monarchs were collected by Knight (1998) during November 1994 and 1995.

The most striking difference among the groups is in the lipid content, Mexican migrant monarchs have two to eight times more lipids than Georgia, Florida and Cuban migrants at the end of October and November. This shows that Georgia, Florida and Cuban monarchs may not have had the necessary lipids to arrive and survive at the Mexican overwintering grounds (Table 3-10). It is possible, however, to argue that Georgia and North Florida monarchs are on their way to the Mexican overwintering places. On the other hand, migrant monarchs collected in October in Texas (Walford 1980 and Alfonso 1996) have a significantly higher quantity of lipids than those from the two southeast localities. Texas migrant monarchs have 112 (S.D=5) to 120 (S.D=5) mg of fat (Walford 1980 and Alfonso 1996). Texas, Georgia and Florida are located at the similar latitudes and the migrant monarchs were collected at similar times, so it is

expected that they will have similar amounts of lipids if they will have a chance to survive the overwintering period in Mexico. However, the migrant monarchs in Georgia and St. Marks do not have these lipids reserves, but rather, have 4 to 13 times less lipid reserve than the Texas migrants (Table 3-15). The migrants in Georgia and North Florida in November have a similar amount or less lipid reserves than the remigrants (migrants that spend their winter in the colonies in Mexico and are coming back to USA) in March. These remigrants have an average 26 mg (Alonso 1996) in comparison to 20 mg found in monarchs in Georgia and 22.6-28.7 mg found in migrants in St. Marks (Table 3-15). In addition, migrant monarchs arrive in the overwintering colonies at the end of October and November, the same time Georgia and St. Marks monarchs were collected in these locations

Urquhart (1987) states referring to the migrants from the more eastern portion of North America monarchs "… Upon reaching the Gulf of Mexico they follow the coastline westward, passing through Florida, Mississippi, Louisiana, and Texas. They finally orient their course to the southwest, eventually arriving at the overwintering site in Mexico…." He suggested (1987) that these monarchs were pushed away from their typical migration in southwest direction by strong winds out of the west during the fall.

The results of this work combined with that of Knight (1998) and Alonso (1996) do not support Urquhart's hypothesis. This hypothesis requires that migrant monarchs at the same latitude and time will have the same amount of lipids. However, was that migrant monarchs collected in October in Texas have a significantly higher amount lipids than St. Mark monarchs collected in October and monarchs collected in Georgia in November. Texas, St. Marks, and Georgia are roughly located at the same latitude. Urquhart's

Table 3-15. Mean and standard deviation of wing length, wing condition and fat content of females and males migrant monarchs collected in Georgia (Brower et al. without publishing), San Blas peninsula (Knight without publishing), St. Marks, (Knight without publishing), Sanibel island (Knight without publishing), Miami (Knight 1998), Cuba, and Mexico (Vanhook 1996). The lipid content and lean mass of migrant monarchs in Mexico was obtained from Alonso (1996).

	N	Wing length	Wing condition	Lipid content	Lean mass
Georgia monarchs in November 1980					
Females/males	46	---	---	20 (4)	169(4)
San Blas monarchs in January of 1988					
Females	8	5.2 (0.1)	2.1 (0.3)	14.3 (8.5)	144.6 (9.4)
Males	5	5.0 (0.3)	2.1 (0.2)	8.7 (3.8)	133.9 (26.8)
St. Marks monarchs in October of 1994					
Females	43	5.1 (0.3)	1.9 (0.5)	28.7(24.3)	144.1(23)
Males	51	5.1 (0.2)	1.7 (0.5)	22.6(13.2)	165.9 (20.3)
Sanibel monarchs in November of 1995					
Females	3	5.2(0.0)	2.5(0)	74.8(55.5)	144.6 (9.4)
Males	8	5.0(0.2)	2.5(0.5)	9.8(2.2)	133.9 (26.8)
Cuba monarchs in November of 1993 and 1997					
Females	10	4.8 (0.3)	3 (0.6)	25.3 (20.2)	98.1(29.8)
Males	24	5.0 (0.3)	2.5 (0.6)	15.9 (17.8)	105.6 (22.0)
Miami monarchs in November of 1994 and 1995 (*Asclepias syriaca* butterflies only)					
Females	21	5.2 (0.1)	2.7 (0.8)	31.0 (32.4)	150.6 (15.4)
Males	8	5.3 (0.3)	2.4 (0.6)	27.0 (31.1)	149.0 (28.6)
Mexico monarchs in November (collected at the overwintering colonies)					
Females	592	5.17 (2.1)	1.6 (0.7)[1]	---	---
Males	306	5.23 (2)	1.6 (0.7) [2]	---	---
Females/males	100	5.2 (0.2)	---	133 (5)[3]	170 (3)[3]

[1] The sample size was 593
[2] The sample size was 311
[3] Overwintering females and males collected on November of 1993 (Alonso 1996).

hypothesis also can not explain, why monarchs migrate consistently to South Florida (Knight 1998) and to Cuba. Last, his hypothesis will require that the years when migrant monarchs travel following this most eastern portion of the United States, there will be strong winds coming from the northwest, and this was not the case for the years: 1980, 1988, 1994, 1995, 1996 and 1997 (http://www.nhc.noaa.gov/tracks/).

Of the three migrant groups, from Florida, Cuba, and Mexico, the Cuban migrants on average have the smallest wings and wings in the poorest condition. The lean mass was lower for Cuban migrants than for anyother group of migrants monarchs analyzed here, suggesting great physiological stress. Lean mass is measured primarily in protein content, and their low mean mass could suggest that Cuban migrant monarchs are transforming proteins into carbohydrates as a last resource of energy.

Environment and Phenotypic Response

The present work suggests that migrant monarchs, *D.p.plexippus*, that migrate through Florida and Cuba respond to the wide variety of spatial and temporal environments that they experience to during their migration with a highly variable phenotype. This highly variable phenotypic response results in a reproductive advantage over a genetically fixed one, allowing the butterflies to be better synchronized to the highly variable conditions during their migration South. This high variability phenotypic response will result in greater fitness because environmentally induced phenotypes are more likely to reflect the conditions to which the migrant monarchs are exposed at particular time and space than genetically fixed phenotypic responses. A variable phenotypic response to a heterogeneous environment by the monarchs can be favored by natural selection, since individuals will be more likely to reproduce and survive if their phenotypes are better tuned to the prevailing environment.

A more variable phenotypic response is seen in the Florida -Cuban migrant monarchs that are exposed to a more heterogeneous environment, as compared to migrants that go to Mexico. Monarchs that arrive in Mexico are apparently exposed to more homogeneous spatial and temporal conditions than migrants that arrive in Cuba, and as a result their phenotype is more homogeneous (Table 3-15) than the Florida-Cuban ones. The natal ground of the majority (95%) of the Mexican migrant monarchs is the United States Midwest and surrounding areas (Wassenaar and Hobson 1999), in contrast to, the natal grounds of the Cuban migrant monarchs, which are southeastern Canada and all of the eastern of United States (Chapter 2). Besides this spatial variation that Mexican and Cuban migrant monarchs exhibit, there is a temporal variation as well. Mexican migrants monarchs have a smaller window of time for their migration than Floridian-Cuban monarchs. Mexican migrant monarchs leave the United States no later than early November in their travel to Mexico. In contrast, migrant monarchs arrive in Miami from late October to the middle of December (Knight 1998). With regard to Cuban migrant monarchs, it is known that they arrive in November at this island, however whether they arrive in other months is not known.

This spatial and temporal variations between Mexican and Florida-Cuban migrant monarchs is translated into phenotypic differences between the two groups (Table 3-15). These phenotypic differences include: behavior (the two groups have distinctive migratory routes and destinations), difference in reproductive stages (Mexican migrants are in reproductive diapause and the majority Florida and Cuban migrant monarchs are not), and differences in wing condition, lean mass and lipid content (Table 3-15).

However these differences are not only seen between Mexican and Florida-Cuban migrants but within Mexican, Florida and Cuban migrants. Alonso's work (1996) shows that there is a relationship between amount of lipids and the migratory behavior of Mexican migrant monarchs. Mexican migrant monarchs overwinter in Oyamel forests where microclimatic conditions are suitable for their five month overwintering period (Calvert et al. 1993). However, a small number of migrant monarchs break out of their overwinter period and exhibit active behaviors, such as flights to close by water and nectar sources (Master et al. 1993). The inactive and nectaring Mexican migrant monarchs vary in their lipid content, prior to their remigration back to the United States in March (Alonso 1996). Inactive migrant monarchs in the overwintering colonies have more than double the lipid content of migrant monarchs that are nectaring in the flowers (Table 3-16).

Table 3-16. Mean and standard deviation of wing length, wing condition and fat content of females and males migrant monarchs collected in March, Mexico (Alonso1996). Wing condition data was not available.

Mexico overwintering female and males monarchs observed in March

	N	Wing length	Lipid content	Lean mass
Inactive	94	5.21	56 (3)	168 (2)
Flowers	100	5.13	21 (2)	157 (2)

Alonso (1996) found that inactive clustered monarchs on the Oyamel trees had significantly higher amounts of lipid mass, water content and, lean mass and had larger wings than flower-visiting migrant monarchs during their overwintering period. These flower-visiting monarchs in March are in such poor condition that it is probable that they can not migrate back to the United States (Alonso 1996) (Table 3-12). One piece of

evidence to support this is the higher amount of lipids that remigrant monarchs have in the southern United States compared to flower-visiting migrant monarchs collected in March at the overwintering site. The remigrants have a lipid content of 26 mg (2 mg S.D) in the southern United States, compared to the 21 mg (2 mg S.D) of the nectaring migrant monarchs in Mexico. This suggests that flower-visiting monarchs do not have enough lipids to remigrate to the United States (Alonso 1996).

Monarchs that migrate through the Florida Peninsula are unique in terms of the variety of behaviors and phenotypes. Some Floridian migrant monarchs roost and spend the winter around the roosting area (San Blas peninsula), others roost for short periods of time and continue their migration (to St. Marks), others stay active and hybridize with the resident monarch population (Knight 1998), and others arrive in Cuba. Monarchs that follow this Floridian route have significantly lower amounts of lipids and Cuban migrants have the lowest lean mass.

This link between phenotype and migratory behavior of Mexican migrant monarchs, can be seen within the two Cuban migrant populations as well: Guanahacabibes and San Antonio. All migrant monarchs sampled in Guanahacabibes are coming from the Midwest and surrounding area (regions 2 and 3) as are 95% of the butterflies that migrate to Mexico (Wassenaar and Hobson 1998). In contrast, migrant monarchs in San Antonio are coming from all the eastern part of North America and southeastern Canada (Chapter 2). Migrant males in Guanahacabibes have significantly larger wings ($p=0.001$) and wing in better condition ($p=0.002$) than migrant males monarchs in San Antonio (Table 3-4). The comparison was only made between males since only one female was found in Guanahacabibes. These two migrant populations

differ in their wing shape as well. Migrant monarchs in Guanahacabibes generally have a longer and narrower wing in contrast to the relatively short, rounded wings of the migrants in San Antonio. Long and narrow wings are more efficient for flight (Gibo and Pallet 1979), allowing the butterfly to fly to more distant areas, such as Guanahacabibes. The forewing tip also differs in the two populations. Migrant monarchs in Guanahacabibes have a more pointed forewing tip, in contrast to the more rounded forewing tip of the San Antonio monarchs. Broader wing tips cause more turbulence (drag) (Futuyma 1998). Therefore, more drag will result in shorter distances covered by the butterfly. The distance from San Antonio to Guanahacabibes is not very significant (261 km) compared to the total migration distance, however for a migrant butterfly with low lipid content (23 mg) (Table 3-7), the extra kilometers could be significant. Figure 3-8 summarizes the phenotypic differences between migrant populations from Guanahacabibes and San Antonio.

The phenotypic differences between the two migrant populations in Cuba, inGuanahacabibes and San Antonio, are accompanied by different migratory behaviors. It is possible that Guanahacabibes migrants do not stay on the island; they perhaps continue their travel to the Yucatan Peninsula, 100 miles away. This is supported by the absence of monarch larvae in the Guanahacabibes area, and the presence of few host plants, *A. curassavica*.Only two plants were observed, an observation supported by the local people of the area. Field work carried out at Guanahacabibes, revealed migrant monarchs clumped together on the westernmost portion of the peninsula, closest to theYucatan Peninsula, and it appears that they fly in that direction. Urquhart (1987) reported monarchs flying to the Yucatan Peninsula from the ocean. Hernandez (pers.comm), who

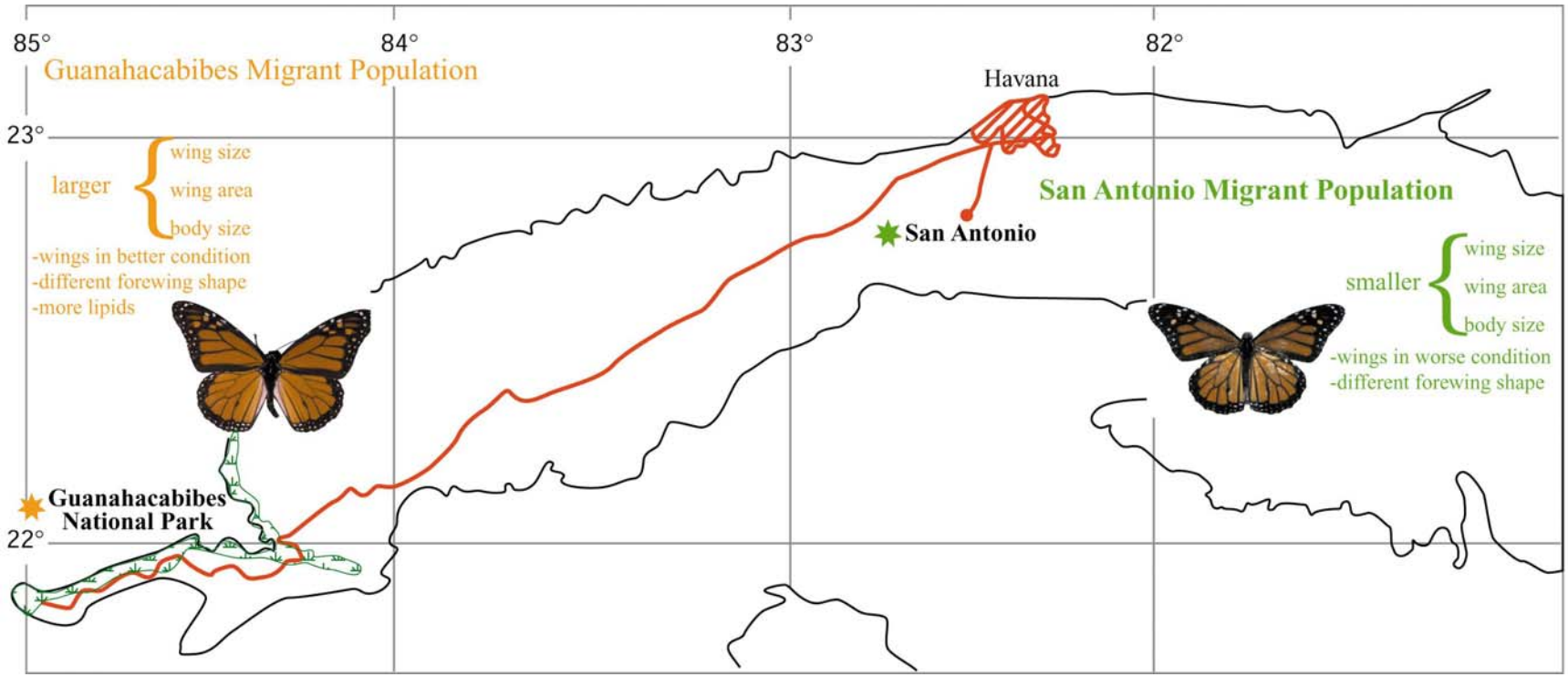


Figure3-8. Phenotypic differences between Cuban migrant populations in two different locations: Guanahacabibes and San Antonio

observed and collected the migrant monarchs during November ’1993, also stated that the monarchs appeared on the Yucatan Peninsula.

Monarchs appear in Guanahacabibes peninsula after cold fronts during the last three months of the year. Guanahacabibes itself does not support a permanent resident population (Alfredo, Guanahacabibes National Park manager and pers.obs). Migrant monarchs in Guanahacabibes were males (N=13), with the exception of one female.

It is possible that different phenotypes between migrant males and females can explain the male bias in the sex ratio in Cuban monarch populations. More than twice as many males as females apparently migrate to Cuba. Migrant Cuban males have significantly larger wings than females ($p=0.016$; Table 3-2) and they are different in angle α ($p=0.002$; Table 3-11). This again may explain why more males than females are able to reach more distant areas such as Guanahacabibes.

Data presented in this work show that Cuban migrant monarchs are not a homogeneous group in terms of phenotypic traits that could be potentially important in their migration. Migrant monarchs from San Antonio are more similar in phenotypic traits to the resident monarchs present in November than to the migrant monarchs from Guanahacabibes. San Antonio migrants and Cuban residents are very similar in wing length, body size, wing condition, fat content and lean weight (Table 3-5). Wing shape, too, is more similar between these two groups (Figure 3-5) than to the Guanahacabibes one. In contrast, migrants from San Antonio and Guanahacabibes are significantly different in their wing size, body size, and wing condition (Table 3-4). This suggests that destinations of migrant monarch are linked to their phenotype; this is important since San Antonio is closer to the Florida peninsula, where it is suspected they are coming from.

Guanahacabibes is 274 km away in straight line from San Antonio. This distance can be potentially critical since migrants from Guanahacabibes and San Antonio have very low amounts of lipids (Table 3-7). It is possible that no San Antonio or Guanahacabibes monarchs, with their low lipid contents, can likely survive the overwintering period in Mexico. Alonso (1996) also found that Mexican migrant monarchs that differ significantly in phenotypic traits, such as the amount of lipids, differ in their migratory behavior too. Alonso suggests that flower-visiting monarchs do not have enough lipid reserves to remigrate to the southern breeding areas in the United States.

CHAPTER 4
WHAT DO MIGRANT MONARCHS DO IN CUBA?

Fall monarchs east of the Rockies migrate in two different directions, one to Mexico and the other to the Florida peninsula and some further to Cuba. Migrant monarchs that arrive in Mexico (late October and November) overwinter in the Oyamel forest for almost five months, and in the spring remigrate to the United States. However little is known about the migrant monarchs that follow the Florida route and until now nothing is known about the monarchs that go to Cuba. Knight (1998) suggests that some migrant monarchs arrive in South Florida, in particular to the Miami area, hybridize with the resident Caribbean monarch populations; and then some continue south to Cuba. Her work with migrant monarchs in Miami suggests that these monarchs will not remigrate in the spring to the United States.

But, what do migrant monarchs do when they arrive in Cuba? I hypothesized that a scenario similar to the one in Miami occurs in Cuba, that is, some migrant monarchs that arrive in Cuba hybridize with the resident population and others continue to other areas of the insular and continental Caribbean, like the Yucatan peninsula. This hybridization hypothesis requires that (1) at least some of the migrant monarchs that arrive in Cuba are reproductively active or became so, and (2) it will be possible to find phenotypic evidence of this hybridization, such as intermediate phenotypes between the North American subspecies (*Danaus plexippus plexippus*) and the Cuban residents (*Danaus plexippus megalippe*) captured in November. In contrast, resident Cuban monarchs in March when, according to my preliminary results as well as Knight's work (1998), will

not encounter North America monarchs, and Cuban monarchs will look more like *D.p.megalippe*; however it is possible that North American genes will stay in Cuba in the possible hybrids. The analyses of Cuban monarchs captured during four years in Cuba in November show that Canadian and United States monarchs migrate on a regular basis (all the sampled years) to this Caribbean island (Chapter 2). In contrast, the analyses of ten monarchs captured in March show that migrant monarchs were not present in March in this Caribbean island, implying that (1) they move to another areas of Cuba and/or the Caribbean, (2) they already died, and/or (3) the remigrate back to the United States through South Florida. This last scenario is not supported by Knight's work with migrant monarchs around Miami. Migrant monarchs arrive in Miami from the end of October through the beginning of December (Knight 1998) and probably some of them go to Cuba later on. Knight did not find migrants monarchs around Miami in March. This is supported as well by reports of Cuban scientists, who said, "…Occasionally, (*D. p. plexippus*) is collected at the end of the year, when they come from the United States…" (Alayo and Hernandez 1981), and apparently they are absent after that time.

However, it is possible that intermediate phenotypes observed in Cuba and other Caribbean islands (Brown and Heineman 1972; Williams et al., 1942; pers. obs.), are not the result of the hybridization between these two subspecies, but the result of phenotypic plasticity (polyphenism), in which discrete morphs are produced as seasonal changes of temperature and rain occur in Cuba.

The arrival of the North American monarch to the Caribbean and its hybridization with the resident population of *D. p. megalippe* has long been suspected. Williams (1941), found individuals that had an intermediate phenotype between *D.p. plexippus* and

D. p. megalippe in Cuba, Jamaica, Haiti and some of the Virgin Islands, suggesting that hybridization had been occurring between these two subsspecies. However there is no agreement when this hybridization took place. Brown and Heineman (1972) suggest that a hybridization of these two subspecies occurred in Jamaica, but they think this hybridization was restricted to the Wisconsin glacial stage …"Much of this mixing (between *D. p. plexippus* and *D. p. megalippe)* is undoubtedly the result of the southward movement of *D. p. plexippus* deep into the northern parts of the tropics, probably during the peak of the Wisconsin glacial stage".

In contrast Urquhart's (1987) work suggests that this hybridization is still happening. Urquhart (1987) suspects that the diversity of wing patterns in South and Central America is due to their current hybridization. He crossed female *plexippus* and male *megalippe*, and female *megalippe* and male *plexippus* (he did not mention where the specimens were collected). The offspring showed the same variation of wing pattern as the specimens observed in these areas (South Atlantic, South and Central America).

To test this hybridization scenario, I gathered the following data: 1) the reproductive stage of the North American monarchs that arrive in Cuba, 2) body size and wing color comparisons between migrant and resident Cuban monarchs captured in March and November. Resident Cuban monarchs captured in March, according to my hypothesis, are not exposed to the direct influence of North American butterflies. As a result Cuban resident monarchs captured in March are going to look more like the typical *D. p. megalippe*. However during November when migrant monarchs are present in this Caribbean island and hybridize with the resident Cuban monarch population, the phenotypes for resident Cuban monarchs are going to be more variable than the resident

Cuban monarchs of March. Some of them will look like *D. p. megalippe* and others will exhibit intermediate phenotypes between the North American and Caribbean subspecies. Two phenotype traits, were taken into account (besides reproductive status): wing size and color wing pattern.

If migrant monarchs in Cuba were reproductively active (presence of eggs), it implies that they broke their reproductive dormancy (a condition that is characteristic of migrant monarchs). This will result in them staying in Cuba (not returning to the United States) and reproducing with the resident populations until they die, probably by the end of December. This contrasts with the over wintering monarchs in Mexico, which are in reproductive diapause at the time of their arrival.

The migrant monarchs (*D. p. plexippus*) differ significantly in body size (Arango 1996), wing shape and color wing patterns compared to the tropical sedentary subspecies, (*D. p. megalippe)*. *D. p. plexippus* is significantly larger and the tip of its forewing has a more acute angle than the Caribbean one. The elongated spots in the apex of this subspecies are pink-fulvous, rather than white that we also find in the *D. p. megalippe*. The apical spots, close to the end of the cell of the forewing are a pink-fulvous in the North American subspecies, compared to smoky (and in some cases black) in the tropical monarch subspecies. If hybridization between these two subspecies occurs in Cuba (and possibly in other Caribbean areas), it may be possible to find a wide range of individuals between the typical *D. p. plexippus* and *D. p. megalippe* in terms of their wing size and wing coloration.

A tentative option besides hybridization that may explain the diversity in wing color in the Cuban monarch is environmental cues, such us seasonal changes in

temperature and day length. This seasonal phenotypic plasticity (polyphenism), does not produce a continuous spectrum of phenotypes, but rather results in a few (usually a pair) of well distinguished phenotypes (Bradshaw, 1965). In Cuba we find two distinctive monarch phenotypes, one represented by *D. p. plexippus* and the other by *D. p. megalippe*. It is possible that these two phenotypes are the result of seasonal phenotypic plasticity to the well-marked rain-dry Cuban seasons

The adaptive value of the phenotypic response depends on the availability of some environmental cue(s) during the preadult stage that predicts the environmental conditions for the adult (Shapiro 1976). These cues are typically temperature and day length in temperate areas, and humidity and/or temperature in tropical areas (McLeod 1984; Brakefield 1987; Brakefield and Reitsma 1991). All Cuba is located in the tropical region; the exact location of la Havana is 23^{o} 8' North latitude and 82^{o} 22' West longitude. Cuba is subject to important fluctuations in temperature and rain during the year. Temperatures during July (i.e., summer) range from 27 ^{o}C to 28 ^{o}C; the temperatures in January (i.e., winter), range from 21 ^{o}C to 24 ^{o}C. January has a record for the lowest temperature of 1 ^{o}C (Atlas de Cuba 1978). The arrival of cold fronts to Cuba which reach their peak during January and February, are largely responsible for the drop in temperature during the Cuban "winter" and probably assist the migrant monarchs in their movement to Cuba and other Caribbean islands. The amount of rain during the year also has important fluctuations. During the rainy season (May to October), the amount fluctuates from 800 to 1600 mm . During the dry season (November to April), the amount of rain, ranges from 200 to 600 mm (Atlas de Cuba 1978) (Table 4-1).

Table 4-1. Land and water mean temperatures, number cold fronts (mean) and precipitation in the dry (November-April) and wet period (May-October) (Atlas de Cuba 1978).

Seasons	Land temperature (C^o)	Water temperature (C^o)	Cold fronts (average number)	Precipitation (mm)
Dry season November - April	21- 24	26	173	200-600
Rainy season May - October	27 -28 ^{o}	29	22	800-1600

If environmental conditions are responsible for the phenotype exhibited by Cuban resident monarchs, and not hybridization, we expect that butterflies from both subspecies raised in the four different conditions (Table 4-2) will be distinctive in their phenotype. Especially butterflies in conditions A and C (conditions of the tropical region) will have more distinctive phenotypes than butterflies raised in conditions B and D (conditions of the summer in the temperate regions).

Methods

Monarch Collection and Sampling Sites

Monarchs were collected in three places in the western part of Cuba: San Antonio de los Banos (31 km or 19 miles southwest of la Havana, Havana Province), Zapata Swamp (Matanzas Province), and Guanahacabibes Peninsula (the most western portion of Cuba, Pinar del Rio Province). The collection of monarchs was done in November of 1995, ‘96 and ‘97 and March 1995. I also analyzed fifteen mounted monarchs that Hernandez (Guanahacabibes, Nov 1993) donated during the visit that Knight and I made to Cuba in March 1995. A complete description of localities, maps, and collection methods is discussed in Chapter 2.

Measurements and Chemical Analysis

Before any laboratory analysis was performed on a butterfly, a picture of it was taken, and the right forewing length was measured. Then, the right forewing and hind wing of each butterfly were saved for isotopic determination of carbon ($\delta^{13}C$) and deuterium (δD), analyses that were made to determine if the butterfly was a migrant or not. This was done in addition to TLC, the aim of which was to determine if the monarch was a resident or a migrant (see Chapter 2 for a complete description). Finally, females were dissected to determine their mating status, this was done following VanHook's methodology (1996).

Different Conditions Experiments

To test if seasonal polyphenism rather than hybridization is responsible for wide variety of monarch wing patterns in Cuba observed in November, individual *D. p. megalippe* as well as *Danaus plexippus plexippus* (migrants) were reared under two different temperature conditions, 21^0 C and 31^0 C, combined with two day length conditions, 12-12 and 16-8 hours of light and dark, respectively. These four conditions (A, B, C and D) were designed to mimic natural conditions encountered by Cuban residents, migrant monarchs, and the offspring of these two groups, which could result in the two distinct monarch phenotypes encounter in Cuba. Resident monarchs, as well as the descendents of the migrant monarchs that hatch in Cuba, were exposed to equal light and dark hours during the day and a variety of temperatures depending of the altitude where the monarchs live (conditions A and C). In the tropics I observed monarchs from the sea level (Cuba) to 1700 m (Choachi area, East of Bogota, Colombia). Salazar and Velez collected a monarch in Manizales-Colombia at 2150 m. It is also possible to encounter monarchs at higher elevation than to sea level in Cuba such as mountain ranges

like Pico Turquino (1972 m) in Sierra Maestra, the most eastern part of the island (Atlas de Cuba 1978).

Condition B, 16-8 light-dark and 31^0 C, represents summer in lower latitudes (close to the Equator) and late summer in higher latitudes. Condition C, 12-12 light-dark and 21^0 C reproduces conditions found by monarchs in the spring in temperate regions and tropical regions (middle altitudes). And finally condition D, 16-8 light-dark and 21^0 C mimics summer conditions in the early summer in higher latitudes. The temperatures in conditions B and D were selected to be between the normal maximum and minimum in July in the northeast United Sates (Environmental data Service 1968).

The two subspecies, *D. p. plexippus* and *D. p. megalippe* were raised in four environmental conditions, where light-dark hours and temperature were varied (Table 4-2). The two subspecies were fed with *Asclepias curassavica*, a common host plant for *D. p. megalippe* but not for *D. p. plexippus. Asclepias curassavica* t is much more toxic than the common host plant for the migrant monarch, *A.syriaca*.

Table 4-2. Four different conditions where the two subspecies, D. p. plexippus and D. p. megalippe were raised.

Conditions	Temperature (C)	Light-dark hours
A	31	12-12
B	31	16-8
C	21	12-12
D	21	16-8

Five gravid females of *D. p. megalippe* were collected (August) around Pereira, Colombia, South America, and males (N=3) and females (not gravid, N=6) of *D. p. plexippus* were collected on the beginning of September in Minnesota. On their arrival at Gainesville these butterflies were released into a porch insectary, where they ovoposited on individual milkweed plants free of eggs and cover by a mesh. Afterwards the eggs

were removed by cutting off small pieces of the plants and placed them in a ½ pint clear plastic container. These plastic containers were placed in the four different controlled chambers: chamber A, 12-12 light-dark and 31^0 C; chamber B, 16-8 light-dark and 31^0 C; chamber C, 12-12 light-dark and 21^0 C and chamber D, 16-8 light-dark and 21^0 C (Table 4-2). The mortality for the five larvae stages as well for the pupae was recorded.

Cuban migrant monarchs are born during the summer (as well as Mexican migrants ones), and it is expected that they will mainly be exposed to temperatures and day length represented by conditions B and D at their egg, larvae and pupae stages. These butterflies were raised and treated after their eclosion in the same way as the butterflies in the hybridization experiment that is explained below.

Hybridization Experiments

The hybridization experiments were done crossing males and females of *D. p. megalippe* collected around Pereira, Colombia in August (South America) with females and males of *D. p. plexippus* collected in Minnesota in September (United States). Virgin females were released with the males of the other subspecies in a large screen porch, filled with nectar and host plants (*Asclepias curassavica*). After the females mated, they were put individually in a small silk organza bag on an *A.curassavica* plant (free of other monarch eggs). The eggs were removed from the plant by cutting off small pieces of the plant and then placed in ½ pint clear plastic containers. These plastic containers were maintained in an air-conditioned laboratory at approximately 23^0 C, on a 12-12-daylight cycle. Each container had approximately 10 eggs and a few milkweed leaves. When the larvae hatched they were transferred to the four different conditions. Each day the containers in the four conditions were cleaned and new leaves of *A.curassavica* were added. The different instars were moved to new containers, as they grew. After they had

eclosed from the pupal case and their wings dried, the butterflies were killed, a pictured was taken and finally the butterflies were mounted.

Results

Condition experiments

North American Monarchs- Danaus plexippus plexippus

North American monarchs raised in the four different conditions show variations in wing coloration patterns (Figure 4-2, Figure 4-3, Figure 4-4 and Figure 4-5), however, these patterns can not be clearly correlated with any particular condition, except for condition A. Color wing patterns of North American butterflies raised in condition A (Figure 4-2), resemble wing patterns typical of *Danaus plexippus megalippe,* that is, wing veins strongly marked, a general darker color of the wings, partial absence of the double line of white spots that border the fore and hind wings. One of the most relevant aspects is the presence of an extra white spot in some of the butterflies, a white spot that is present in *Danaus erippus* (Figure 4-1)*,* the sister species of *D.plexippus*. This white spot is in the forewing, at the base of a double line of white spots, close to the apex of the wing.

The North American butterflies raised in all four conditions were significantly smaller than their parents (5.16, SD=0.17; N=8), especially butterflies in condition A (Table 4-3). Survival percentages vary too for the different environmental conditions. Butterflies in conditions A and B have lower survival rates than butterflies in conditions B and C for *D. p. plexippus* (Table 4-3). This survival is for all five larvae stages and pupae, that is, percentage of adults produced from all the first larvae that were initially put in that particular condition.

Figure 4-1. *Danaus erippus*, sister species of the monarch butterfly *D.plexippus*. Picture courtesy of Myrian Medina..

Table 4-3. Mean wing size (cm) and standard deviation for North American butterflies raised in four different environmental conditions. Conditions that were determined by temperature and day length (DL). The parents of these butterflies have an average wing size of 5.16 cm (SD= 0.17; N=8).

.	T(C0)	DL	N	Mean (cm)	S.D.	Survival
Condition A	31	12/12	5	4.57	0.035	9.0
Condition B	31	16/8	9	4.74	0.18	9.0
Condition C	21	12/12	20	4.8	0.27	39.0
Condition D	21	16/8	18	4.73	0.18	53.0

The statistical comparison of wing size between *D.p.plexippus* raised in conditions that simulated the tropics (conditions A and C), to butterflies raised in conditions that simulate the conditions that migrant monarchs encounter (conditions B. and D) was not performed since sample size for conditions A and B were small as a result of their significant low survival.

Figure 4-2. North American butterflies, *Danaus plexippus plexippus*, raised in condition A: 12-12 light-dark hours and 31^0 C. Notice the extra white spot present in the bottom of the double white lines close to the apex of the forewing. This is very clear in bottom female and very faint in the male.

Figure 4-3. North American butterflies, *Danaus plexippus plexippus*, raised in condition B: 16-8 light-dark hours and 31^0 C.

Figure 4-4. North American butterflies, *Danaus plexippus plexippus*, raised in condition C: 12-12 light-dark hours and 21^0 C.

Figure 4-5. North American butterflies, *Danaus plexippus plexippus*, raised in condition D: 16-8 light-dark hours and 21^0 C.

Colombian Monarchs- *Danaus plexippus megalippe*

Colombian monarchs, especially females, in conditions A and B have a general darker color, (Figure 4-6 and Figure 4-7) than the ones in conditions C and D (Figure 4-8 and Figure 4-9). An extra white spot at the bottom of a double line of white spots at the apex of the forewing is present in one female of condition A. The same white spot is present in one North American female raised in condition A (Figure 4-2), a spot that is characteristic of the monarch sister's species, *Danaus erippus* (Figure 4-1). Butterflies in conditions C and D (Figure 4-8 and Figure 4-9) are more similar to North American butterflies, however they have important differences especially at the tip of the forewing. The forewing tip of these butterflies is darker in comparison to the smoky orange color of the North American butterflies, and the double spot line close to the tip is white, instead of the typical light orange of North American butterflies. The inner line of white spots at the apex of the forewing of Colombian butterflies is less conspicuous and in some cases hardly visible, this is especially apparent in conditions C (Figure 4-8) and D (Figure 4-9).

The proximal spot line of the double spot lines at the apex of the forewing is nearly visible in two Colombian males in condition C (Figure 4-8). The absence of this inner line of spots is characteristic of *Danaus plexippus portoricensis* (Clark), a subspecies from Puerto Rico.

The wing size of *D. p. megalippe* butterflies in conditions A and B is less than that of their female breeders; in contrast, *D. p. megalippe* butterflies in conditions C and D are similar in size or larger than their female parents (4.53, SD=0.22; N=5; Table 4-4).

Figure 4-6. Colombian butterflies, *Danaus plexippus megalippe*, raised in condition A: 12-12 light-dark hours and 31^{0} C.

Figure 4-7. Colombian butterflies, *Danaus plexippus megalippe*, rised in condition B: 16-8 light-dark hours and 31^{0} C.

Figure 4-8. Colombian butterflies, *Danaus plexippus megalippe*, raised in condition C: 12-12 light-dark hours and 21^0 C.

Figure 4-9. Colombian butterflies, *Danaus plexippus megalippe*, raised in condition D: 16-8 light-dark hours and 21^0 C.

The survival rate for Colombian butterflies under the four different conditions varies greatly. The survival of Colombian butterflies in conditions A and B are two and five times higher than North American butterflies in conditions A and B respectively. However survival rates for Colombian butterflies are lower than those North American butterflies in conditions C and D (Table 4-3 and Table 4-4).

Table 4-4. Mean wing size and standard deviation for Colombian butterflies *(Danaus plexippus megalippe*) raised in four different environmental conditions. These conditions were determined by temperature and day length (DL). The mean wing size of the female breeders of these butterflies was 4.53, SD=0.22; N=5.

	T (^{o}C)	DL	N	Mean (cm)	S.D.	Survival
Condition A	31	12/12	9	4.37	0.22	20.0
Condition B	31	16/8	21	4.33	0.31	49.0
Condition C	21	12/12	8	4.49	0.16	33.0
Condition D	21	16/8	6	4.61	0.12	23.6

Danaus plexippus megalippe raised in conditions A and C, conditions experienced by the monarchs in the tropics, have not significant larger wings than monarchs raised in conditions B and D, conditions experienced by migrants monarchs, Table 4-5.

Table 4-5. Mean, standard deviation, as well as a comparison in wing size between *Danaus plexippus megalippe* in conditions A/C and B/D. These data were analyzed with a Wilcoxon one-sample test (non parametric test).

Condition	N	Mean (cm)	STDV	Z-Value	P-Value
A/C	17	4.43	0.20	-0.16	0.4375
B/D	27	4.40	0.31		

Frequency of Female Mating

Three of the four Cuban female resident monarchs collected in San Antonio in March 1995 were mated individuals and one multiply mated. The monarch that was not mated, had medium-poor wing condition (condition 3), which implies that its mating

condition was not the result of its recent eclosion. This virgin female, plus the few monarchs observed (10) around La Havana area during a six day visit to the island in March of 1995, could be the result of scarce host plants and dry conditions. At the time of collection of the resident females in San Antonio and areas close to La Havana, few host plants, *A. curassavica*, were present and the general conditions for Central Cuba were dry to very dry. In contrast, eight of the nine resident monarchs collected in San Antonio in November ’1997 were multiple mated.

Of the female monarchs collected in the same locality in November ’1997 and determined as migrants by isotopic and TLC analyses (Chapter 2), all except one (that was not mated) were multiple mated. This unmated monarch was in reproductive diapause. Two of these multiple mated migrant monarchs were observed ovipositing on *A. curassavica* plants. Of the monarchs collected in San Antonio in November 1995 and 1996 and analyzed only with TLC (Chapter 2), only two females were classified as migrants by this technique, and both were mated (Table 4-6).

Table 4-6. Mating frequency as determined by bursa copulatrix dissections and spermatophore counts for resident female monarchs collected in March 1995 and migrant monarchs collected in November 1997 in San Antonio. The determination of the monarchs from March 1995 and November 1997 as resident and migrants was done through isotopic and TLC analyses; however, monarchs captured in November 1995 and November 1996 were only analyzed with TLC. This technique only identified two female migrants from the 114 monarchs collected (Chapter 2).

Site	Date	N	No. and Frequency of Spermatophores						
			Mean (S.D)	Range	0	1	>2	% mated	% mult. mated
Residents									
San Antonio	March '95	4	1 (0.8)	0-2	1	2	1	75	25
San Antonio	Nov '97	9	2.5 (1.4)	1-6	0	1	8	100	89
Migrants									
San Antonio	Nov '97	8	2.1 (0.9)	0-3	1	0	7	87	87
Zapata Swamp	Nov '95	1	-	-	0	1	0	-	-
San Antonio	Nov '96	1	-	-	0	0	1	-	-
Total		23							

Hybridization Experiments

The hybrids between North American males and Colombian females (NC hybrids) (Figure 4-10), as well as Colombian and North American males (CN hybrids) (Figure 4-11) are more similar in their wing color to the resident monarchs captured on the island in November (Figure 4-12) than to resident monarchs captured on the island in March (Figure 4-13). There is not a significant difference between NC hybrids and CN hybrids in their wing size ($Z=1.94$, $p=0.0521$). However, the mean wing size of the CN hybrids is larger than NC hybrids (Table 4-7), suggesting a possible maternal effect.

Table 4-7. Mean, standard deviation, as well as a comparison in wing size between NC hybrids and CN hybrids. These data were analyzed with a Wilcoxon two-sample test (non parametric test).

Hybrid	N	Mean (cm)	STDV	Z-Value	P-Value
NC	24	4.69	0.17	1.94	0.0521
CN	15	4.78	0.23		

Resident monarchs collected in March and November are significantly different in their wing size as well (Z=-2.38, p= 0.024), with November monarchs significantly larger than those in March (Table 4-8).

Table 4.8. Wing length of resident monarchs collected in San Antonio in March '1995 and November '1997. Because the N<30, these data were analyzed with a Wilcoxon two-sample test (non parametric test) with the t-approximation.

	N	Mean (cm)	STDV	Z-Value	P-Value
Resident-March	10	4.48	0.24	-2.38	0.0245
Resident-Nov	18	4.75	0.35		

These differences between March-November monarchs are consistent with my hybridization hypothesis between *D.p.plexippus* and *D.p.megalippe*. The CN cross and the NC cross results are consistent with this hypothesis, since the two crosses produce individuals with larger wings

Figure 4-10. Hybrids of North American males and Colombian females (NC hybrids).

Figure 4-11. Hybrids between Colombian males and North American females (CN hybrids).

Figure 4-12. Resident Cuban monarchs collected in November.

Figure 4-13. Resident Cuban monarchs collected in March.

Discussion

The majority of migrant monarchs from United States and Canada migrate to Mexico, however some of them migrate to Cuba and possibly other areas of the Caribbean as well (Chapter 2). What migrants do in Mexico have been extensively studied ever since their overwintering colonies were discovered by Urquhart in the 1970's. In contrast what migrants do in Cuba is not known since this migration route was never studied until the present work. My preliminary work suggest that migrant monarchs in Cuba follow two different strategies: (1) some of these migrants use Cuba as a bridge before reaching other Caribbean grounds such as the Yucatan Peninsula and Puerto Rico, and (2) the others reproduce and hybridize with the resident Cuban monarch population.

The first strategy, Cuba as a bridge, is consistent with Urquhart's recovery of one migrant monarch in Cuba, four migrants in the Yucatan Peninsula, two in Hispaniola and one in Puerto Rico (Urquhart 1987). In addition, he observed in the Yucatan Peninsula monarchs coming from the sea (Cuba?) at the end of the year. My own data suggests that at least some of these migrants fly through Cuba but do not stay in this Caribbean island. This is based on two observations. First, migrant monarchs in Cuba are in reproductive diapause, suggesting that they will continue their migration since there are not any over wintering in known locations in Cuba. Second, in the Western Cuba peninsula of Guanahacabibes, one of the locations where migrant monarchs were collected, no larva host plants were observed (with the exception of two small *Asclepias curasavica* plants) and only one Cuban resident monarch was observed. The presence of a permanent Cuban resident monarch population in Guanahacabibes would require the availability of enough host plant, a resource that the descendants of migrant monarchs could use; however, this resource is not available. Additional observations of Hernandez (pers.. comm. of the

collector of the monarchs of 1993 in this locality) and scientific personnel of the Biosphere Reserve, reported that monarchs are seen only at the end of the year and that after within a few days of their arrival they disappear, suggesting that they move on to other areas. They confirmed as well the absence of a Cuban monarch resident population. The migrant monarchs that I personally collected were concentrated in the tip of Guanahacabibes, Cape San Antonio (the closest point of Cuba to The Yucatan Peninsula), and no resident monarch population was observed.

The second strategy of Cuban migrants, reproduction and hybridization with the Cuban monarch resident population, is supported by: (1) the active reproductive stage of many of the migrant monarchs (90%), and (2) the similar wing sizes and color patterns of Cuban resident monarchs collected in November (Table 4-8 and Figure 4-9) and from the controlled cross of *D. p. plexippus* and *D. p. megalippe* (Table 4-7; Figure 4-10 and Figure 4-11), supporting a possible hybridization between the two subspecies. These similarities between the Cuban migrants (Figure 4. 14) and Cuban resident monarchs collected in March (Figure 4-13) are not so apparent.

Knight's (1998) work in South Florida found a similar scenario to the one I found in Cuba, that is, arrival of migrant monarchs at the end of the year and the hybridization of some of these migrants with the resident population. Knight studied two populations of monarchs in Miami in Dade County, Florida, during 1994 through 1995. She collected during these two years, in the months of October and November, 326 monarchs in these two locations. She found that between 16% to 62% were migrants in the locations where she worked. The majority of these migrants were reproductively active, suggesting that they hybridize with the Miami resident population. Knight shows that migrant monarchs

arrive in the Miami area at the end of the year, but they do not remigrate during March and April through South Florida. I found a similar pattern in Cuba also for migrant monarchs. They arrive in Cuba but do not remigrate back during the spring, suggesting that migrant monarchs that migrate through South Florida and Cuba, do not return to North America and as a result, are "lost" from the North American monarch population. Knight'swork and my work is consistent with Walker's nine-year work in butterfly migration in the Florida Peninsula. He monitored butterfly migration during the spring, summer and autumn, from March 1979 through December 1988, near Gainesville, Florida. He found monarchs migrating southward during the fall, and none remigrating northward in the spring (Walker 1991).

My data support the hybridization hypothesis and the important role that environmental conditions play in the monarch phenotype. However, my data do not support the idea that environmental conditions are the driving force behind the phenotypic differences between Cuban resident monarchs collected in March and November in Cuba. But, they suggest that environmental conditions could be an important force in the phenotype of subspecies that some scientists recognize in the insular Caribbean, such *Danaus plexippus portirricensis* (Clark) of Puerto Rico. This subspecies is characterized by the reduction or absence of the internal line of white spots in the apex of the forewing. Individuals of *D. p. megalippe* raised in condition C exhibit this characteristic (Figure 4-7). Condition C (21^0 C and day lengths (DL) of 12/12), is found in Puerto Rico and Cuba, where this subspecies is also observed.

Figure 4-14. Migrant Cuban monarchs collected in November.

The different conditions have an effect over the phenotype of the two monarch subspecies, *D. p. plexippus and D. p. megalippe*, however these phenotypic traits do not affect these two subspecies in the same manner. These phenotypic traits are survival rate, wing pattern and wing size. Conditions A and B had the strongest effect in both subspecies in regards to the three phenotypic traits studied here. The survival for *D. p. plexippus* in condition A and B were the lowest for that subspecies, suggesting that temperatures of 31^0 C are lethal for many larvae. The larger survival rate for *D. p. plexippus* corresponds to condition D, 21^0 C and DL of 16/8, conditions that migrants will encounter in their migration south. For *D. p. megalippe* condition A had the lowest survival rate for this subspecies, suggesting that temperatures of 31^0 C and day lengths (DL) of 12/12 are stressful for this subspecies. However condition B, 31^0 C and DL of 16/8 has the largest survival rate for that subspecies, suggestion that the lowest survival rate in condition A for *D. p. megalippe*, can be attributed to the DL of 12/12.

The different conditions affect significantly the wing size of *D. p. plexippus* and *D. p. megalippe,* however, not in the same degree. For *D. p. plexippus,* all the experimental conditions affect negatively the wing size, that is the wing size mean was always significatively smaller than the breeders 5.16 cm (SD =0.17; N=8) in the four conditions, especially for butterflies in condition A. Besides temperature and DL, *A. curassavica*, the food plant source could have an important effect in their wing size as well in the survival rate. *A. curassavica,* which is very toxic, is the most common natural host plant for *D. p. megalippe* but not for *D. p. plexippus.* The mean wing size of tropical monarchs, *D. p. megalippe*, is reduced and more variable for conditions A and B. However the mean wing

size for *D. p. megalippe* butterflies in condition D are larger than their mother breeders and the largest for the four conditions.

Environmental conditions produce very visible effects in wing coloration in the two subspecies as well. Some *D. p. plexippus* butterflies in condition A (31^0 C and DL of 12/12), as well as some *D. p. megalippe* butterflies, show an extra white spot in the forewing at the base of a double line of white spots close to the apex of the wing. This extra spot is present in the sister species of the monarch butterfly, *Danaus erippus*. The consideration of *D.erippus* as a different species from *D.plexippus* has been a matter of controversy. This species, *D.erippus,* is present in eastern Brazil, Uruguay, Paraguay, Argentina, Bolivia, Chile and southern Peru (Ackery and Vane-Wright 1984), countries where temperatures of 31^0 C and day lengths of 12/12 are very common since big portions or the entire countries are in the Tropics. It is possible that the presence of this extra spot in some (all?) "*D.erippus*" can be the result of environmental conditions. The sensitivity of *D.plexippus* to this particular environmental condition can contribute to the confusion of trying to sort out the question if *D.erippus* and *D.plexippus* are a different species or not.

CHAPTER 5
GENERAL CONCLUSION

Monarchs from the eastern United States have been thought to migrate only to Mexico; however, a new migratory route to Cuba was unveiled. This research shows that Canadian and North American monarchs from east of the Rockies migrate in November to at least three different areas in western Cuba (Chapter 2). These areas were San Antonio de los Banos, Zapata Swamp and Guanahacabibes Peninsula (Figure 5-1).

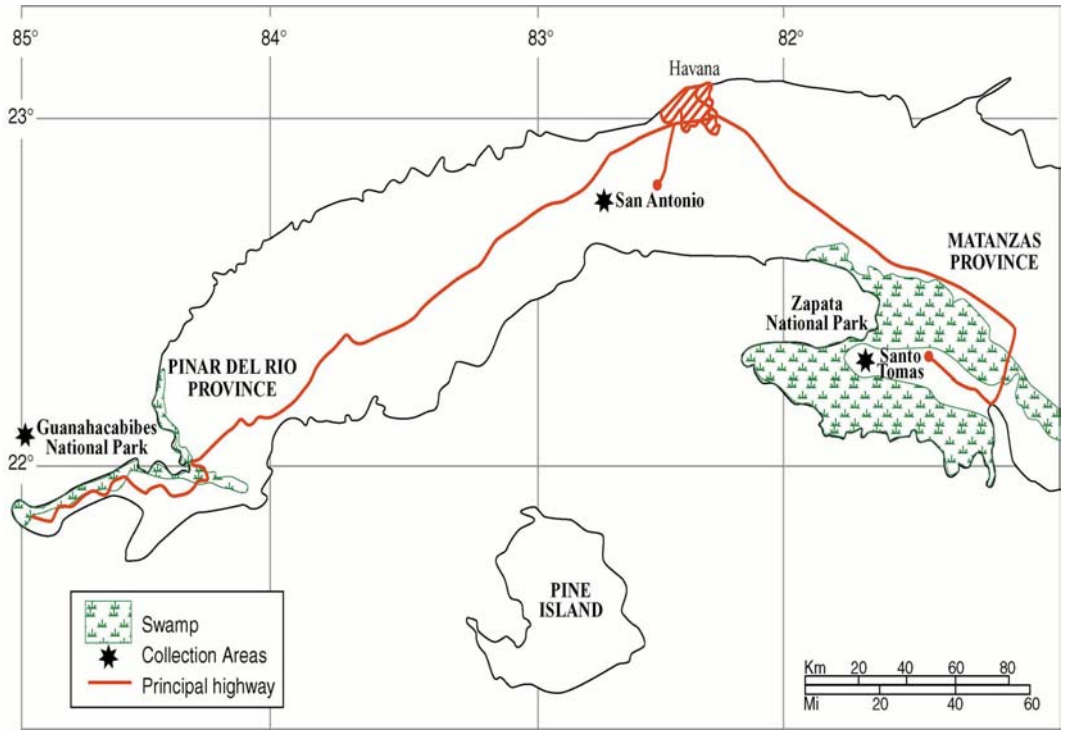


Figure 5-1. Western Portion of Cuba showing the collecting localities San Antonio, Zapata Swamp, and Guanahacabibes.

In order to know if the butterflies were Cuban resident or migratory, two techniques were used: thin Layer Chromatography (TLC) and Stable Isotopes (carbon and deuterium). The fat content of the butterflies as well their reproductive stage was

analyzed too. The results show that some North American and Canadian monarchs do not migrate to Mexico, and instead go to Cuba. This migration to Cuba occurred during all the four years of this project (1993, 1995, 1996, and 1997). The sample of November 1993 was donated by a Cuban scientist, Luis Roberto Hernandez. The preliminary isotopic results show that migrant monarchs that go to Cuba came from a broad geographical range, southeast Canada and along all eastern United States, in contrast to the 95% of the monarchs that go to Mexico came from the Midwest and surrounding areas (Chapter 2).

But why do some migrant monarchs migrate to Cuba instead of Mexico? Various explanations are explored to why and when this migration to Cuba have been occurred, and a new explanation is proposed. I proposed that migratory routes and final destinations of the migrant monarchs are linked to phenotypic traits that could be functionally important in their migration. If true, this implies that the migrant monarchs that arrive in Cuba are significantly different in their phenotypic characteristics from the migrants in Mexico; these phenotypic characteristics are wing size and shape, butterfly lipid, and lean weight. Monarchs that migrate to Cuba increase their survival and reproductive opportunities migrating to this Caribbean island, opportunities that would be slim or non-existent if they had migrated to Mexico. My results support this hypothesis (Chapter 3).

The preliminary results show that North American monarchs that migrate to Cuba hybridize with the resident population, resulting in the inclusion of their individuals and their genes into the Caribbean, specifically into the Cuban population. This can potentially explain the presence of intermediate phenotypes in Cuba and other areas of the Caribbean. Phenotypes that have described in some cases as subspecies, such us

Danaus plexippus portorricensis. Preliminary results suggest too, that monarchs that migrate to Cuba, do not return to the United States, as Mexican migrants do (Chapter 4).

Future Research

This work shows that North American monarchs migrate to Cuba; but, do North American monarchs migrate to other areas of Cuba (eastern Cuba) and the insular and continental (such Yucatan peninsula) Caribbean? The results of the tagging program developed by Urquhart (1987), suggest that this can be happening. Urquhart (1987) reported the arrival of *D.p. plexippus* butterflies in the insular and continental Caribbean: four in the Yucatan peninsula, two in Hispaniola, one in Jamaica , one in Puerto Rico, one in the lesser Antilles, and one as far south as Trinidad only a few miles from the South American continent. In the case that monarchs migrate to other areas of the Caribbean as Urquhart's data suggest, are these migrants different in phenotypic traits from the Mexican migrants, as the western Cuban migrants are?

An other area of future research is to see if differences in destinations of migrant monarchs as well as difference in phenotypic traits between Mexico and Cuba, are accompanied by differences in their genetic structure. Since the genetic structure of populations depends on the mating behavior of their individuals and the magnitude of the genetic flow with other populations, Cuban and Mexican migrant from the eastern population offer a good opportunity to determine if different migratory strategies, exhibit by these two groups of migrants, are accompanied by differences in their genetic structure, allelic diversity, and heterozygosity.

The study of the genetic structure of these group of migrants can give some insight too, into the genetic differences linked to the different subspecies of the monarch, (1) *Danaus plexippus plexippus* in the North American

continent and (2) *Danaus plexippus megalippe* a subspecies from some areas of the Caribbean (such as Cuba), because many North American-Canadian monarchs that migrate to Cuba hybridizes with the resident Cuban population.

LIST OF REFERENCES

Ackery, P. R. and Vane-Wright, R. I. 1984. Milkweed butterflies: their cladistics and biology. British Museum of Natural history. Cornell University Press. Ithaca, New York.

Alayo, P. D. and Hernandez. L. R. 1987. Atlas de las mariposas diurnas de Cuba (lepidoptera: Rhopalocera), Editorial Cientifico-Tecnica, La Habana.

Alonso, A. 1996. Biology and conservation of overwintering monarch butterflies in Mexico. Ph.D. dissertation, University of Florida, Gainesville, Florida.

Alonso, A., Rendon, E., Montesinos, E. and Brower, L. P. 1997. Use of lipid reserves by monarch butterflies overwintering in Mexico: implications for conservation. Ecological Applications 7 (3), 934-947.

Arango, N. 1996. Stabilizing selection in migratory butterflies: a comparative study of queen and monarch butterflies. M.S. thesis, University of Florida, Gainesville, Florida.

Atlas de Cuba. 1978. Instituto Cubano de Geodesia y Cartografia. La Habana.

Beall, G. and Williams, C. B. 1945. Geographical variation in the wing length of *Danaus plexippus* (Lep. Rhopalocera). Proceedings of Royal Entomological Society of London 20: 65-76.

Bookstein, F. L. 1982. Foundation of morphometrics. Annu. Rev. Eco. Systs. 13:451-470.

Bowles, N. 1996. Wildlife reports. Butterflies. British Wildlife 7(3):186-187.

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13:115-155.

Brakefield, P. M. 1987. Tropical and wet season polyphenism in the *Melanitis leda* (Satyrinae): Phenotypic plasticity and climatic correlates. Biol. J. Linn. Soc. 31:175-191.

Brakefield, P. M. and Reitsma, N. 1991. Phenotypic plasticity, seasonal climate and the population biology of *Bicyclus* butterflies (Satyridae) in Malawi. Ecol. Entomol. 16:291-303.

Brower, L.P. 1984. Chemical defense in butterflies. In Vane-Wright, R. I. and P. R. Ackery (eds). The biology of butterflies. Academic Press, London.Ackery, P. R. and R. I. Vane-Wright. 1984. Milkweed Butterflies: Their Cladistics and Biology. Ithaca: Cornell University Press.

Brower, L. P. 1985. New perspectives on the migration biology of the monarch butterfly, *Danaus plexippus* L. *In* Rankin, M. A. (ed). Migration: Mechanisms and Adaptive Significance. Austin, Texas: University of Texas, 748-785.

Brower, L. P. 1995. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857-1995. Journal of the Lepidopterists' Society 49:304-385.

Brower, L. P. 1996. Monarch butterfly orientation: missing pieces of a magnificent puzzle. The Journal of Experimental Biology 199: 93-103.

Brower, L. P. and Calvert, W. H. 1985. Foraging dynamics of bird predators on overwintering monarch butterflies in Mexico. Evolution 39: 852-868.

Brower, L. P. and S. B. Malcolm 1991. Animal migrations: endangered phenomena. American Zoologist 31: 265-276.

Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P. and Holland, M. M. 1984. Plant-determined variation in the cardenolide content, thin layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus*, reared on the milkweed, *Asclepias eriocarpa* in California. Journal of Chemical Ecology 8:579-633.

Brower, L. P., Seiber, J. N., Nelson, C. J., Tuskes, P. and Lynch, S. P. 1982. Plant determined variation in the cardenolide content, thin layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus*, reared on the milkweed, Asclepias speciosa in California. J. Chem. Ecol. 10:601-639.

Brown, F. M. and Heineman, B. 1972. Jamaica and its butterflies. E.W. Classy Limited, London.

Calvert, W. H. and Lawton, R. O. 1993. Comparative phenology of variation in size, weight, and water content of eastern North American monarch butterflies at five overwintering sites in Mexico. *In* Malcolm S. B., and M. P. Zalucki (eds). Biology and Conservation of the Monarch Butterfly. Los Angeles: Publications of the Los Angeles County Museum of Natural History, 299-307.

Calvert, W. H., Malcolm, S. B., Gendinning, J. I., Brower. L. P., Zalucki, M. P., Hook, T. V., Anderson, J. B. and Snook, L. C. 1989. Conservation biology of monarch butterfly overwintering sites in Mexico. Vida Silvestre Neotropical 2:38-48.

Cenedella, R. J. 1971. The lipids of the female monarch butterfly *Danaus plexippus* during fall migration. Insect Biochemistry 1:244-247.

Chaplin, S. B. and Wells, P. H. 1982. Energy reserves and metabolic expenditures of monarch butterflies overwintering in southern California. Ecological Entomology 7: 249-256.

Christopher, R. A. and J. A.Waters. 1974. Fourier series as a quantitative descriptor of miospore shape. Journal of Paleontology 48:697-709.

Clark, A. H. 1941. Notes on some North and Middle American Danaid butterflies. Proceedings of the U.S. National Museum. 90: 531-542.

Davey, P. 1999. Backtrack for the 1999 monarch *Danaus plexippus* (Linn.) influx. Atropos 9:17-19.

Dingle, H. 1996. Migration: The Biology of Life on the Move. Oxford University press, New York.

Dingle, H. and Arora, G. 1973. Experimental studies of migration of bugs of the genus *Dysdercus*. Oecologica 12:119-140.

Environmental Data Service. June 1968. Climatic Atlas of the United States, U.S Department of Commerce. Environmental Science Services Administration. Environmental Data Service. Silver Springs, Maryland.

Ferson, S., Rohlf, F. J. and Koehn, R. K. 1985. Measuring shape variation of two-dimensional outlines. Systematic Zoology 34(1):59-68.

Futuyma, D. J. 1998. Evolutionary Biology. Sinauer Associates, Inc. Sunderland, Massachusetts.

Garrido, O. H. 1988. La migracion de las aves en Cuba. Donana 0: 1-47.

Gibo, D. L. and Pallett, M. J. 1978. Soaring flight of monarch butterflies, *Danaus plexippus* (Lepidoptera:Danaidae), during the late summer migration in southern Ontario. Canadian Journal of Zoology 57:1393-1401.

Gibo, D. L. 1981. Altitudes attained by migrating monarch butterflies, *Danaus p. plexippus* (Lepidoptera: Danaidae), as reported by glider pilots. Canadian Journal of Zoology 59: 571-572.

Gonzalez, H., McNichol, M. K., Hamel, P.B., Acosta, M., Godinez, E., Hernandez, J., Rodriguez, D., Jackson, J. A., Grego, C.M., McRae, R.D y Sirois, J. 1992. A cooperative bird-banding project in Peninsula de Zapata, Cuba, 1988-1989. In Ecology and Conservation of Neotropical Migrant Landbirds. Hagan J.M. and Johnston D.W. Eds., Smithsonian Institution Press, pp 131-142. Washington D. C.

Gonzalez, H., Pozas, G. y Gonzalez, F. 1982. Aspectos reproductivos y densidad poblacional de *Torreornis inexpectata inexpectata* (Aves: Fringillidae) en la Cienaga de Zapata, Cuba. Ciencias Biologicas.

Herman, W. S. 1985. Hormonally mediated events in adult monarch butterflies. Pages 779-815 in M. A. Rankin, editor. Migration: mechanism and adaptive significance. Contributions in Marine Science, Supplement 27. University of Texas, Austin, Texas.

Hobson. K. A. and Wassenaar, L. I. 1999. Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologica 120:397-404.

Ivie, M. A., Philips, T. K. and Johnson, K. A. 1990. High altitude aggregations of *Anetia briarea* Godart on Hispaniola (Nymphalidae: Danainae). Journal of the Lepidopterist' Society 44:209-214.

Jackson, J. E. 1991. A user's guide to principal components. Wiley, New York.

Kanz, J. E. 1977. The orientation of migrant and non-migrant monarch butterflies, *Danaus plexippus* (L.). Psyche 84(2):120-141.

Kingsolver, J. G. 1985. Butterfly engineering. Scient. Am. 253:106-113.

Kitching, R. L. and Scheermeyer, E. 1993. The comparative biology and ecology of the Australian Danaines. In S. B. Malcolm and M. P. Zalucki, eds. Biology and conservation of the monarch butterfly. Los Angeles: Publications of the Los Angeles County Museum of Natural History.

Knight, A. 1998. A population study of monarch butterflies in North-Central and South Florida. Master Thesis. University of Florida, Gainesville, Florida.

Kuhl, F. P., and Giardina, C. R. 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing 18:236-258.

Leon and Alain (Religious Brothers). 1953. Flora de Cuba. Vol. II. Dicotiledoneas; Casuarinaceas a Meliaceas. La Habana.

Leon and Alain (Religious Brothers). 1957. Flores de Cuba. Contribuciones ocasionales del Museo de Historia Natural del Colegio de la Salle. 4:198-217. La Habana.

Lugger, O. 1890. On the migration of the milkweed butterfly. Proc. Entomol. Soc. Wash 1:256-258.

Malcolm, S. B., Cockrell, B. J. and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. Journal of Chemical Ecology 15: 819-853.

Malcolm, S. B., Cockrell, B. J. and Brower, L. P. 1993. Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration? *In* Malcolm, S. B., and M. P. Zalucki (eds). Biology and Conservation of the Monarch Butterfly. Los Angeles: Publications of the Los Angeles County. Museum of Natural History, 253-267.

Masters, A. R. 1993. Temperature and thermoregulation in the monarch butterfly. *In* Malcolm, S. B., and M. P. Zalucki (eds). Biology and Conservation of the Monarch Butterfly. Los Angeles: Publications of the Los Angeles County Museum of Natural History, 147-156.

Miller, G. E. 1990. Body size and diet quality in the genus *Cydia* (Tortricidae). J. Lep. Soc. 44:113-142.

Moranz, R. 1996. Geographic and temporal variation of the cardenolide-based chemical defenses of the queen butterfly in relation to host plant cardenolides. M.S. thesis, University of Florida, Gainesville, Florida.

Nelson, J. 1996. The Monarch *Danaus plexippus* (L.) influx into Britain and Ireland in October 1995. Atropos 1:5-10.

Norberg, U. M. 1981. Allometry of bat wings and legs and comparison with bird wings. Phil. Trans. R. Soc. Ser. B 292, 359-398.

Rainer, J. M.V. 1987. Form and function in avian flight. Curr. Ornith. 5:1-66.

Rohlf, F. J. and Archie, J. W. 1984. A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera:Culicidae). Systematic Zoology 33(3):302-317.

Scheermeyer. E. 1993. Overwintering of three Australian danaines: *Tirumala hamata*, *Euploea tulliolus*, and *E. core corinna*. In S. B. Malcolm and M. P. Zalucki, editors. Biology and Conservation of the monarch Butterfly. Los Angeles. Publications of the Los Angeles County. Museum of Natural History, 345-353.

Seiber, J. N., Brower, L. P., Lee, S. M., McChesney, M. M., Cheung, H. T. A., Nelson, C. J. and Watson, T. R. 1986. Cardenolide connection between overwintering monarch butterflies from Mexico and their larval foodplant, *Asclepias syriaca*. Journal of Chemical Ecology 12: 1157-1170.

Sein, F. 1929. Una invasion de mariposas. Rev. Agr. Puerto Rico 22:169-170.

Shapiro, A. M. 1976. Seasonal polyphenism. Evol. Biol. 9: 259-333.

Skinner, B. and Parson, M. S. 1998. The immigration of Lepidoptera to the British isles in 1994. Entomologist's Rec. J. Var 110:1-19.

Urquhart, F. A. 1960. The Monarch Butterfly. Toronto: University of Toronto Press.

Urquhart, F.A. 1987. The monarch butterfly: international traveler. Nelson-Hall editors.

Urquhart, F. A. and Urquhart, N. R. 1976. Migration of butterflies along the Gulf Coast of northern Florida. Journal of the Lepidopterists' Society 30: 59-61.

Van Hook, T. 1996. Monarch Butterfly mating ecology at a mexican overwintering site: proximate causes of non-random mating. Ph.D. dissertation, University of Florida, Gainesville, Florida.

Walford, P. 1980. Lipids in the life cycle of the monarch butterfly (*Danaus plexippus*). Senior Honor Thesis. Amherst College, Amherst, Massachusetts.

Walker, T. J. 1980. Migrating Lepidoptera: Are butterflies better than moths? Insect Behavioral Ecology 63:79-98.

Wang, H. Y. and Emmel, T. C. 1990. Migration and overwintering aggregations of nine danaine butterfly species in Taiwan (Nymphalidae: Danaidae). J. Lepid. Soc. 44:216-228.

Wassenaar, L. I. and Hobson, K. H. 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence. Proc. Natl. Acad. Sci**.** 95, 15436-15439.

Wetmore, A. 1926. Observations on the birds of Argentina, Paraguay, Uruguay and Chile. Bulletin United States National Museum 133:22.

Wicander, R. and Monroe, J. S. 1993. Historical Geology: Evolution of the Earth and Life through Time. Second Edition. West Publishing Company. St. Paul, Michigan.

Williams, C., Cockbill, G. F., Gibbs, M. E. and Downes, J. A. 1942. Studies in the migration of Lepidoptera. Transactions of the Royal Entomological Society of London. 92: 101-282.

Woodson, R. E. 1954. The North American species of *Asclepias* L. Annals of the Missouri Botanical Garden 41: 1-211.

Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

BIOGRAPHICAL SKETCH

I was born at the end of the sixties in Bogota, Colombia, South America. My parents Andre and Magola were at that time two professors at the Universidad Nacional, a university were the 1960s' ideas —system challenge, peace, and free love— were strongly felt. My house was also full of different ideas, languages and foods, since my parents came from very different backgrounds. My mom is a sociologist from Colombia and my father an electrical engineer from Belgium. As a result, my childhood was surrounded by classical and Beatles music, electrical machines and all kinds of books, and a special passion for learning. However, my passion turned in a different direction than my parents'. Mine was for nature. As far as I can remember I always liked to be outside, looking, and touching flowers, animals, and trees.

This desire to be close to nature took me to study biology at the Universidad de los Andes, Bogota. After I earned my master's degree in Colombia, I studied the effects of fragmentation on a Mullerian complex of butterflies in the Andes mountains. Now, I am studying the migration of the monarch to Cuba. I will continue studying the monarchs through a postdoctoral position at the University of Leiden, Holland.