

Directional and stabilizing selection on wing size and shape in migrant and resident monarch butterflies, *Danaus plexippus* (L.), in Cuba

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The majority of migrant monarchs (*Danaus plexippus*) from the eastern USA and south-eastern Canada migrate to Mexico; however, some of them migrate to Cuba. Cuban migrants hatch in south-east Canada and eastern USA, and then engage in a southern trip of 4000 km to this Caribbean island. In Cuba, these migrants encounter resident monarchs, which do not migrate, and instead move between plant patches looking for nectar, mating partners and host plants. These differences in flight behaviour between migrant and resident Cuban monarchs may have resulted in different selective pressures in the wing size and shape. Two modes of selection were tested, directional and stabilizing. In addition, wing condition was compared between these two groups. Monarchs were collected for 4 years in Cuba and classified as resident or migrant using two independent techniques: Thin-layer chromatography and stable hydrogen and stable carbon isotope measurements. Wing size was measured and wing condition was rated in the butterflies. Fourier analysis and wing angular measurements were used to assess wing shape differences. Migrants have significantly longer wings than residents, thus supporting the action of directional selection on wing size. In addition, directional selection acts on wing shape; that is, migrant females differ significantly from resident females in their wing angles. However, the results do not support the action of stabilizing selection: there was no significant variance between migrant and resident monarchs in their wing size or shape. Also, migrant females and males differed in wing condition as a result of differences in flight behaviour. In conclusion, eastern North American monarchs offer a good opportunity to study the selective pressures of migration on wing morphology and how different migratory routes and behaviours are linked to wing morphology and condition. © 2007 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2007, 92, 605–616.

ADDITIONAL KEYWORDS: Fourier analyses – *megalippe* – wing angles – wing condition – wing morphology.

INTRODUCTION

Millions of eastern North American monarchs, *Danaus plexippus plexippus* (Linnaeus) (Fig. 1), migrate during the autumn each year from their breeding ranges to overwintering colonies in central Mexico (Brower & Malcolm, 1991; Alonso, 1996). The breeding ranges for many (95%) of these migrant monarchs are the Midwest USA and surrounding areas (Wassenaar & Hobson, 1998). After hatching, the migrants start their journey southward, during which they accumulate lipid reserves that are critical for their survival during their overwintering months in the Oyamel fir forests (Alonso, 1996). During

migration these monarchs travel, on average, 4000 km at altitudes greater than 1 km, engaging in varied flight behaviours (Gibo, 1991). Migrant monarchs arrive from late October and throughout November at the Mexican colonies, where they remain in a semi-dormant stage until March, when they start to mate before re-migrating back to the USA. During this spring re-migration, the descendants of the Mexican migrants feed on freshly emerging milkweed plants (Herman, 1985; Brower & Malcolm, 1991; Malcolm, Cockrell & Brower, 1993).

Not all eastern North American monarchs migrate to Mexico, however. Some move eastward, toward the Atlantic coast of the USA (Urquhart, 1987) and south, arriving in southern Florida (Knight, 1998) and Cuba (Dockx *et al.*, 2004). Comparisons of reproductive

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Figure 1. North American female monarch, *Danaus plexippus plexippus*, collected in November 1997 in Cuba. Notice the difference in wing coloration and body size with the resident subspecies (see Fig. 2). The vertical strings were used to immobilize the butterfly.

stage, lipid content and wing condition between Mexican (Alonso, 1996), south Florida (Knight, 1998) and Cuban (Dockx, 2002) migrant monarchs show that Florida and Cuban monarchs are part of the same migratory group that fly through this southern peninsula to the Caribbean. The existence of two different migratory routes for eastern North American monarchs, Mexico and Florida–Cuba, is supported as well by the differences in the natal grounds of Mexican and Cuban monarchs. Cuba migrants come from south-eastern Canada and the eastern USA (Dockx *et al.*, 2004); in contrast, the majority of Mexican migrants (95%) come from the Midwest and surrounding areas (Wassenaar & Hobson, 1998). Cuban migrants arrive in November and do not overwinter there as their Mexican counterparts do. Instead, some of them mix with the resident Cuban population, and others continue to other areas of the insular and continental Caribbean (Dockx *et al.*, 2004). Support for the mixing of these groups of monarchs came from the active reproductive status of the migrant monarchs, observations of migrants checking plants to oviposit, and the intermediate phenotypes of these two subspecies when migrants are present on the island (Dockx, 2002). When migrant monarchs arrive at Cuba, they encounter a permanent resident monarch population, *Danaus plexippus megalippe* (Hübner) (Fig. 2) that does not migrate, and breeds all year round. These butterflies are localized in open grasslands where their host plant, *Asclepias* spp., is present, especially *Asclepias curassavica* (Williams *et al.*, 1942; Alayo & Hernandez, 1987; J. Salazar, pers. comm.; C. Dockx, pers. observ.). The Cuban resident monarchs fly short distances, very close to vegetation and look for mates and nectaring flowers. The distinctive flight behaviours between

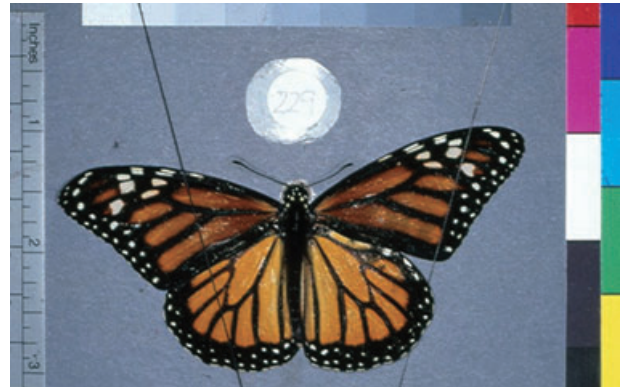


Figure 2. Resident female monarch, *Danaus plexippus megalippe*, collected in November 1996 in Cuba. The vertical strings were used to immobilize the butterfly.

migrant and resident Cuban monarchs may have resulted in different selective pressures that, in turn, could be reflected in different aspects of their wings.

The action of two modes of selection will be addressed in this paper, directional and stabilizing selection. Directional selection occurs when one extreme phenotype is the fittest; in this mode of selection, the proportion of individuals with extreme values of the trait will increase, resulting in displacement of the population mean. Stabilizing selection occurs when an intermediate phenotype is the fittest; this mode of selection does not necessarily change the mean but it may reduce the variance of the trait (Endler, 1986).

WING SIZE AND WING CONDITION

Wing size has been reported to differ between migrant and resident monarchs (Beall & Williams, 1945; Arango, 1996). Arango (1996) compared migratory and non-migratory populations of four butterfly taxa, including the monarch butterfly, and found that migrant butterflies have larger and less variable forewing length than conspecific resident butterflies. Based on the results of Arango (1996) and Beall & Williams (1945), I hypothesized that Cuban migrant monarchs have been subject to (1) directional selection that would result in longer wings and (2) stabilizing selection that would reduce wing variation.

I also expected that migrants would have wings in better condition than residents, as a result of differences in flight behaviour.

WING SHAPE

The importance of wing shape in flight performance in animals such as birds and bats is known (Norberg, 1981; Rayner, 1987), and it is expected that wing

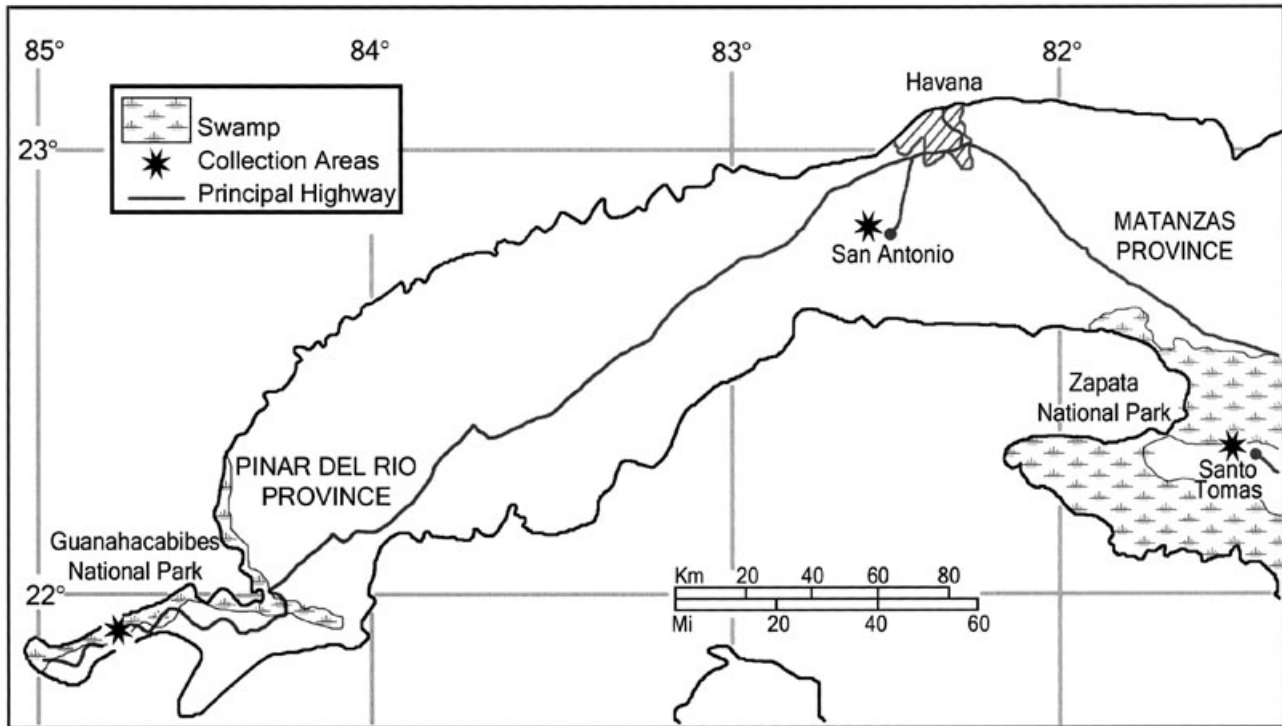


Figure 3. Map of western Cuba, showing the two monarch sample collecting localities: San Antonio and Guanahacabibes. Ecological Applications' copyright.

shape also plays a significant role in the flight of migrant vs. resident monarchs. Cuban migrant monarchs, *D. p. plexippus*, fly long distances and use soaring and gliding during their migration (Gibo, 1991). Many birds that engage in soaring and gliding have long and narrow wings (Hilty & Brown, 1986; National Audubon Society, 2001), as do man-made gliders; therefore, it was expected that migrant monarchs would have more slender wings than the resident subspecies, *D. p. megalippe*. I expected wing angles (quantified through wing angle measurements) in migrant monarchs to be more extreme as a result of directional selection and to be less variable than resident monarchs as a result of stabilizing selection.

PROBLEM AND HYPOTHESIS

In this paper, I analysed wing size and shape differences between migrant and resident Cuban monarchs in order to study the effects of directional and stabilizing selection. Wing condition was compared in these two groups as well. It was expected that Cuban migrants would (1) have longer mean, and less variable, wing length; (2) have more slender wings than resident monarchs; and (3) have wings in better condition than resident monarchs.

METHODS

Monarchs were collected in November of 1995, 1996 and 1997 in two locations in the western part of Cuba: San Antonio de los Baños (22°8'N, 82°4'W) and the Guanahacabibes Peninsula (21°8'N, 85°0'W) (Fig. 3). Fourteen other mounted monarchs collected by Hernandez in Guanahacabibes in November 1993 were also included in this work. A complete description of localities, dates, the number of individuals collected and collection methods can be found in Dockx (2002).

MEASUREMENTS AND CHEMICAL ANALYSIS

Before any laboratory analysis was performed on a specimen, the butterfly was photographed against a grey background under standardized light conditions, using a circular flash attached to the camera lens, at a speed of 16 hundredths of a second and at a distance of 20 cm from the specimen. The camera was a Leica 35 mm with Provia SLR 34 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,



Figure 4. Triangulation of the right forewing and the two measured angles: α and β . This butterfly is a migrant male, *Danaus plexippus megalippe*, collected in November 1997 in Cuba.

and in some cases wing sections absent) in increments of 0.5.

Because Cuba has a permanent resident population of monarchs, it was necessary to determine the natal grounds of the monarchs; that is, if they were migrant or resident. The natal origins of butterflies captured in Cuba were determined using two independent chemical techniques: cardenolide fingerprinting using thin-layer chromatography (TLC; Roeske *et al.*, 1975; Brower, 1984; Malcolm, Cockrell & Brower, 1989) and stable isotopic measurements of carbon ($\delta^{13}\text{C}$) and hydrogen (δD) (Wassenaar & Hobson, 1998). A complete description of the methodology and the results can be found in Dockx *et al.* (2004).

WING SHAPE ANALYSIS

Wing shape analysis was carried out using Fourier analysis and angular wing measurements. The final digitalization (used in the Fourier analysis) values and wing angles measurements were taken after the techniques were standardized. The complete description of Fourier analyses can be found in Dockx (2002) (full-text version at <http://purl.fcla.edu/fcla/etd/UFE1001134>).

ANGULAR WING MEASUREMENTS

Because the PCA analyses and casual inspection of the wings revealed that there were significant differences in wing shape, three angles of the right forewing were measured: α , β and λ (Figs 4, 5). Because angles can only be measured between two lines, lines were traced along each contour of the wing using wing landmarks, and angles α and β were measured (Fig. 4). However, this was not possible for angle λ ,



Figure 5. Circular section at the right forewing tip, and the angle λ formed between a hypothetical tangent to this circle and the line labelled X. The yellow dot represents the centre of the circle and r shows its radius. X and r are perpendicular to each other, and X is always smaller than r .

because the forewing tip does not have a good landmark. A circular section was drawn in the tip following its margin, and angle λ was formed between a hypothetical tangent to this circle and a line labelled X (example shown in Fig. 5). The derivation of the formula used to calculate λ is as follows.

The circle equation of radius r and centre 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:

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

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:

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

STATISTICAL ANALYSES

To test the action of directional selection, the means of the four variables (wing length, angles α , β and λ) were compared between the different populations using Wilcoxon's non-parametric test (Wilcoxon, 1945). To test the action of stabilizing selection, the variance of these four variables between populations was compared using the Siegel–Tukey test (Siegel & Tukey, 1960). Non-parametric tests were used

Table 1. Wing length and wing condition compared between migrant and resident monarchs that were collected in the two locations in Cuba during November in 1993, 1995, 1996 and 1997. Ten Cuban resident monarchs collected in San Antonio during March 1995 were also included. The independent variable was migratory vs. resident. One-tailed Wilcoxon test was applied

Migrant/resident	<i>N</i>	Wing (cm) mean (SD)	Wing condition mean (SD)
Migrant	88	4.83 (0.30)	2.61 (0.69)*
Resident	47	4.60 (0.36)	2.86 (0.82)†
Wilcoxon <i>Z</i> -value		-3.42	1.71
<i>P</i> -value		0.0003	0.04
Siegel–Tukey <i>Z</i> -value		-0.22	-0.08
<i>P</i> -value		0.41	-0.47

*The sample size was 92; †the sample size was 48. SD, standard deviation.

because these four variables did not follow a normal distribution, and they could not be transformed to have one. A one-tailed or two-tailed test was applied depending on the hypothesis. The chosen significance value for the Wilcoxon test (*Z*) and Siegel Tukey (*Z*) test was 0.05.

RESULTS

WING LENGTH

Migrants had significantly longer wings (longer mean length) than resident monarchs ($Z = -3.42$, $P < 0.0003$; Table 1). However, migrant females ($N = 33$) had smaller wing lengths than males ($N = 55$; $Z = -2.25$, $P < 0.01$; Table 2). In contrast, the wing size of resident females and males did not differ ($Z = -0.31$, $P > 0.38$; Table 2).

Migrant male monarchs collected in Guanahacabibes ($N = 14$) had significantly longer wings ($Z = 4.10$, $P < 0.0001$; Table 3) than migrant males collected in San Antonio ($N = 41$). However, this particular result must be considered with caution, as 13 of the 14 Guanahacabibes butterflies were caught in a single field season.

There were no significant differences of variance in the right forewing length between migrant and resident monarchs (Table 1), migrant males and females (Table 2), and migrant males from the two locations (Table 3). In spite of the absence of significant statistical differences between groups, there were some patterns worth mentioning. Migrant monarchs collected in Guanahacabibes ($N = 14$) had the lowest mean wing length variation [standard deviation (SD) and coefficient of variation (CV); Table 4]. In contrast,

Table 2. Wing length and wing condition compared between migrant and resident males and females collected in November 1993, 1995, 1996 and 1997 in Guanahacabibes and San Antonio. Ten resident monarchs captured in San Antonio in March were included in the analyses. One-tailed Wilcoxon test was used

	<i>N</i>	Wing (cm) mean (SD)	Wing cond mean (SD)
Migrant			
Females	33	4.74 (0.31)	2.85 (0.69)*
Males	55	4.88 (0.28)	2.46 (0.66)†
Wilcoxon <i>Z</i> -value		-2.25	2.70
<i>P</i> -value		0.01	0.003
Siegel–Tukey <i>Z</i> -value		0.57	0.03
<i>P</i> -value		0.28	0.49
Resident			
Females	20	4.58 (0.36)	3.0 (0.81)
Males	27	4.61 (0.36)	2.8 (0.83)‡
Wilcoxon <i>Z</i> -value		-0.31	1.0
<i>P</i> -value		0.38	0.1
Siegel–Tukey <i>Z</i> -value		-0.20	1.04
<i>P</i> -value		0.42	0.15

*The sample size was 34; †the sample size was 58; ‡the sample size was 28. SD, standard deviation.

Table 3. Wing length and wing condition compared between migrant males collected in Guanahacabibes and San Antonio during November in 1993, 1995, 1996 and 1997. The independent variable was the locality. Two-tailed test was applied

Location of collection	<i>N</i>	Wing (cm) mean (SD)	Wing cond. mean (SD)
Guanahacabibes	14	5.14 (0.14)	2.32 (0.32)
San Antonio	41	4.76 (0.27)	2.51 (0.73)*
Wilcoxon <i>Z</i> -value		4.10	-0.54
<i>P</i> -value		< 0.0001	0.59
Siegel–Tukey <i>Z</i> -value		-0.97	3.06
<i>P</i> -value		0.33	< 0.002

*The sample size was 44. SD, standard deviation.

Cuban resident monarchs captured in San Antonio ($N = 36$) in November had the greatest wing length variation (SD and CV; Table 4).

WING CONDITION

Wing condition was significantly different between resident and migrant monarchs ($Z = 1.71$, $P < 0.04$; Table 1); migrant monarchs have wings in better con-

Table 4. Mean right forewing length (cm) for migrant and resident monarchs collected in November 1993, 1995, 1996 and 1997 from San Antonio (San Anto.) and Guanahacabibes (Guanaha.). Males and females were included

Migrant/resident	Location	Date	<i>N</i>	Wing (cm) mean (SD)	Range	CV
Migrant	Guanaha.	Nov 1993 and 1995	14	5.14 (0.14)	4.9–5.5	3.4
	San Anto.	Nov 1995, 1996 and 1997	73	4.76 (0.27)	4.0–5.4	5.7
Resident	Guanaha.	Nov 1993	1	5.20 (–)		
	San Anto.	March 1995	10	4.48 (0.24)	4.1–4.9	5.4
	San Anto.	Nov 1995, 1996 and 1997	36	4.61 (0.37)	3.55–5.15	8.1
Total			134	4.75 (0.34)	3.55–5.55	7.1

CV, coefficient of variation; SD, standard deviation.

Table 5. 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 1993, 1995, 1996 and 1997 from San Antonio (San Anto.) and Guanahacabibes (Guanaha.)

Migrant/resident	Location	Date	<i>N</i>	Wing condition (cm) mean (SD)	Range	CV
Migrant	Guanaha.	Nov 1993 and 1995	14	2.33 (0.31)	1.5–2.5	13.23
	San Anto.	Nov 1995, 1996 and 1997	77	2.66 (0.74)	1.0–4.5	27.66
Resident	Guanaha.	Nov 1993	1	2.5 (–)		
	San Anto.	March 1995	10	3.0 (0.93)	1.0–4.0	30.37
	San Anto.	Nov 1995, 1996 and 1997	37	2.8 (0.81)	1.5–4.5	28.67
Total			139	2.7 (0.75)	1.0–4.5	27.70

CV, coefficient of variation; SD, standard deviation.

dition than resident ones. In addition, comparison of the wing condition of migrant female ($N = 34$) vs. migrant male ($N = 58$) monarchs revealed that they also differed significantly ($Z = 2.70$, $P < 0.003$; Table 2); migrant males had wings in better condition than females. Additionally, migrant male monarchs collected in Guanahacabibes had significantly less variation in wing condition than migrant males from San Antonio ($Z = 3.06$, $P < 0.002$; Table 3). The mean, SD, range of values and CV for wing condition for the migrant and resident monarchs is show in Table 5.

WING SHAPE

Wing shape was encoded by nine harmonics and four coefficients (a, b, c and d) for each harmonic, which resulted in 36 harmonic coefficients (Dockx, 2002) (full-text version at <http://purl.fcla.edu/fcla/etd/UFE1001134>). These 36 Fourier coefficients were the input data set for performing principal components analysis (PCA). The 'broken stick expectation' (gn; null hypothesis), accounts for the proportion of variance expected for each component by chance alone. Because observed variance for the first four principal components was higher than the variance expected

Table 6. Eigen-values of the correlation matrix and variance for the first four principal components (Prin) for 103 migrant and resident monarchs collected in Cuba. 'Broken stick' accounts for the proportion of variance expected for each component by chance alone

Component	Eigen-value	Observed variance	Broken stick value (gn)
Prin 1	11.58	0.32	0.11
Prin 2	5.75	0.16	0.08
Prin 3	3.45	0.09	0.07
Prin 4	2.66	0.07	0.06

by chance (gn), only these four components were retained (Table 6). The 36 initial components were reduced to four components, indicating a high correlation between the coefficients, suggesting patterns in the wing shape. The first principal (Z1) component accounted for 32% of the total variance, the second (Z2) for 16.0%, the third (Z3) for 9% and the fourth (Z4) for 7% of the total variation. These four components together explain 64.0% of variation in the data. Because PCA analysis and casual inspection of the

Table 7. Angle comparison (α , β and λ) between migrant and resident monarchs collected in the two locations in Cuba during November in 1993, 1995, 1996 and 1997. Ten Cuban resident monarchs collected in San Antonio during March 1995 were also included. The independent variable was migrant vs. resident. Two-tailed Wilcoxon test was applied

Migrant/resident	<i>N</i>	Angle α mean (SD)	Angle β mean (SD)	Angle λ mean (SD)
Migrant	66	39.97 (2.71)	113.42 (2.69)	70.62 (3.29)*
Resident	43	40.05 (2.79)	113.07 (3.05)	69.13 (3.78)†
Wilcoxon <i>Z</i> -value		0.05	-1.57	-1.81
<i>P</i> -value		0.96	0.11	0.07
Siegel–Tukey <i>Z</i> -value		0.53	-0.10	-1.49
<i>P</i> -value		0.59	0.92	0.13

*The sample size was 64; †the sample size was 41.
SD, standard deviation.

Table 8. Angle comparison (α , β and λ) between migrant and resident female monarchs collected in the two locations in Cuba during November in 1993, 1995, 1996 and 1997. Ten Cuban resident monarchs collected in San Antonio during March 1995 were also included. The independent variable was migratory vs. resident. Two-tailed Wilcoxon test was applied

Females	<i>N</i>	Angle α mean (SD)	Angle β mean (SD)	Angle λ mean (SD)
Migrant	26	38.32 (2.42)	114.31 (2.01)	70.81 (3.20)*
Resident	18	38.95 (2.89)	114.27 (3.76)	67.40 (3.41)
Wilcoxon <i>Z</i> -value		0.84	-0.99	-2.82
<i>P</i> -value		0.40	0.32	0.005
Siegel–Tukey <i>Z</i> -value		-1.11	-2.44	-0.23
<i>P</i> -value		0.27	0.01	0.82

*The sample size was 24.
SD, standard deviation.

migrant and resident butterfly wings (Figs 1, 2) suggested wing shape differences, angular measurements were compared between the different groups.

ANGLE MEASUREMENTS

Wing angle measurements for α , β and λ were not significantly different between migrant ($N = 66$) and resident ($N = 43$) monarchs (Table 7). However, when migrant and resident monarchs were divided according to their sex and locality, differences emerged.

Angle λ differed between migrant ($N = 26$) and resident ($N = 18$) females ($Z = -2.82$, $P < 0.005$; Table 8, Figs 1, 2). In contrast, the three angle measurements did not differ between migrant ($N = 40$) and resident ($N = 25$) males (Table 9). Angles α and β did not differ between resident females ($N = 18$) and males ($N = 25$), but angle λ did differ significantly between the sexes ($Z = -2.51$, $P < 0.01$; Table 10). A comparison of migrant males and females from Guanahacabibes could not be made because only one resident female was captured in this locality. There

were significant differences in angles α ($Z = -4.1$, $P < 0.0001$; Table 10) and β ($Z = 2.33$, $P < 0.02$; Table 10) between migrant males ($N = 40$) and females ($N = 26$), but not in angle λ . The same pattern was found for migrant male ($N = 26$) and female ($N = 26$) monarchs collected in San Antonio; they had different α angles ($Z = 2.91$, $P < 0.004$; Table 11) and β angles ($Z = -2.35$, $P < 0.02$; Table 11), but not different λ angles. Angle α in migrant male monarchs collected in Guanahacabibes ($N = 14$) during November 1993 and 1995 was significantly longer than in migrant males collected in San Antonio ($N = 26$) during November 1995, 1996 and 1997 ($Z = 2.19$, $P < 0.03$; Table 12). However, as noted previously, these results should be interpreted with caution, because 13 of the Guanahacabibes males were captured in a single field season.

DISCUSSION

WING SIZE

The hypothesis concerning wing length stated that migration would act as a directional selection force,

Table 9. Angle comparison (α , β and λ) between migrant and resident male monarchs collected in the two locations in Cuba during November in 1993, 1995, 1996 and 1997. Ten Cuban resident monarchs collected in San Antonio during March 1995 were also included. The independent variable was migratory vs. resident. Two tailed Wilcoxon test was applied

Male	<i>N</i>	Angle α mean (SD)	Angle β mean (SD)	Angle λ mean (SD)
Migrant	40	41.04 (2.35)	112.84 (2.94)	70.50 (3.37)
Resident	25	40.84 (2.48)	112.21 (2.11)	70.48 (3.55)*
Wilcoxon <i>Z</i> -value		-0.76	-1.12	0.23
<i>P</i> -value		0.45	0.26	0.82
Siegel–Tukey <i>Z</i> -value		1.02	0.86	-1.27
<i>P</i> -value		0.31	0.39	0.20

*The sample size was 23.

SD, standard deviation.

Table 10. Angle comparison (α , β and λ) between migrant and resident males and females collected in November 1993, 1995, 1996 and 1997 in Guanahacabibes and San Antonio. Two-tailed Wilcoxon test was applied

	<i>N</i>	Angle α mean (SD)	Angle β mean (SD)	Angle λ mean (SD)
Migrant				
Females	26	38.32 (2.42)	114.31 (2.01)	70.81 (3.2)*
Males	40	41.04 (2.35)	112.84 (2.94)	70.50 (3.37)
Wilcoxon <i>Z</i> -value		-4.10	2.33	0.41
<i>P</i> -value		< 0.0001	0.02	0.68
Siegel–Tukey <i>Z</i> -value		0.13	1.21	-1.01
<i>P</i> -value		0.90	0.23	0.31
Resident				
Females	18	38.95 (2.89)	114.27 (3.76)	67.40 (3.41)
Males	25	40.84 (2.48)	112.21 (2.11)	70.48 (3.55)†
Wilcoxon <i>Z</i> -value		-1.95	1.38	-2.51
<i>P</i> -value		0.05	1.17	0.01
Siegel–Tukey <i>Z</i> -value		-1.08	-0.34	0.29
<i>P</i> -value		0.28	0.73	0.77

*The sample size was 24; †the sample size was 23.

SD, standard deviation.

increasing wing size to a certain optimum in Cuban migrants, and that stabilizing selection would reduce its variation. Evidence that supports the action of directional selection on wing size is that migrants have significantly longer wings than resident monarchs. Our results support this hypothesis: Cuban migrant monarchs have longer wings than resident monarchs (Table 1, Figs 1, 2). The same trend is also found in migrants and residents in Miami (Knight, 1998) and the Americas (Beall & Williams, 1945; Arango, 1996). In contrast, the lack of significant differences in wing size variance between migrants and residents does not support the effect of stabilizing selection on Cuban migrant monarchs. The literature

offers a mixed picture about stabilizing selection on eastern North American monarchs. Arango (1996) compared forewing size variation in five migratory subpopulations of monarchs (autumn–central migrants, autumn–eastern migrants, overwintering, summer and spring migrants) and found that only autumn–central migrants had a significantly higher CV, suggesting that stabilizing selection was not operating in this particular group of migrants. In contrast, Van Hook's (1996) extensive work with overwintering monarchs ($N = 898$) does not support the action of stabilizing selection for wing length. This indicates that stabilizing selection is working in diverse ways in eastern North American monarch populations.

Table 11. Angle comparison (α , β and λ) compared between migrant females and males collected in San Antonio during November in 1993, 1995, 1996 and 1997. The independent variable was migrant vs. resident. Two-tailed Wilcoxon test was applied

Migrant	<i>N</i>	Angle α mean (SD)	Angle β mean (SD)	Angle λ mean (SD)
Males	26	40.40 (2.40)	112.71 (2.99)	70.83 (3.39)
Females	26	38.32 (2.42)	114.31 (2.01)	70.81 (3.20)*
Z-value		2.91	-2.35	0.22
P-value		0.004	0.02	0.82
Siegel–Tukey Z-value		-0.90	-0.96	-1.08
P-value		0.37	0.34	0.28

*The sample size was 24.
SD, standard deviation.

Table 12. Angle comparison (α , β and λ) compared between migrant males collected in Guanahacabibes and San Antonio during November in 1993, 1995, 1996 and 1997. The independent variable was the locality. Two-tailed Wilcoxon test was applied

Location	<i>N</i>	Angle α mean (SD)	Angle β mean (SD)	Angle λ mean (SD)
Guanahacabibes	14	42.22 (1.78)	113.07 (2.93)	69.89 (3.39)
San Antonio	26	40.40 (2.4)	112.71 (2.99)	70.83 (3.39)
Z-value		2.19	0.30	-0.11
P-value		0.03	0.76	0.91
Siegel–Tukey Z-value		0.05	-0.67	-0.44
P-value		0.96	0.50	0.66

SD, standard deviation.

Migrant males collected in Guanahacabibes had longer and less variable wing length than the migrants collected in San Antonio (Table 3). It was observed that Guanahacabibes migrants gathered on the most western point at a lighthouse, and when the winds blew from the south-west or west they flew off (lighthouse resident's comments). The proximity of western Cuba to the Yucatan and the report from Urquhart (1987) of four Canadian migrant monarchs captured in the Yucatan peninsula suggests that the Yucatan is a probable destination for these monarchs. It is possible that longer wings (and bodies) allow these butterflies to arrive at the western portion of the island and then continue to the Yucatan. Wing length is a good estimator of body size in Lepidoptera (Miller, 1990), and longer body sizes show a positive association with migratory capacity in the hemipteran genera *Oncopeltus* and *Dysdercus* (Dingle & Arora, 1973); thus, it is probable that this association exists in migrant monarchs as well.

In San Antonio, not only migrant males (as in Guanahacabibes), but migrant females were also captured. Many (90%) of the San Antonio migrant

females were reproductively active and were observed checking patches of *Asclepias curassavica*, the host plant. This is evidence for the argument that migrant monarchs in San Antonio mix with the resident population. This possible mix could explain: (1) the longer and more variable wing size of resident monarchs in November when migrants are present on the island, and smaller and less variable wing sizes in March when the migrants are apparently not present (Table 4); (2) the lack of significant difference in wing length between the resident monarchs captured in San Antonio in November and the migrant monarchs (Table 13); and (3) the similarity in the mean of angles α , β and λ between migrant and resident monarchs captured in San Antonio in November (Table 14).

Mixing is also possibly occurring between migrant and resident monarchs in the Miami area (Knight, 1998). Based on evidence that the majority of migrant monarchs, as well as resident ones, were reproductively active, and that resident monarchs had more variable wing size, Knight concluded that a permanent resident population of monarchs mixes with the

migrant monarchs from October and throughout December. Another line of evidence for the occurrence of mixing between the migrant and resident monarchs in south Florida is the high level of fluctuating asymmetry in the wings of monarchs collected there (Arango, 1996). Fluctuating asymmetry is defined by Van Valen (1962) as small, random, non-directional deviations from a perfect symmetrical trait. An increase of fluctuating asymmetry has been reported between species hybrids compared with their parent species (Graham & Felley, 1985; Learly, Allendorf & Knudson, 1985), which would support the hypothesis that mixing is occurring between these two monarch populations.

WING CONDITION

The wing condition differed between migrant and resident monarchs (Table 1) as a result of differences

Table 13. Wing length and wing condition compared between migrant and resident monarchs collected in San Antonio during November in 1995, 1996 and 1997. One-tailed Wilcoxon test was performed

Migrant/resident	<i>N</i>	Wing (cm) mean (SD)	Wing condition mean (SD)
Migrant	73	4.76 (0.27)	2.66 (0.74)*
Resident	36	4.61 (0.37)	2.82 (0.81)†
Z-value		-1.63	0.70
P-value		0.05	0.48
Siegel–Tukey Z-value		-0.32	0.74
P-value		0.37	0.23

*The sample size was 77; †the sample size was 37. SD, standard deviation.

Table 14. Angle comparison (α , β and λ) between migrant and resident monarchs collected in November 1993, 1995, 1996 and 1997 from San Antonio (San Anto.), and Guanahacabibes (Guanaha.). Males and females were included

Migrant/resident	Location	Date	<i>N</i>	Angles (mean and SD)		
				Angle α	Angle β	Angle λ
Migrant	Guanaha.	Nov 1993 and 1995	14	42.22 (1.78)*	113.07 (2.93)	69.89 (3.39)
	San Anto.	Nov 1995, 1996 and 1997	52	39.36 (2.6)	113.51 (2.65)	70.82 (3.26)†
Resident	Guanaha.	Nov 1993	1	40.50 (–)	112.50 (–)	83.33 (–)
	San Anto.	March 1995	10	41.74 (1.93)	112.03 (1.71)	63.74 (11.5)
	San Anto.	Nov 1995, 1996 and 1997	32	39.59 (2.93)	113.41 (3.36)	69.54 (3.97)‡
Total			109	40.00 (2.73)	113.28 (2.83)	70.04 (3.54)§

*Only migrant males were collected in Guanahacabibes.

†The sample size was 50; ‡the sample size was 30; §the sample size was 105. SD, standard deviation.

in flight behaviours: migrants fly long distances at high altitudes (Gibo, 1991), and residents fly shorter distances at lower altitudes, usually very close to vegetation, making wing damage more likely in the latter group. Resident monarchs fly close to vegetation, checking for plants on which to nectar and oviposit.

Migrant females had wings in worse condition than migrant males (Table 2); many of these Cuban migrant females (90%) were reproductively active and were observed flying close to the vegetation checking plants, making wing damage more probable.

WING SHAPE

The results support the action of directional, but not of stabilizing, selection on the monarch wing shape. In addition, migration combined with differences in flight behaviours between the sexes and populations are important selective pressures on wing shape (the same selective pressures that appear to be acting on wing size).

Angle λ (wing tip), but not angles α and β , differed significantly between migrant (Fig. 1) and resident (Fig. 2) females (Table 8). Migrant females (Fig. 1) had a longer and narrower forewing tip, in contrast to the relatively short, rounded forewing tip of the residents (Fig. 2). The shorter and broader wing tips of resident monarchs favour slow, controlled and agile flight (Betts & Wootton, 1988) between vegetation patches. In contrast, migrant females have more elongated wing tips to reduce turbulence and drag (Betts & Wootton, 1988; Futuyma, 1998) during their long migration south and to enhance gliding. This would enable the migrant female to fly farther, as migratory monarchs rely heavily on gliding during migration (Gibo & Pallett, 1978). A similar wing pattern (elongated wing tip) is found in birds such as seabirds that soar and glide

(Harrison, 1983; National Audubon Society, 2001). In contrast, resident and migrant males did not have significant differences in any of the three angles (Table 9). One possible explanation for this lack of differentiation is that both migrant and resident males glide above the vegetation patrolling for females. Migrant males and females (Table 10) did not differ in angle λ , but did have significant differences in angles α and β . These differences in wing shape between migrant males and females can be explained by the opposing selective pressures on wing design in migrant females. Wings in migrant females are used not only for long flights at high elevations, but also for short and lower elevation flights. At the end of migration, or upon reaching their destination, females fly close to vegetation checking for nectar and ovipositing, and also evading male pursuit by flying and crawling through dense vegetation where males cannot find them (Pliske, 1975). Migrant males, however, spend more time above the vegetation, patrolling for females.

In contrast to migrants, resident males and females did not have differences in angles α and β , but did have significant differences in angles λ (Table 10). This suggests the action of directional selection for longer values of angle λ for butterflies that use gliding, such as migrants and resident males (to a lesser degree), and directional selection for longer values of angle α for butterflies that fly longer distances, such as the Guanahacabibes migrant males. Preliminary observations of Guanahacabibes migrants in conjunction with the data of Urquhart (1987) suggest that this group of monarchs continues to other areas of the continental Caribbean.

Eastern North American migrant monarchs offer a good opportunity to study (1) the role of natural selection in wing size and shape in different monarch populations; and (2) wing morphology differences amongst monarch populations that differ in migratory routes and migratory behaviour and their resident populations.

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