Lack of genetic differentiation among widely spaced subpopulations of a butterfly with home range behaviour

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We examine seven geographically separate subpopulations of *Heliconius charithonia*, a butterfly with well-documented home range behaviour, in Miami–Dade County, Florida, for genetic differentiation using cellulose acetate electrophoresis. These subpopulations exhibit little genetic variation (percent polymorphic loci = 27, average heterozygosity = 0.103) especially in comparison to populations of the same and related species from mainland South America. Allele frequencies do not differ among the subpopulations in south Florida and estimates of Wright's fixation index (F_{ST}) support that there is no detectable genetic differentiation among them. This result supports an earlier finding that the dispersal ability of *Heliconius* butterflies may be underestimated. However, it is unlikely that increased dispersal ability alone could account for the lack of genetic differentiation observed among subpopulations separated by almost 80 km. Given the likely effective population size of these subpopulations ($N_e = 205$) and the average generation time of this species in the subtropics (in the range of 30–90 days), this lack of genetic differentiation is best explained by current or very recent gene flow following a stepping-stone model. Furthermore, this result provides evidence that the current extensive degree of habitat fragmentation surrounding the city of Miami does not limit gene flow among urban subpopulations of *Heliconius charithonia*.

Keywords: allozyme electrophoresis, gene flow, genetic differentiation, *Heliconius charithonia*, stepping-stone model, urban fragmentation.

Introduction

Butterflies of the genus *Heliconius* (Lepidoptera: Nymphalidae) are common organisms in New World tropical and subtropical forests. This genus is well represented throughout South and Central America, into Mexico, and the southern United States. *Heliconius* butterflies are a major focus of research due to their longevity, Mullerian mimicry, use of pollen as a nutritional supplement, unpalatability to predators, aposematic coloration, pupal-mating system, gregarious roosting, striking coevolution with plants of the genus *Passiflora*, inter- and intraspecific wing colour-pattern variation, and home-range behaviour (Turner, 1971; Ehrlich & Gilbert, 1973; Gilbert, 1975; Gilbert, 1991).

Results of mark–recapture studies suggest that dispersal rates in *Heliconius* butterflies are low, apparently as a result of home-range behaviour. *Heliconius* butter-

flies use specific foraging routes by which they return daily to sites of adult host plants (for nectar and pollen) and larval host plants (for egg laying and mating) (Turner, 1971; Ehrlich & Gilbert, 1973; Cook *et al.*, 1976; Mallet & Jackson, 1980; Quintero, 1988). Additionally, *Heliconius* butterflies exhibit marked fidelity to night roosts (Turner, 1971; Brown, 1981; Waller & Gilbert, 1982; Mallet, 1986a). These behaviours suggest that *Heliconius* butterflies travel within a limited range throughout their lifetimes. Limited range, in turn, suggests that gene flow between geographically separated populations is likely to be low.

Mallet (1986b), however, found that dispersal distances of *Heliconius* butterflies may be substantially underestimated. Prereproductive dispersal is common in insects, so Mallet investigated movement of marked *Heliconius erato* individuals from the site of pupal eclosion. He found that most dispersal occurs before the first capture of an individual in a typical mark–recapture study. Thus, individuals may disperse some distance (an average of 296 m in *H. erato*; Mallet, 1986b) before they exhibit home-range behaviour. Additionally, one individual

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moved 1000 m between the sites of eclosion and first capture, and 820 m between sites of first and second capture. This example shows that extended dispersal is possible for *Heliconius* butterflies. Mallet (1986b) used his dispersal data to estimate rates of gene flow between populations of *H. erato*. Using estimates of neighbourhood deme size, Mallet predicted Wright's fixation index values (F_{ST} values) would range between 0.02 and 0.04, relative to 100 local neighbourhoods. Values this low indicate little genetic differentiation between populations.

In this study we analyse the effect of butterfly dispersal on gene flow by measuring allele frequencies in several widely spaced subpopulations of *H. charithonia* in south Florida. From these allele frequencies we calculate Wright's (1951) *F*-statistics, as well as a number of standard population genetics statistics to determine whether subpopulations separated by distances much greater than the proposed dispersal distance exhibit significant genetic differentiation. In addition, we compare our results with other studies to determine (1) whether our measure of genetic variability (heterozygosity) for *H. charithonia* is typical of the genus *Heliconius* and butterflies in general, (2) what factors might be responsible for any observed patterns of heterozygosity within the subfamily Heliconiinae, and (3) whether our estimated pattern of gene flow corresponds to that of other insect species in which both dispersal and gene flow have been estimated. Finally, we consider our results in the context of the extensive human population growth, which has led to a highly fragmented natural landscape in south Florida, in order to determine what effect this has had, and potentially will have, on the pattern of gene flow in this area.

Materials and methods

Between April 1997 and January 1998, we collected 20 adult *H. charithonia* for allozyme electrophoresis from each of seven subpopulations in Miami–Dade County, Florida (Fig. 1). Five of the sites are remnant patches of native forest (either pineland or tropical hardwood hammock), one site was Gumbo Limbo Hammock in Everglades National Park, and the final site was a suburban backyard. Butterflies were transported live to the laboratory, where they were frozen and stored at −80°C until processing for electrophoresis.

Tissue extracts were prepared by grinding one-quarter to one-half of the abdomen of each butterfly in 250 μL of extraction buffer (Hagen & Scriber, 1991), and the extracts were then centrifuged for 6 min at 9000 × g. Supernatant from the centrifuged extracts was applied to thin layer cellulose acetate plates ('Titan III' [94 by 76 mm], Helena Laboratories, Beaumont, TX) for electrophoresis and these were then run for approxi-
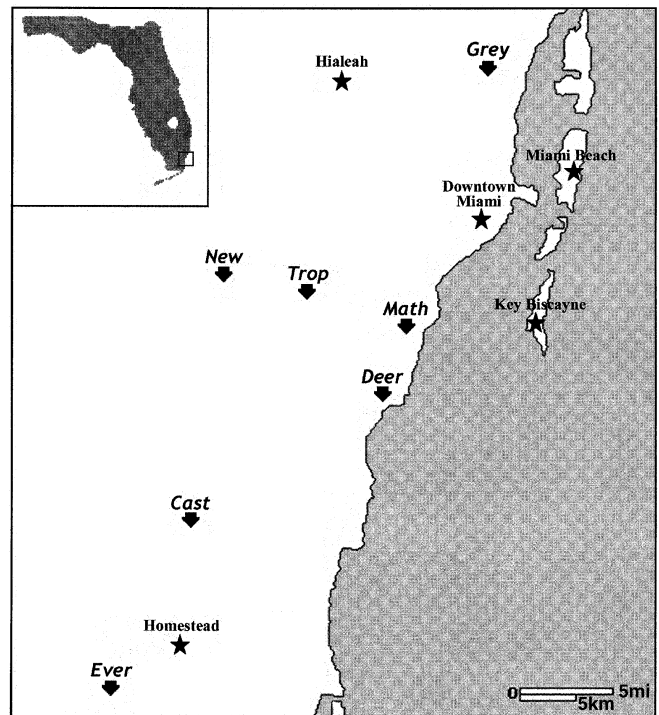
Fig. 1 Map of the seven subpopulations of *Heliconius charithonia* included in this study (each indicated by an arrow). Math, Matheson Hammock; Deer, Deering Estate; Cast, Castellow Hammock; Grey, West Greynolds Park; Trop, Tropical Park; Ever, Everglades National Park; New, residential backyard.

mately 40 min at 200 V using a Tris–glycine buffer. After electrophoresis, the plates were stained for the appropriate enzyme (Table 1) following standard recipes (Harris & Hopkins, 1978; Richardson *et al.*, 1986) scaled to approximately 2 mL total volume, and applied to plates as overlays mixed 1 : 1 with 1.5% agar solution following Hebert & Beaton (1993).

After an initial survey for variation at 15 loci, 11 were found to be consistently scorable. Of these, three were found to be polymorphic in a sample of 24 individuals, 12 of which were captured from areas outside the study sites and 12 were randomly selected study individuals. The remaining eight loci were monomorphic. Enzyme names, abbreviations, and E.C. numbers for the 11 loci scored are given in Table 1. The allelic state of each individual was determined for the three polymorphic loci, and percentage polymorphic loci (%P), average alleles per locus (APL), and Nei's (1978) unbiased estimate of average heterozygosity ($\bar{H}$) were calculated for each of the seven subpopulations.

Since larger areas of habitat are expected to support larger *Heliconius* populations, and larger populations may exhibit greater genetic variation, average heterozygosity was compared to the size of each of the

Table 1 Enzymes, abbreviations, and E.C. numbers for the 11 loci scored in *Heliconius charithonia*. Note, the eight monomorphic loci were determined by screening a random sample of 24 individuals

Enzyme	Locus	E.C. number	Poly/ Mono morphic*
Phosphoglucomutase	*Pgm*	5.4.2.2	Poly
Phosphoglucose Isomerase	*Pgi*	5.3.1.9	Poly
Hydroxybutyrate Dehydrogenase	*Hbdh*	1.1.1.30	Poly
Malate Dehydrogenase	*Mdh*	1.1.1.37	Mono
Fumarate Hydratase	*Fum*	4.2.1.2	Mono
Peptidase	*Pep*	3.4.11	Mono
Mannose-6-Phosphate Isomerase	*Mpi*	5.3.1.8	Mono
Isocitrate Dehydrogenase	*Idh*	1.1.1.42	Mono
6-Phosphogluconate Dehydrogenase	*6Pgdh*	1.1.1.44	Mono
Aspartate Aminotransferase	*Aat*	2.6.1.1	Mono
Arginine Phosphokinase	*Apk*	2.7.3.3	Mono

*Polymorphic locus determined as frequency of most common allele < 95%.

localities from which the individuals were taken using Spearman's rank correlation test. The localities, which are primarily urban parks, were ranked in size by visually comparing their boundaries on a city map.

The degree of genetic subdivision was estimated by calculating Wright's fixation index (F_{ST}) for each of the three polymorphic loci using the method of Weir & Cockerham (1984). The three estimates were then combined into one jackknife mean F_{ST}. F_{ST}, which ranges from 0 to 1, indicates the amount of genetic subdivision between subpopulations (values near 0 indicate little genetic subdivision and values near 1 indicate substantial genetic subdivision). Additionally, Wright's inbreeding coefficient (F_{IS}), which measures the

reduction in heterozygosity of an individual due to nonrandom mating within its subpopulation, and Wright's overall inbreeding coefficient (F_{IT}), which measures the reduction in heterozygosity of an individual relative to the total population, were calculated for each of the three polymorphic loci. A chi-squared test was used to determine if the allelic frequencies differed significantly among subpopulations. Allelic frequencies at each locus for each subpopulation were also tested against Hardy–Weinberg expectations. Calculation of heterozygosities, *F*-statistics, and tests for deviation from Hardy–Weinberg equilibrium were performed using the program TFPGA (Miller, 1997).

Results

Allele frequencies at the three polymorphic loci for each subpopulation are reported in Table 2. There were no significant deviations from Hardy–Weinberg expectations at these loci in any subpopulation. Average alleles per locus (APL) and average heterozygosity ($\bar{H}$) for each subpopulation are given in Table 3. Since the subpopulations shared the same three polymorphic loci as well as the same eight monomorphic loci, the percentage polymorphic loci for each subpopulation was invariant at 27.3%. Average alleles per locus varied slightly among subpopulations, primarily due to two rare alleles. An *S*- allele at the *Pgm* locus was present in one individual in each of the Deer and Trop subpopulations. Additionally, an *F+* allele at the *Pgi* locus was present in one individual in the Ever subpopulation (Table 2). Average heterozygosity varied slightly among subpopulations, however, there was no correlation between average heterozygosity and size of each habitat patch ($r_s = 0.571$, P > 0.05).

Across all subpopulations, the loci produced similar values of F_{ST}, ranging from −0.0121 to 0.0070 with a jackknife mean of 0.0029, which is not significantly

Table 2 Allele frequencies at the three polymorphic loci scored for each subpopulation of *Heliconius charithonia*. Alleles are designated as *F+* (fastest migrating allele), *F* (fast migrating allele), *M* (moderate migrating allele), *S* (slow migrating allele), and *S–* (slowest migrating allele) with respect to the other alleles. $N = 20$ for each locus at each site (number of alleles = 2*N*)

Locus	Allele	Math	Deer	Cast	Grey	Trop	Ever	New	χ^2	P
Hbdh	*M*	0.850	0.875	0.875	0.900	0.950	0.925	0.875	2.99	0.811
	S	0.150	0.125	0.125	0.100	0.050	0.075	0.125		
Pgm	*F*	0.150	0.175	0.200	0.200	0.200	0.225	0.050	14.98	0.243
	M	0.800	0.800	0.700	0.800	0.750	0.650	0.875		
	S	0.050	—	0.100	—	0.025	0.125	0.075		
	S–	—	0.025	—	—	0.025	—	—		
Pgi	*F+*	—	—	—	—	—	0.025	—	13.84	0.311
	F	0.150	0.250	0.125	0.175	0.050	0.150	0.075		
	M	0.600	0.500	0.575	0.500	0.775	0.600	0.600		
	S	0.250	0.250	0.300	0.325	0.175	0.225	0.325		

Table 3 Average alleles per locus (APL) and Nei's (1978) unbiased estimate of average heterozygosity ($\bar{H}$) for each of the seven subpopulations of *Heliconius charithonia*

Subpopulation	APL	$\bar{H}$
Math	1.45	0.107
Deer	1.45	0.109
Cast	1.45	0.116
Grey	1.36	0.104
Trop	1.55	0.080
Ever	1.55	0.113
New	1.45	0.091

Table 4 The inbreeding coefficient (F_{IS}), overall inbreeding coefficient (F_{IT}) and fixation index (F_{ST}) for each of the three polymorphic loci, the jackknife mean of each, and the 95% confidence interval as determined by bootstrapping over loci. These *F*-statistics were estimated using the method of Weir & Cockerham (1984)

Locus	F_{IS}	F_{IT}	F_{ST}
Hbdh	−0.0310	−0.0435	−0.0121
Pgm	−0.0608	−0.0534	0.0070
Pgi	−0.0087	−0.0049	0.0038
Mean	−0.0265	−0.0235	0.0029
95% C. I.	−0.0087 to	−0.0049 to	0.0070 to
	−0.0608	−0.0534	−0.0121

different from 0 (Table 4). Therefore, at most only a fraction of a percentage of the overall genetic diversity of these subpopulations results from variation among subpopulations (Table 4). In support of this conclusion, a chi-squared analysis revealed that the allele frequencies for the three polymorphic loci were not significantly different among the seven subpopulations (Table 2). F_{IS} values were small but consistently negative (Table 4), indicating that at each of the three polymorphic loci there was an excess of heterozygotes. Given the negative values of F_{IS} and the very small values of F_{ST}, F_{IT} is also negative for all three polymorphic loci (Table 4). As with F_{IS}, these negative values indicate that there was an excess of heterozygotes at each of the three polymorphic loci for the entire population.

Discussion

Our results suggest that subpopulations of *H. charithonia* in south Florida contain relatively little genetic variation and are not genetically subdivided. Our results differ in some respects from a previous allozyme study of *Heliconius* butterflies. Turner *et al.* (1979) reported that 47% to 76% of the 17 loci they studied in eight species of Heliconiine butterflies were polymorphic. Here, only 27% of the loci were polymorphic. Although this result could be due to the smaller number of loci studied (11 vs. 17), it could also indicate an overall reduction in genetic variation in this species in south Florida compared with other species in Central and South America. Furthermore, the mean average heterozygosity within subpopulations was 10.3% in this study whereas Turner *et al.* (1979) found that average heterozygosity ranged from 9% to 24% for the eight Central and South American species (Fig. 2). Packer *et al.* (1998) found that average heterozygosity of 56 lepidopteran species (not including any *Heliconius* species) was 10.5%, indicating an increased level of genetic variability in the Central and South American species as compared to south Florida subpopulations of *H. charithonia*.

The low heterozygosity of *H. charithonia* in this study is probably due to the geographical location of these subpopulations on the peninsula of Florida, where the influx of genetic variation from dispersing individuals is presumably less than in the inland areas of South and Central America. This explanation is strongly supported by comparing the average heterozygosity for these subpopulations to that of other *H. charithonia* populations and to that of its sibling species, *H. peruvianus* (Jiggins & Davies, 1998). Populations of *H. charithonia* from the Caribbean islands of Jamaica and Cuba have heterozygosities identical to that of the south Florida subpopulations (Fig. 2) and the extent of geographical isolation on these Caribbean islands is probably similar to the isolation experienced by the subpopulations on the Florida peninsula. In contrast to these isolated populations, *H. charithonia* from the South American mainland country of Ecuador exhibit considerably greater heterozygosity (Fig. 2). As expected, populations of *H. peruvianus* have a heterozygosity intermediate to the mainland and island populations of *H. charithonia* (Fig. 2). The genetic variation of *H. peruvianus* is less than that of parapatric populations of *H. charithonia* probably as a result of its limited geographical distribution, yet the heterozygosity of *H. peruvianus* is greater than that of the island and Florida populations as a result of occasional genetic input due to *H. peruvianus*/*H. charithonia* hybridization (as documented by Jiggins & Davies, 1998). Notice that this scenario is partially mirrored by that of *H. himera*, a recently derived taxon in the same region of Ecuador and Peru. Like *H. peruvianus*, *H. himera* exhibits a slightly decreased (although not significantly so) heterozygosity in comparison to its widespread parapatric sister species *H. erato* (Jiggins *et al.*, 1997).

Our results indicate that there is no genetic differentiation among the seven subpopulations of *H. charithonia*. This is most likely due to the homogenizing effect of
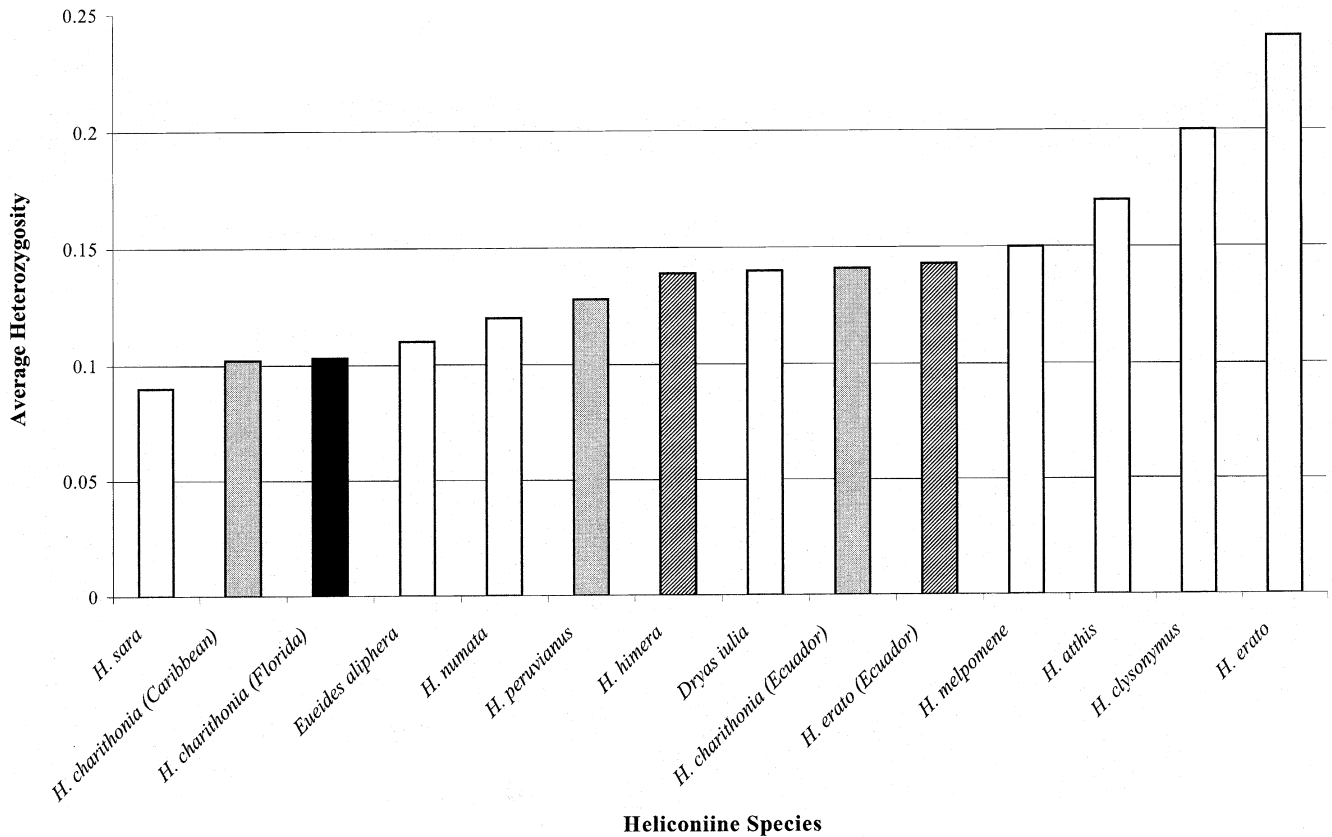
Fig. 2 Average heterozygosity for the eight Heliconiine species studied by Turner *et al.* (1979) (white), *H. erato* and *H. himera* studied by Jiggins *et al.* (1997) (diagonal lines), *H. charithonia* and *H. peruvianus* studied by Jiggins & Davies (1998) (grey), and *H. charithonia* from this study (black).

gene flow. Support for this conclusion comes from the negative values of inbreeding coefficients, indicating an excess of heterozygotes. If subpopulations were genetically isolated, we would expect to find evidence for inbreeding (e.g. a deficiency of heterozygotes indicated by positive inbreeding coefficients).

In their review of literature on genetic variation in natural insect populations, Peterson & Denno (1998) identify a number of factors which could potentially influence gene flow among insect populations. A major factor, obviously, is the dispersal behaviour of insects. Thus, the dispersal-gene flow hypothesis holds that there should be a positive correlation between the extent of dispersal and levels of gene flow. Indeed, such a correlation was found (Peterson & Denno, 1998). Given this, our results suggest that, at a spatial scale of 5–80 km, dispersal of individuals is a potential explanation for the low genetic differentiation observed among the subpopulations of *H. charithonia*.

This finding supports that of Mallet (1986b), who showed that dispersal distances in *Heliconius* butterflies probably have been substantially underestimated by mark–recapture studies. Mallet's estimate of F_{ST}, based

on neighbourhood deme size, was 0.02–0.04. Our estimate of F_{ST} for *H. charithonia* falls well below this range. Although this finding supports the hypothesis that *Heliconius* butterflies disperse a greater distance than was once believed based on their home range behaviour, Mallet found that even after taking early dispersal into account, the average distance travelled remained relatively low. Yet, we have shown that rates of gene flow remain high at distances of up to almost 80 km between subpopulations. Although it is likely that *H. charithonia* individuals disperse even greater distances than those determined for *H. erato*, it remains doubtful that increased movement alone can account for this finding.

In other insect systems, in which both dispersal and gene flow distances are known, gene flow typically occurs over greater distances than those exhibited by dispersing individuals (e.g. McCauley & Eanes, 1987; Rosenberg, 1989; Michalakis *et al.*, 1993; Peterson, 1995, 1996). In many instances, these results are not explained by direct gene flow between populations, but rather by genes being dispersed in a 'stepping-stone' fashion, whereby continued gene flow between neigh-

bouring populations spreads genes greater distances than the dispersal ability of the individuals might predict (Kimura, 1953). Therefore, rather than individuals carrying genes directly between two distant populations, these genes are indirectly transported through geographically intermediate populations.

Although this pattern of gene flow occurring over a larger distance than the dispersal of individuals is common and supports a stepping-stone model of gene flow, other explanations are possible. With few exceptions, allozymes are considered to be selectively neutral, but if this were not the case natural selection for particular allelic states could have a homogenizing effect on subpopulations. Additionally, if these subpopulations represent fragments of a recently continuous population, the allele frequencies might still remain similar among them. Pashley (1989) and Bossart & Pashley Prowell (1998) warn against making inferences of extensive gene flow based solely on allozyme data, on the grounds that if no differences between groups are detected, high levels of gene flow cannot be assumed because markers may not be refined enough to distinguish current gene flow from gene flow that ceased perhaps hundreds or thousands of years ago. Although this is a fair warning, we believe that we can attribute the lack of genetic differentiation observed here to current or very recent gene flow given the relatively low density of individuals in local *H. charithonia* populations and the potentially rapid generation time for this species in the subtropics.

Based on Quintero's (1988) data for a population of *H. charithonia* in Puerto Rico, we estimate N_e to be approximately 205, after accounting for population size fluctuations and a consistent male-biased sex ratio in the adult stage. Estimating generation time is a bit more complex due to the potentially extended life span of *Heliconius* butterflies (Gilbert, 1972 and Ehrlich & Gilbert, 1973.) and the life-long reproduction exhibited by females exposed to sufficient pollen resources (Dunlap-Pianka *et al.*, 1977). However, the minimum generation time, measured as the mean time for a female to hatch, grow, pupate, mate and lay an egg of her own is approximately 30 days in this genus. Measured in the same way, the maximum generation time can potentially exceed five months if females are exposed to unlimited pollen. In the subtropical area of south Florida the primary pollen resources used by *Heliconius* species in South and Central America (*Psiguria* and *Gurania* vines of the family Cucurbitaceae) are absent and therefore pollen resources are likely to be limited. Therefore, we estimate the maximum generation time to be in the neighbourhood of 90 days and the average is probably on the lower end of this range (approximately 45 days).

Considering the effect of genetic drift alone, we can predict how long it will take to reach various degrees of genetic differentiation using the relationship $F_{ST} = 1 - \left(1 - \frac{1}{2(N_e+1/2)}\right)^t$ (Hartl & Clark, 1989). Although F_{ST} has a theoretical maximum of 1, observed values are usually much less than this. Wright (1978) suggested that F_{ST} values which exceed 0.25 indicate very great genetic differentiation. Given complete isolation, two subpopulations should exhibit genetic divergence of the order of $F_{ST} = 0.3$ in the span of 12 to 36.1 years, depending on the estimate of generation time. Likewise, two subpopulations should exhibit a moderate level of differentiation ($F_{ST} = 0.1$) in the span of 3.6 to 10.7 years or a low degree of genetic differentiation ($F_{ST} = 0.05$) in the span of 1.7 to 5.2 years. In the case of *H. charithonia* in south Florida, the F_{ST} among all subpopulations is essentially zero and all pairwise F_{ST} comparisons are below 0.05. Therefore, if gene flow had ceased among any of these subpopulations one thousand, one hundred, or even 20 years ago there would be some detectable level of differentiation today. Interestingly, the history of urbanization in south Florida suggests that if urban fragmentation could limit gene flow in this species, it should have already happened and we should see the resulting genetic differentiation by now.

This study was not carried out in a typically natural area, but rather in the suburban areas surrounding the city of Miami. In these areas, the population of *H. charithonia* is subject to urban fragmentation. Therefore, the seven subpopulations in this study are not geographically separated solely by a region of suitable habitat, but rather, by an expanse of developed land and major roadways in many cases. Heavy drainage and development of the areas around the city of Miami began in the 1920s, and by 1975 only 4% of Miami Rockland forest remained outside of Everglades National Park (Shaw, 1975). Thus, fragmentation of this habitat occurred relatively recently but was swift and extensive. In light of this fragmentation, it is interesting to note that gene flow remains high among subpopulations. Apparently fragmentation of the natural habitat has not reached such a level as to restrict gene flow among many local neighbourhoods. Given the stepping-stone model by which gene flow is presumably occurring, it is likely that subpopulations that occur in open urban lots, small parks, and residential backyards provide the means for gene flow among the larger natural areas studied here such as Matheson Hammock, the Deering Estate, and Everglades National Park.

Although current levels of habitat fragmentation may not affect gene flow, continued human growth and expansion in Miami–Dade County could have an

increasing impact on these subpopulations. More development could potentially cut certain paths of gene flow and effectively separate the now continuous population into two or more gene-exchanging units.

Acknowledgements

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