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THE HISTORICAL BIOGEOGRAPHY OF TWO CARIBBEAN BUTTERFLIES (LEPIDOPTERA: HELICONIIDAE) AS INFERRED FROM GENETIC VARIATION AT MULTIPLE LOCI

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Abstract.—Mitochondrial DNA and allozyme variation was examined in populations of two Neotropical butterflies, *Heliconius charithonia* and *Dryas iulia*. On the mainland, both species showed evidence of considerable gene flow over huge distances. The island populations, however, revealed significant genetic divergence across some, but not all, ocean passages. Despite the phylogenetic relatedness and broadly similar ecologies of these two butterflies, their intraspecific biogeography clearly differed. Phylogenetic analyses of mitochondrial DNA sequences revealed that populations of *D. iulia* north of St. Vincent are monophyletic and were probably derived from South America. By contrast, the Jamaican subspecies of *H. charithonia* rendered West Indian *H. charithonia* polyphyletic with respect to the mainland populations; thus, *H. charithonia* seems to have colonized the Greater Antilles on at least two separate occasions from Central America. Colonization velocity does not correlate with subsequent levels of gene flow in either species. Even where range expansion seems to have been instantaneous on a geological timescale, significant allele frequency differences at allozyme loci demonstrate that gene flow is severely curtailed across narrow ocean passages. Stochastic extinction, rapid (re)colonization, but low gene flow probably explain why, in the same species, some islands support genetically distinct and nonexpanding populations, while nearby a single lineage is distributed across several islands. Despite the differences, some common biogeographic patterns were evident between these butterflies and other West Indian taxa; such congruence suggests that intraspecific evolution in the West Indies has been somewhat constrained by earth history events, such as changes in sea level.

Key words.—Allozymes, island, mitochondrial DNA, phylogeography, population genetics, West Indies.

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Comparative phylogeography based on DNA sequences can reveal the influence of ecology, dispersal ability, and earth history on the evolutionary process (Bermingham and Moritz 1998). A particularly powerful approach is to examine patterns of genetic variation in an archipelago, where discrete insular populations and geographic structure facilitate phylogenetic characterization (Avise 1994). With an emerging body of data concerning the phylogeography of West Indian species (Seutin et al. 1993, 1994; Klein and Brown 1994; Bermingham et al. 1996; Lovette et al. 1998; Ricklefs and Bermingham 1999; Malone et al. 2000) it is becoming possible to take a comparative approach to the evolution of intraspecific variation in the Caribbean. These studies have demonstrated that processes at the population level can be very dynamic, leading to sometimes unexpected and complex patterns of genetic variation.

Most preceding investigations of the West Indian fauna have used mitochondrial DNA (mtDNA) and focused on vertebrates. Relatively few genetic studies have examined insects or surveyed both mitochondrial and nuclear variation. Here we present data on West Indian butterflies using mtDNA sequences and allozyme frequencies. While the DNA sequences are more amenable to phylogenetic analysis, the allozyme data allow an assessment of population structure that is based on multiple loci (i.e., one that is representative of the entire genome). Together, mtDNA and allozymes can provide mutual corroboration of patterns, or they can reveal important discordance. For example, disagreement among maternally inherited mtDNA and bisexually inherited nuclear loci could demonstrate different dispersal tendencies among the sexes.

It has been suggested that species might differentiate relatively slowly over evolutionary time, especially with respect to their ecological niche (Ricklefs and Latham 1992). Niche conservatism was invoked by Peterson et al. (1999) to explain why the geographic ranges of various birds, mammals, and butterflies could be predicted from the ecological characteristics of their sister taxa at the species but not the family level. Here we examine the evolution of intraspecific variation in two heliconiid butterflies, *Dryas iulia* and *Heliconius charithonia*. The Heliconiidae form a relatively tight clade (Brower 1994a) and although *D. iulia* and *H. charithonia* are not sister species, they occupy similar habitats and appear to have broadly similar ecologies (e.g., they share a larval food plant, *Passiflora suberosa*) and dispersal abilities (Smith et al. 1994).

Given the similarities between these two butterflies, one might expect to find common biogeographic patterns in their population genetic structure. Species show congruent patterns of intraspecific variation in regions where their ranges overlap for two principal reasons: (1) vicariance due to common earth history events (Rosen 1976; Bermingham and Avise 1986) or (2) conservatism in the evolution of dispersal ability among related species (Page and Lydeard 1994). We tested the hypothesis that these butterflies would exhibit similar patterns of intraspecific variation predicted by both the evolutionary conservatism and vicariance models. We also explored the prediction made by evolutionary conservatism that

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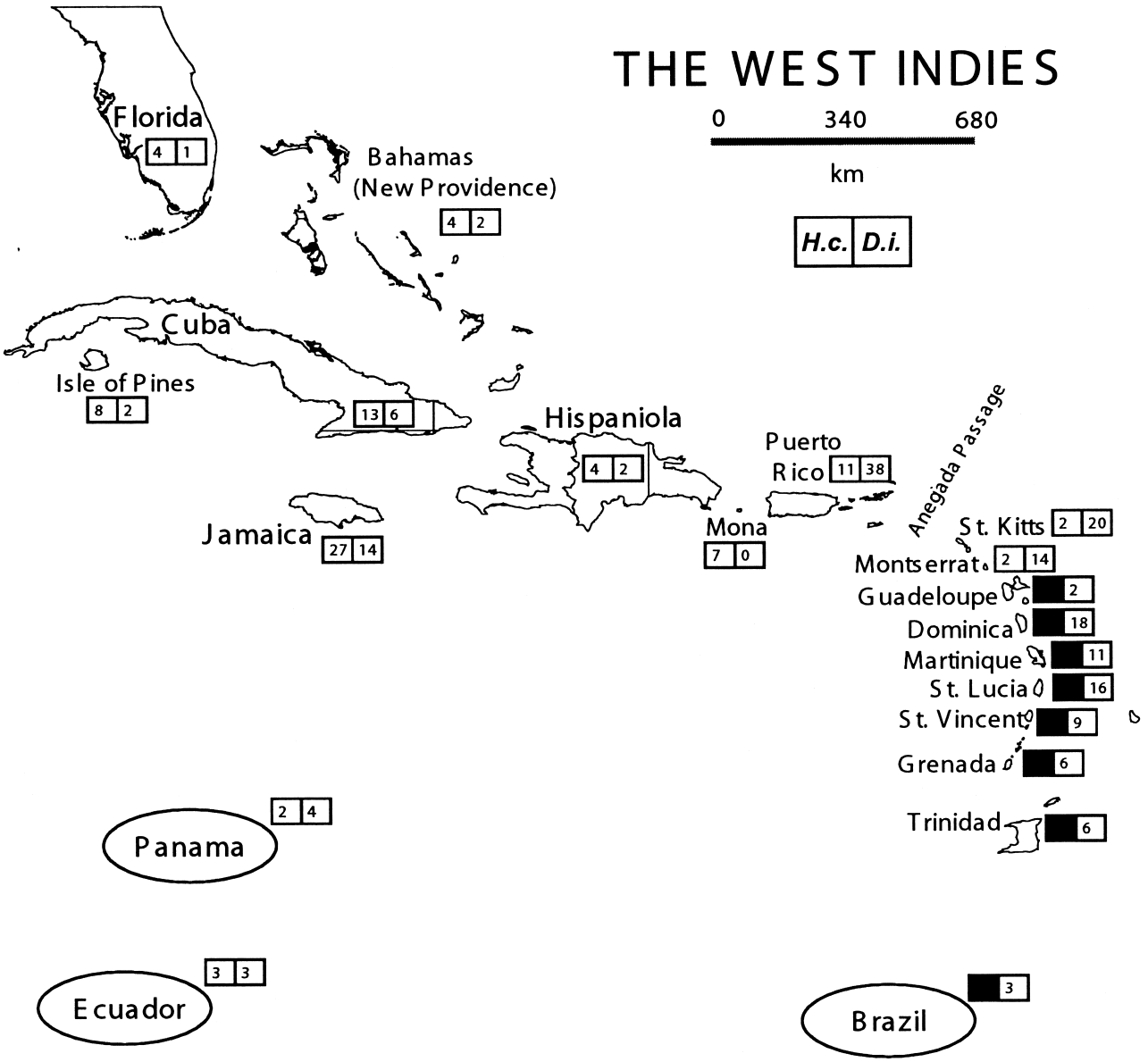

FIG. 1. Distribution map and sampling localities for *Heliconius charithonia* and *Dryas iulia*. A filled box indicates the distributional absence of the species, and an open box indicates that the species is present and the number of mtDNA haplotypes sequenced or screened by PCR-RFLP. *H. charithonia* is presented in the box on the left and *D. iulia* on the right.

closely related species would have more patterns in common with each other than they do with more distantly related taxa, particularly Antillean birds (Bermingham et al. 1996; Ricklefs and Bermingham 1999) and lizards (Roughgarden 1995).

MATERIALS AND METHODS

Collections and Experimental Design

Heliconius charithonia is distributed from the southern United States, through Central America, the Greater Antilles, and northwestern South America; it is absent from northeastern South America, Trinidad, and the Lesser Antilles south of Montserrat. Six subspecies have been described, five of which are restricted to the West Indies while only one occurs on the mainland. A sibling species, *H. peruviana*, is found in southern Ecuador and Peru (Jiggins and Davies 1998). *Dryas iulia* is distributed throughout the continental neotropics, south Florida, and the West Indies. Based on differences in wing pattern among insular populations, there are 12 subspecies of *D. iulia* in the West Indies (Smith et al. 1994).

Butterflies were sampled on three trips to the West Indies during 1991–1994. *Heliconius charithonia* was obtained from nine islands and three mainland localities, and *D. iulia* from 15 islands and four continental localities (Fig. 1). Allozyme electrophoresis was performed on the abdomens using cellulose acetate plates (Helena Laboratories Inc., Beaumont, Texas). Each species was screened for 36 different enzyme systems yielding 21 loci in *D. iulia* and 25 in *H. charithonia* (Table 1). The recipes for the stains were minor modifications

TABLE 1. Allozyme loci used and buffer system (PB, phosphate, TGB, tris glycine).

Name	Buffer	EC number	Number of loci	
			D. iulia	*H. charithonia*
Phosphogluconate dehydrogenase, decarboxylating (6PGD)	PB	1.1.1.44	0	1
Aconitate hydratase (ACO)	PB	4.2.1.3	0	2
Adenylate kinase (AK)	PB	2.7.4.3	1	1
Alcohol dehydrogenase (ADH)	TGB	1.1.1.1	1	1
3-hydroxybutyrate dehydrogenase (HBDH)	TGB	1.1.1.30	1	0
Phosphopyruvate hydratase (ENO)	PB	4.2.1.11	1	0
Fumarate hydratase (FUM)	TGB	4.2.1.2	1	1
Glucose-6-phosphate 1-dehydrogenase (G6PD)	PT	1.1.1.49	1	1
Aspartate aminotransferase (GOT)	TGB	2.6.1.1	2	2
Glutathione reductase, NADPH (GR)	TGB	1.6.4.2	1	1
Glyceraldehyde 3-phosphate dehydrogenase, phosphorylating (GAPDH)	PB	1.2.1.12	1	1
Hexokinase (HK)	TGB	2.7.1.1	0	1
Isocitrate dehydrogenase, NADP+ (IDH)	PB	1.1.1.42	2	2
Leucyl aminopeptidase (LA)	TGB	3.4.11.1	3	3
Malate dehydrogenase (MDH)	PB	1.1.1.37	0	1
Malate dehydrogenase, oxaloacetate decarboxylating, NADP+ (ME)	TGB	1.1.1.40	0	2
Mannose-6-phosphate isomerase (MPI)	PB	5.3.18	0	1
Xaa-Pro dipeptidase (PP)	TGB	3.4.13.9	1	0
Glucose-6-phosphate isomerase (PGI)	PB	5.3.1.9	1	1
Phosphoglucomutase (PGM)	TGB	5.4.2.2	1	1
Phosphoglycerate dehydrogenase (G3PD)	PB	1.1.1.95	1	1
L-iditol 2-dehydrogenase (SDH)	PB	1.1.1.14	1	0
Superoxide dismutase (SOD)	TGB	1.15.1.1	1	1
			21	25

of those described in Richardson et al. (1986) and Mallet et al. (1993). For each species, all the scorable loci were run for up to 10 individuals from every island. The rest of the individuals from each island were only screened for polymorphic loci (those for which the frequency of the most common allele was <0.99).

DNA was extracted from thoraxes, and 1600 base pairs of mtDNA, spanning part of cytochrome oxidase subunit I (COI), the entire subunit II (COII), and the intervening tRNA leucine, were sequenced for two individuals from each island and continental locality. At least one of the individuals was sequenced in both directions to improve the reliability of base calling. With two individuals sequenced per site, populations are likely to contain undetected polymorphism and consequently the gene trees might not reflect the population phylogenies. Polymerase chain reaction/restriction-fragment-length polymorphism (PCR-RFLP) assays based on the sequence data were used to assess the probability that a population carries multiple mtDNA lineages (Bermingham et al. 1996). The COI-COII sequences were searched for restriction sites that uniquely identified subsets of evolutionarily unique mtDNA lineages. Occasionally assays may turn up novel RFLP patterns; these are subsequently sequenced and added to the phylogeny of mtDNA sequences. It is important to stress that these PCR-RFLP assays serve one function, namely to test for the sympatric distribution of evolutionarily independent COI-COII mtDNA lineages. The assays provide no information regarding nucleotide diversity.

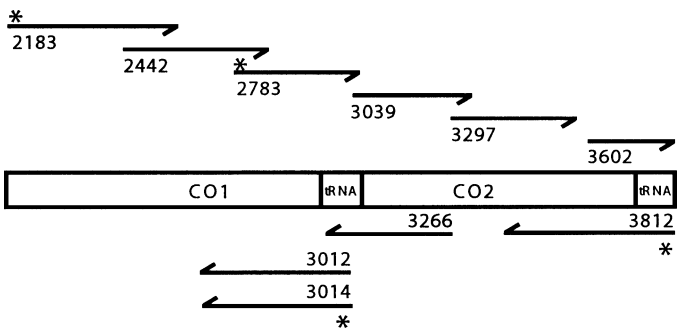

FIG. 2. Primer cartoon. Numbers represent the position of the 3′ end relative to *Drosophila yakuba*. *, external primers used in PCR.

DNA Laboratory Procedures

Thoraxes were lyophilized and ground in liquid nitrogen before genomic DNA was extracted following the protocol described by (Harrison et al. 1987). A 1590 base-pair region of mitochondrial DNA, corresponding to positions 2191–3781 in the *Drosophila yakuba* sequence (Clary and Wolstenholme 1985), was directly sequenced. This region includes half of cytochrome oxidase subunit I (COI), all of tRNA leucine and the entire cytochrome oxidase subunit II (COII). The region was amplified in two sections (Fig. 2) from individual genomic DNA via PCR. Four oligonucleotide primers were used to initiate PCR (Table 2; Fig. 2). For both pairs of primers, DNA was amplified using 25 cycles of the following step-cycle profile: denaturation at 94°C for 45 sec, primer annealing at 54°C for 45 sec and primer extension at 72°C for 60 sec. Amplifications were carried out in a Perkin-Elmer (Foster City, CA) DNA Thermal Cycler 480. Double-stranded DNA was synthesized in 50 μl reactions containing 1 μl DNA, 10 mM Tris (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, 0.01% gelatin, 0.01% NP-40, 2mM dATP, dCTP, dGTP, and dTTP (Sigma, St. Louis, MO), 10 mM of each primer, and 0.25 μl of Amplitaq polymerase (Perkin-Elmer).

The PCR products were separated from unincorporated

TABLE 2. Primers used in PCR and cycle sequencing. Primer names represent their position relative to *Drosophila yakuba.* Nucleotides in parentheses indicate positions for which the primers were redundant.

Primer	Sequence (5′ to 3′)
H3012	AATCCATTACATATAATCTGCC
H3014	TCCAATGCACTAATCTGCCATATTA
H3266	GC(ACT)GGTAAAATAGTTCAAATTAATTC
H3812	CATTAGAAGTAATTGCTAATTTACTA
L2183	CAACATTTATTTTGATTTTTTGG
L2442	CCAACAGGAATTAAAATTTTTAGATGATTAGC
L2783	TAGGATTAGCTGGAATACC
L3039	TAATATGACAGATTATATGTAATGGA
L3297	TGAACTATTTTACC(AGT)GC
L3602	CCTTCTTTAGGAGT(AT)AAAAT(TC)GATGC

primers and dNTPs by electrophoresis in 1.5% low melting point agarose gels run in a Tris-acetate buffer (pH 7.8) containing ethidium bromide (1μg/ml). The single amplification product was cut from the gel and extracted using a Gene Clean II kit (Bio 101, Inc., La Jolla, CA). The purified mtDNA was re-suspended in 25 μl of ddH_2O of which 7 μl was used as a template in a reaction utilizing a Taq DyeDeoxyT Terminator Cycle Sequencing kit (Applied Biosystems, Inc., Foster City, CA). Twenty-five sequencing cycles were carried out in a Gene Amp PCR System 9600 (Perkin Elmer). Sequencing was initiated by preheating the samples to 96°C followed by the following conditions: 96°C for 15 sec, 50°C for 1 sec, and 60°C for 4 min. In addition to the external primers mentioned above, a number of internal primers were also used in the cycle sequencing reaction (Table 2; Fig. 2). The cycle sequencing product was purified over Centrisep columns filled with 780 μl G-50 sephadex. Gels were run on an Applied Biosystems 373A DNA Sequencer. Samples were dried and re-suspended in 3.5 μl of a 5:1 deionized formamide:blue dextran/EDTA (pH 8.0) solution, denatured at 90°C for two min and loaded into 6% acrylamide gels. Gels were run for 12 h at 28 W constant power and we typically collected 350 nucleotides per reaction.

Chromatograms were edited, sequences aligned, and basecalls checked using Sequencher 3.0 (Gene Codes Corporation, Inc., Ann Arbor, MI) Following the verification of each sequence for an individual, alignments were assembled in Sequencher. All DNA sequences were translated to putatively functional peptide sequences representing mtDNA COI and COII genes or a functional tRNA leucine based on comparisons to *Drosophila yakuba*. The nucleotide sequences were checked for reading-frame errors, termination codons, and unlikely nucleotide substitutions and were then assembled as MEGA and NEXUS files for analyses.

Sequence data was surveyed for potential restriction site polymorphisms using the computer program Macvector. Approximately 7–10 μl of amplified product was endonuclease digested without further purification for 3 to 5 h following manufacturer's recommendations. The resulting digested product was visualized using ethidium bromide on a 1.5% TBE minigel containing a 3:1 mixture (low melting point: regular) agarose. Samples displaying unusual fragment profiles or unexpected results were verified by sequencing.

Allozyme Analyses

The allozyme data were processed using the computer program TFPGA 1.3 (Miller 1998). Deviations from Hardy-Weinberg equilibrium were tested using the exact test (Haldane 1954), employing the conventional Monte-Carlo method with 10,000 total permutations to assess significance (Guo and Thompson 1992). The extent of population subdivision was determined using the *F*-statistics option in TFPGA to calculate θ, an estimator of F_{ST} (Weir and Cockerham 1984; Weir 1990) and bootstrapping across loci assessed significance. The arc distance, D_{arc} (Cavalli-Sforza and Edwards 1967) was calculated using the computer program Biosys 1.7 (Swofford and Selander 1989) and unrooted networks were constructed based on D_{arc} using the neighbor-joining (NJ) algorithm (Saitou and Nei 1987) in the computer program MEGA (Kumar et al. 1993). TFPGA was used to create networks using the unweighted pair group method of analysis (UPGMA) based on the modified Roger's distance of Wright (1978). The significance of nodes in the UPGMA networks was assessed by 1000 bootstrap replications across loci (bootstrapping over loci was not possible for NJ networks in either version of MEGA or TFPGA). A more detailed treatment of the allozyme analyses of these and two other Caribbean butterflies will be presented elsewhere (see Davies 1995).

Phylogenetic Analyses

The data partition homogeneity test in PAUP (Swofford 1998) was used to assess whether the three (COI, COII, and tRNA leucine) data partitions showed significantly ($\alpha > 0.05$) discordant trees compared to 100 random partitions of the data. A maximum likelihood (ML) approach was used to reconstruct phylogenetic trees. The most appropriate ML model was chosen using the computer program Modeltest (Posada and Crandall 1998), which evaluates the difference in likelihood between simple and more complex models, beginning by fitting the data to an initial NJ tree and then testing progressively more complex models. As the complexity of the model increases, so do the degrees of freedom, and the most complex model that resulted in a significant increase in likelihood is used in the phylogenetic reconstructions. Maximum likelihood trees were generated from 1000 quartet puzzling steps in PAUP (Swofford 1998).

Populations of *H. charithonia* were rooted with the sibling species, *H. peruviana*. Finding a suitable outgroup for populations of *D. iulia* was less straightforward. Brower's (1994a) phylogenetic study of the Heliconiidae was used as a reference and a source of mitochondrial COII sequences. Brower's COII sequences from *Heliconius demeter, Eueides tales, Heliconius erato,* two 'basal' heliconiids, *Dryadula phaetusa, Dione juno,* and one of our complete COI/II sequences from *H. charithonia* (Montserrat) were used as outgroups for *D. iulia*. All of these species are similarly related to *D. iulia* (the only species in its genus) and the choice of outgroup had no significant impact on the resulting tree. Here we present results using *H. charithonia* (Montserrat) as the outgroup (the only species for which we had both COI and COII sequence).

TABLE 3. Summary of genetic data for *Heliconius charithonia*. *N*, number of individuals examined using allozyme electrophoresis, mtDNA COI/II, mitochondrial sequencing; Dde1 and Msp1, polymerase chain reaction/restriction-fragment-length polymorphism. The frequency of mtDNA haplotypes, identified as Dde1-A or Dde1-B (either by sequencing or by PCR-RFLP), suggests the evolutionary independence of *H. charithonia* in Jamaica. By contrast, the frequency of mtDNA haplotypes identified as Msp1-A or Msp1-B suggests that Hispaniola, Mona, and Puerto Rico are not evolutionarily independent.

Population	Allozymes *N*	mtDNA COI/II	Restriction enzymes		Jamaica vs. rest		Origin of Mona	
			Dde1	Msp1	Jamaica (Dde1-A)	Not Jamaica (Dde1-B)	Hispaniola (Msp1-A)	Puerto Rico (Msp1-B)
H. peruviana	2	3	—	—	—	3	—	—
Ecuador	—	3	—	—	—	3	—	—
Panama, Gamboa	—	2	—	—	—	2	—	—
Florida, Miami	24	4	—	—	—	4	—	—
New Providence	5	4	—	—	—	4	—	—
Cuba, Guanahacabibes	24	2	5	—	—	7	—	—
Isle of Pines	24	2	—	—	—	2	—	—
Jamaica, Point Morant	24	3	24	—	27	—	—	—
Hispaniola, San Cristobal (DR)	22	2	2	4	—	4	4	2
Mona	24	2	5	4	—	7	1	5
Puerto Rico, Rio Guajactaca	24	2	9	—	—	11	—	2
St. Kitts	20	2	—	—	—	2	—	—
Montserrat	25	2	—	—	—	2	—	—
Total	218	33			27	51	5	9

RESULTS

Molecular Characterization

We sequenced a 1600 base-pair region including part of CO-I and all of CO-II for 30 *H. charithonia* individuals sampled from 12 localities, three *H. peruviana* individuals from Ecuador (Table 3; Fig. 1), and 55 *D. iulia* individuals from 20 localities (Table 4; Fig. 1). Twenty-one unique haplotypes were identified in *H. charithonia*, with 88 polymorphic and 77 parsimony informative sites. Mean sequence divergence among haplotypes was 1.1%, transition:transversion ratio (Ts:Tv) was 5.57, and sequences were 74.75% AT rich. Forty-four unique haplotypes were identified in *D. iulia* with 100 polymorphic sites, including 68 that were parsimony-informative. Mean pairwise sequence divergence among haplotypes was 1.3%, Ts:Tv was 4.53, and 74.7% of nucleotides were either A or T.

Partial sequence of 820 base pairs was obtained for the COI gene. Of the 47 polymorphic sites in *H. charithonia*, 22 substitutions occurred at twofold and 25 at fourfold degen-

TABLE 4. Summary of genetic data for *Dryas iulia*. *N*, number of individuals examined using allozyme electrophoresis, mtDNA COI/II or COI, Msp1 and Taq1, polymerase chain reaction/restriction-fragment-length polymorphism. The frequency of mtDNA haplotypes, identified as Msp1-A or Msp1-B (either by sequencing or by polymerase chain reaction/restriction-fragment-length polymorphism) suggests the evolutionary independence of *D. iulia* in the Greater Antilles (GA) and the northern Lesser Antilles (NLA). The frequency of mtDNA haplotypes identified by sequencing or polymerase chain reaction/restriction-fragment-length polymorphism as Taq1-A or Taq1-B similarly indicates the evolutionary independence of *D. iulia* in the NLA from populations in the Central Lesser Antilles (CLA).

Population	Allozymes *N*	mtDNA		Restriction enzymes		GA vs. NLA		NLA vs. CLA	
		COI/II	COI	Msp1	Taq1	GA (Msp1-A)	NLA (Msp1-B)	NLA (Taq1-A)	CLA (Taq1-B)
Brazil	—	2	1	—	—	—	—	—	—
Ecuador	—	3	—	—	—	—	—	—	—
Panama, Gamboa	19	1	2	—	—	—	—	—	—
Trinidad, Simla	7	2	3	—	—	—	—	—	—
Grenada	6	3	2	—	—	—	—	—	—
St. Vincent	9	4	5	—	—	—	—	—	—
St. Lucia	16	12	4	—	—	—	—	—	16
Martinique	11	4	7	—	—	—	—	—	11
Dominica	24	3	1	14	13	—	18	17	—
Guadeloupe	6	2	—	—	—	—	2	—	—
Montserrat	22	2	—	12	—	—	14	—	—
St. Kitts	19	2	1	17	—	—	20	—	—
Puerto Rico, Cayey	18	2	—	36	—	38	—	—	—
Hispaniola, San Cristobal (DR)	14	2	—	—	—	2	—	—	—
Jamaica, Morant Point	21	2	—	12	—	14	—	—	—
Isle of Pines	20	4	—	—	—	4	—	—	—
Cuba, Guanahacabibes	17	2	—	2	—	4	—	—	—
Cuba, Baracoa	—	—	2	—	—	2	—	—	—
New Providence	20	2	—	—	—	2	—	—	—
Florida, Key Largo	—	1	—	—	—	1	—	—	—
Total	249	55	28	93	13	67	54	17	27

erate sites. Fifty-nine of the mutations were third-base substitutions, only one of which led to an amino acid change. The single first-base substitution was silent. In *D. iulia*, there were 60 polymorphic sites, 18 of which were at twofold and 38 at fourfold degenerate sites. The majority of mutations were silent, third-base substitutions. A single first- and three second-base substitutions were found, all of which led to amino acid substitutions.

The complete COII gene was sequenced for both species. In *H. charithonia*, there were 40 polymorphic sites with 19 of the substitutions at twofold and 18 changes at fourfold degenerate sites. The majority of mutations were silent, third-base substitutions. Three first- and two second-base substitutions were observed; two of the first base- and one second-base substitutions caused amino acid replacements. In *D. iulia*, 38 sites were polymorphic with 20 substitutions at twofold and 15 changes at fourfold degenerate sites. The majority of mutations were silent, third-base substitutions; the single second- and two of three first-base changes caused amino acid replacements.

Nucleotide diversity across the two gene regions was very similar in *H. charithonia*: COI (0.057) and COII (0.058). Brower (1994b) also reported similar evolutionary divergences between COI and COII across many heliconiid species (although only a small part of COI was included in the 945bp region). The rate of substitution per nucleotide was somewhat higher in *D. iulia* COI (0.073) than COII (0.053), with the 39% higher diversity values for COI matching exactly the differential observed for the same region of COI/II in *Choristoneura* moths (Sperling and Hickey 1994).

Heliconius charithonia

A data partition homogeneity test failed to demonstrate significant ($\alpha > 0.05$) heterogeneity among the three mtDNA partitions tested (COI, COII, and tRNA leucine) compared to 100 random partitions of the data. The best model of mtDNA sequence evolution for *H. charithonia* according to the ratio likelihood test in Modeltest (Posada and Crandall 1998) was an HKY model (Hasegawa et al. 1985) with a gamma shape parameter of 0.658 and 0.826 invariable sites. The maximum-likelihood tree (Fig. 3) based on this model was derived from 1000 quartet puzzling steps. Only nodes supported by 60% bootstraps of the puzzling steps were considered significant.

The analysis of *Heliconius* identified three principal mtDNA lineages: (1) *H. peruviana*, (2) *H. charithonia simulator*, the subspecies from Jamaica, and (3) all other West Indian and continental subspecies of *H. charithonia*. The three *H. peruviana* individuals formed a distinct monophyletic group, and each individual had similar but unique mtDNA haplotypes. *Heliconius peruviana* mtDNA has diverged from *H. charithonia* by a minimum of 3.3%. The mtDNA haplotype found in all three individuals sampled from Jamaican *H. c. simulator* was very distinct from all other *H. charithonia* haplotypes (average sequence divergence 2.3%). Furthermore, in trees rooted with *Heliconius peruviana*, the Jamaican mtDNA lineage was sister to all other *H. charithonia* haplotypes. Continental *H. charithonia* (from Ecuador and Panama) and all West Indian *H. charithonia* haplotypes, excluding Jamaica, were very similar (average sequence divergence 0.4%) despite an enormous geographic range extending from Ecuador to Montserrat. Relationships were poorly resolved within this group, suggesting that the populations were established over a short period of evolutionary time. The only strongly supported clades were among the mtDNA haplotypes from Mona, Puerto Rico, in which populations of *H. charithonia* seemed to form a distinct evolutionary unit.

Notwithstanding the low levels of genetic divergence observed between mtDNA haplotypes representing the geographically widespread *H. charithonia* clade, the restricted geographic distribution of some haplotypes provided weak evidence (given the small sample sizes) for the evolutionary independence of some populations. For example, the two Montserrat individuals shared an mtDNA haplotype not found elsewhere, and the two St. Kitts individuals possessed a single haplotype that was unique to the island. Finally, eight individuals from Florida, the Isle of Pines, and the Bahamas carried the same mtDNA haplotype.

Based on the sequence data, we used PCR-RFLP analyses of *H. charithonia* to increase the sample of individuals that were haplotyped for each population. The phylogenetic analysis of *H. charithonia* had raised two hypotheses that might be tested using PCR-RFLP and for which a suitable (diagnostic) restriction enzyme could be found. First, was there any evidence of genetic exchange between Jamaica and the other islands (Jamaica vs. rest; Table 3); and second, was Mona part of a unique ''Puerto Rican'' clade, genetically isolated from Hispaniola (origin of Mona; Table 3)?

We typed all 27 individuals from Jamaica with the restriction enzyme *Dde*1 that is diagnostic for the Jamaican haplotype. All Jamaican individuals had the *Dde*1-A haplotype; thus, the frequency of *Dde*1-A in Jamaica is 89% or greater ($P = 0.04$; Table 3), indicating very little gene flow from outside of Jamaica. To determine whether the *Dde*1-A haplotype was found outside of Jamaica, we typed a few more individuals from the neighboring Greater Antilles using *Dde*1 bringing the total number of non-Jamaican *H. charithonia* typed (through sequence or *Dde*1) to 48. Not one of these butterflies outside of Jamaica had the *Dde*1-B haplotype, demonstrating that the Jamaican haplotype does not occur elsewhere at a frequency greater than 7% ($P = 0.03$).

We began surveying populations of *H. charithonia* in Hispaniola and Mona using *Msp*1 to see if there was any evidence of gene flow between the two islands. The analysis quickly revealed introgression, because we found two individuals from Hispaniola that had the supposed ''Puerto Rican'' clade (*Msp*1-B); we also found one individual in Mona that had the supposed Hispaniolan clade (*Msp*1-B). Thus, no further screening was necessary to reject the hypothesis that Puerto Rico and Mona shared a unique haplotype (*Msp*1-B) that was not present in Hispaniola.

The allozyme analysis revealed significant structure among populations of *H. charithonia* ($\theta = 0.32$). An unrooted NJ network based on allozyme-based arc distances is pictured in Figure 4 and is generally concordant with the mtDNA tree. In particular, Mona and Puerto Rico were grouped together by both NJ and UPGMA, as were Montserrat and St. Kitts. Jamaica did not cluster with any other islands but it was

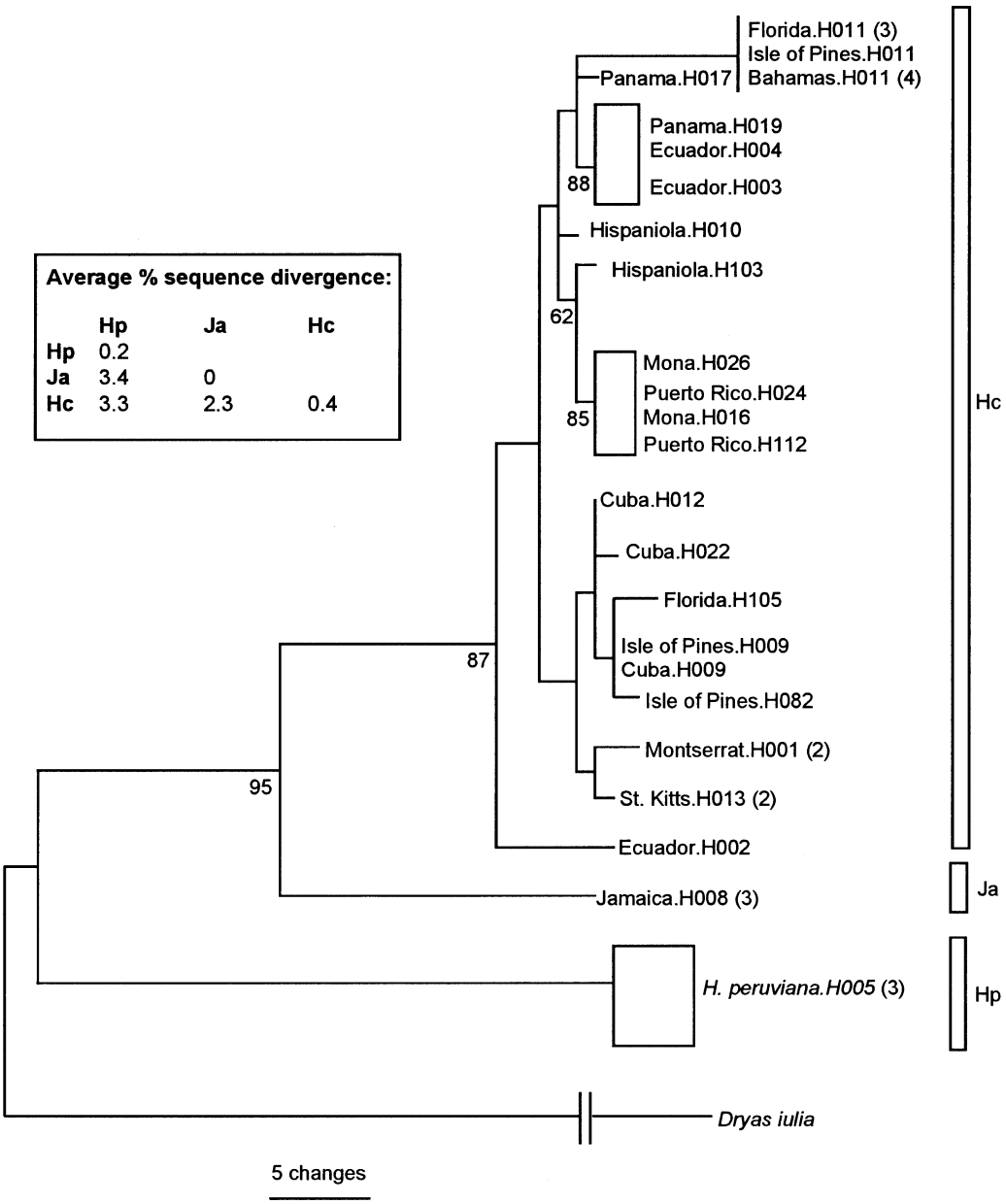

FIG. 3. Relationships among populations of *Heliconius charithonia* inferred from sequences of 1600 base pairs of mitochondrial COI and COII. Maximum likelihood phylogram based on 1000 puzzling steps (figures represent percent support for nodes). Haplotypes are coded Hxxx; multiple individuals from the same location with the same haplotype are indicated with the number of individuals in parenthesis. A box at a tip indicates that there was little support for relationships among those individuals; it represents a number of different haplotypes that form an unresolved clade. The width of a box indicates the level of diversity among its constituent haplotypes.

clearly less distinct from the other *H. charithonia* populations than *H. peruviana*.

Dryas iulia

The data partition homogeneity test of *D. iulia* mtDNA revealed significant heterogeneity ($P = 0.04$) among the three partitions tested (COI, COII, and tRNA leucine) compared to 100 random partitions of the data. However, we observed no significant heterogeneity among the COI and COII genes when we excluded the tRNA leucine partition (which contained only a single parsimony informative site). Thus, mtDNA analysis of *D. iulia* was based on the combined COI and COII sequences. Modeltest (Posada and Crandall 1998) identified the HKY model with a gamma shape parameter of 0.286 and 0.6 invariable sites as the most appropriate basis for the ML puzzling analysis.

The allozyme analysis revealed significant structure among populations of *D. iulia* ($\theta = 0.70$; Fig. 5) and the network of distances (Fig. 5) was consistent with the ML mtDNA tree (Fig. 6). Populations of *D. iulia* in the islands north of Martinique (the Greater Antilles and northern Lesser Antilles) clustered together in terms of allele frequencies and formed a monophy-

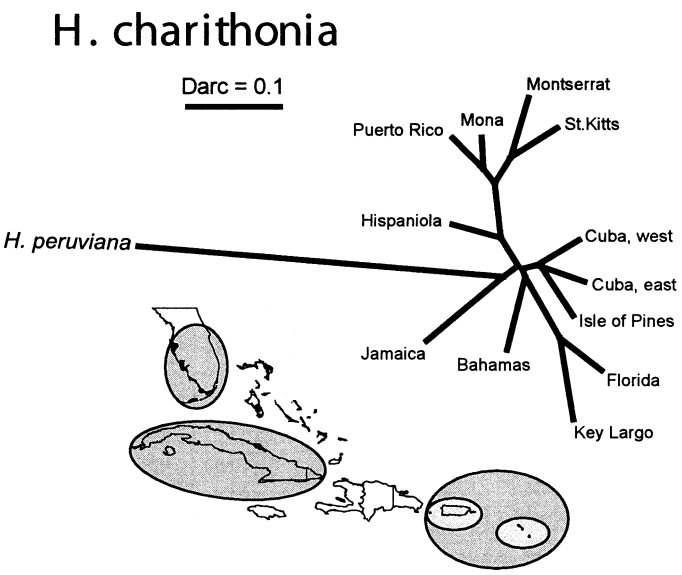

FIG. 4. Relationships among populations of *Heliconius charithonia* based on allozyme data. Unrooted neighbor-joining network based on arc distances. Encircled populations represent those that were grouped together by both NJ analysis and unweighted pair group method of analysis (and in the latter supported by > 60% of the bootstrap replications across loci).

letic group in the mtDNA phylogeny. Within this clade, the northern Lesser Antilles and Greater Antilles were reciprocally monophyletic and differed significantly in allele frequencies (though no differences were fixed). Haplotypes in the Greater Antilles differed on average by 0.75% sequence divergence from those in the northern Lesser Antilles, and none of the haplotypes sequenced were shared between the two groups.

There was little discernable structure among populations of *D. iulia* in the Greater Antilles at either allozyme or mitochondrial loci. Most of the haplotypes sequenced from the Greater Antilles were unique, although (as in *H. charithonia*) one haplotype was found in six individuals from Cuba, the Bahamas, the Isle of Pines, and Florida. Allozyme frequencies were also similar across these populations (Fig. 5). Within the northern Lesser Antilles, the six individuals sequenced from St. Kitts, Montserrat, and Guadeloupe had identical haplotypes that were closely related to, but distinct from, the haplotype observed in the four *D. iulia* from Dominica. The allozymes showed a slightly different pattern, with St. Kitts being slightly more distinct from Montserrat, Guadeloupe, and Dominica.

Together, the Greater Antillean and northern Lesser Antillean clade had diverged strongly from the populations to the south. Populations of *D. iulia* in the central Lesser Antilles, St. Lucia, and Martinique were very distinct from those in the northern Lesser Antilles, both in terms of allozyme frequencies and mtDNA sequence divergence (on average 2–3%). The two central Lesser Antillean populations were not significantly distinct from each other in terms of their allozyme frequencies, but they could be distinguished by mtDNA sequence. However, St. Lucia and Martinique haplotypes were very similar, with average sequence divergence of only 0.41%. We sequenced a minimum of 800 base pairs for each of the 16 *Dryas* individuals collected from St. Lucia. Five distinct haplotypes were present, one of them being found in 68% of individuals. None of the St. Lucia haplotypes were found elsewhere. The 11 individuals sampled from Martinique also carried island-specific mtDNA haplotypes; 10 individuals had identical mtDNA sequences and the only other haplotype differed by just a single substitution.

Both the allozymes and mtDNA showed that the central Lesser Antilles were also differentiated from St. Vincent, Grenada (the southern Lesser Antilles), and the continental populations. In terms of mtDNA sequence divergence, central Lesser Antillean haplotypes were more similar to those in the southern Lesser Antilles (about 1% on average) than they were to those in the northern Lesser Antilles (almost 3% sequence divergence). Continental *D. iulia* and populations in the southern Lesser Antilles were paraphyletic and basal with respect to the other islands. There was no discernable geographic structure in the enormous area over which these butterflies were sampled. Indeed, the only significant clade in this group included individuals from locations as far apart as Brazil, Panama, and Trinidad. The continental haplotypes were the most diverse, however, with average sequence divergence of 0.43% compared to only 0.27% among Greater Antillean haplotypes.

Based on the sequence data, we used PCR-RFLP analyses of *D. iulia* to increase the sample of individuals that were haplotyped for each population. The phylogenetic analysis of *D. iulia* had raised two questions that might be addressed using PCR-RFLP and for which a suitable (diagnostic) restriction enzyme could be found. Was there any evidence of gene flow between (1) the Greater Antilles and the northern Lesser Antilles (GA vs. NLA; Table 4), and (2) between the northern Lesser Antilles and the central Lesser Antilles (NLA vs. CLA; Table 4)? The Greater Antillean clade of *D. iulia* could be distinguished from the northern Lesser Antillean clade using *Msp*1 (Table 4; Fig. 7). Our efforts focused on Puerto Rico as the most likely population of *D. iulia* in the Greater Antilles to show evidence of gene flow with the Lesser Antilles; however, we failed to uncover the northern Lesser Antillean haplotype (*Msp*1-B) among any of 65 Greater Antillean individuals tested (including 38 from Puerto Rico). This demonstrates that *Msp*1-B does not occur in the Greater Antilles at greater than 5% frequency ($P = 0.036$). Similarly, none of the 54 northern Lesser Antillean individuals tested were Greater Antillean *Msp*1-A, indicating that this haplotype has a maximum frequency of 6% ($P = 0.035$) in the northern Lesser Antilles.

Addressing the question of gene flow among the central and northern Lesser Antilles, we were able to type 17 individuals from Dominica, the southernmost island of the northern Lesser Antillean clade (*Taq*1-A), to compare these with the mtDNA haplotypes found in the central and southern Lesser Antilles (*Taq*1-B). Based on all haplotyped individuals, the frequency of the southern *Taq*1-B haplotype is unlikely to exceed 17% in Dominica ($P < 0.05$), and the northern *Taq*1-A is not greater than 11% in Martinique and St. Lucia ($P < 0.05$).

DISCUSSION

Analysis of mtDNA and allozyme variation of *H. charithonia* and *D. iulia* showed that geographic populations of

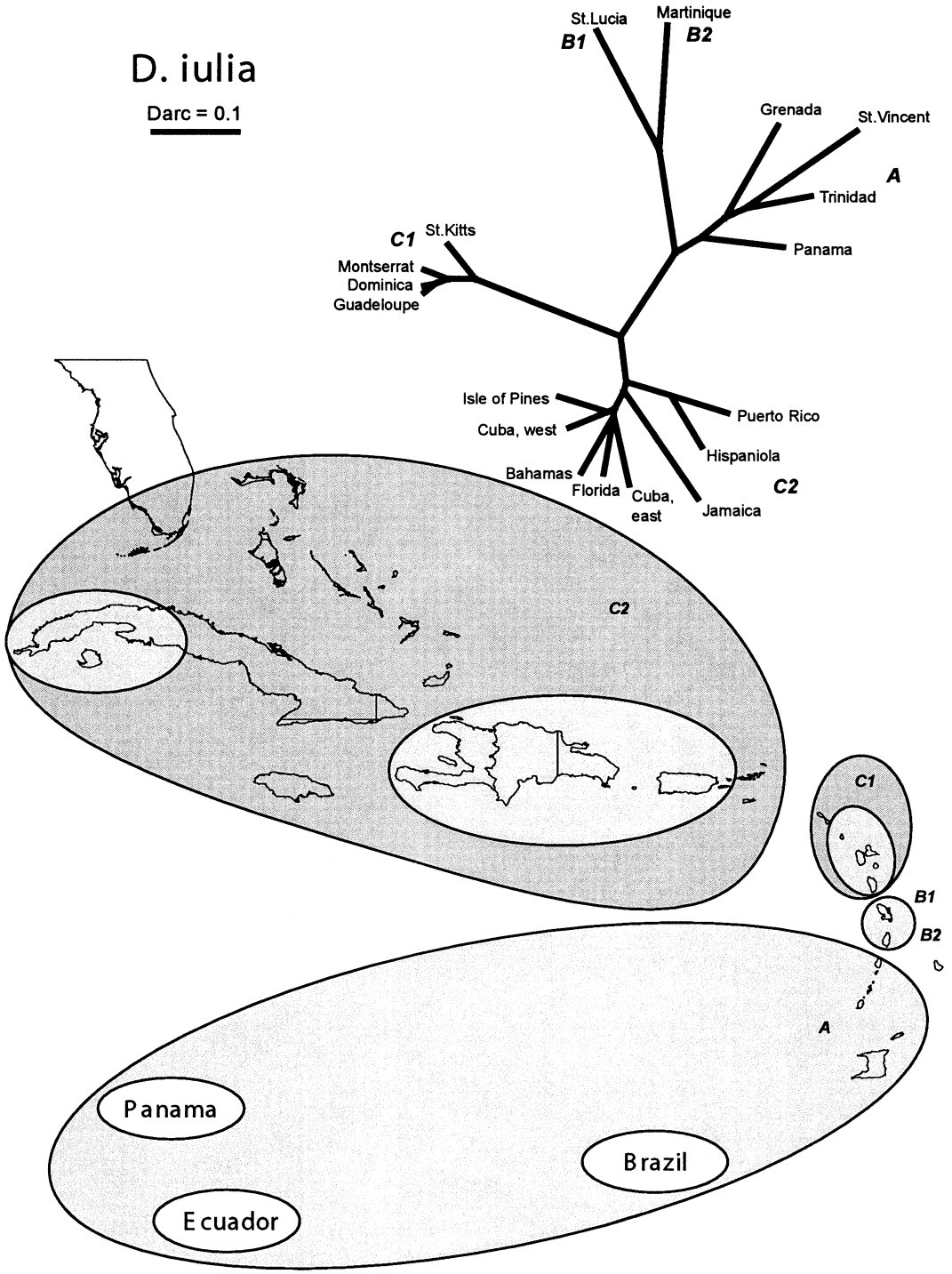

FIG. 5. Relationships among populations of *Dryas iulia* based on allozyme data. Unrooted neighbor-joining network based on arc distances. Encircled populations represent those that were grouped together by both NJ analysis and unweighted pair group method of analysis (and in the latter supported by > 60% of the bootstrap replications across loci).

D. iulia in the West Indies were more evolutionarily distinct than those of *H. charithonia*. Whereas populations of *D. iulia* in the islands north of St. Vincent formed a monophyletic group, West Indian populations of *H. charithonia* were polyphyletic with respect to those on the mainland. The evidence suggests that this is due to a recent founder effect (expansion) in *H. charithonia* rather than high levels of contemporary gene flow between mainland and insular populations. There are no obvious ecological reasons why *H. charithonia* should be absent from the Lesser Antilles south of Montserrat, and distance is clearly not a sufficient barrier because *H. charithonia* has evidently crossed large ocean gaps. The most prob-

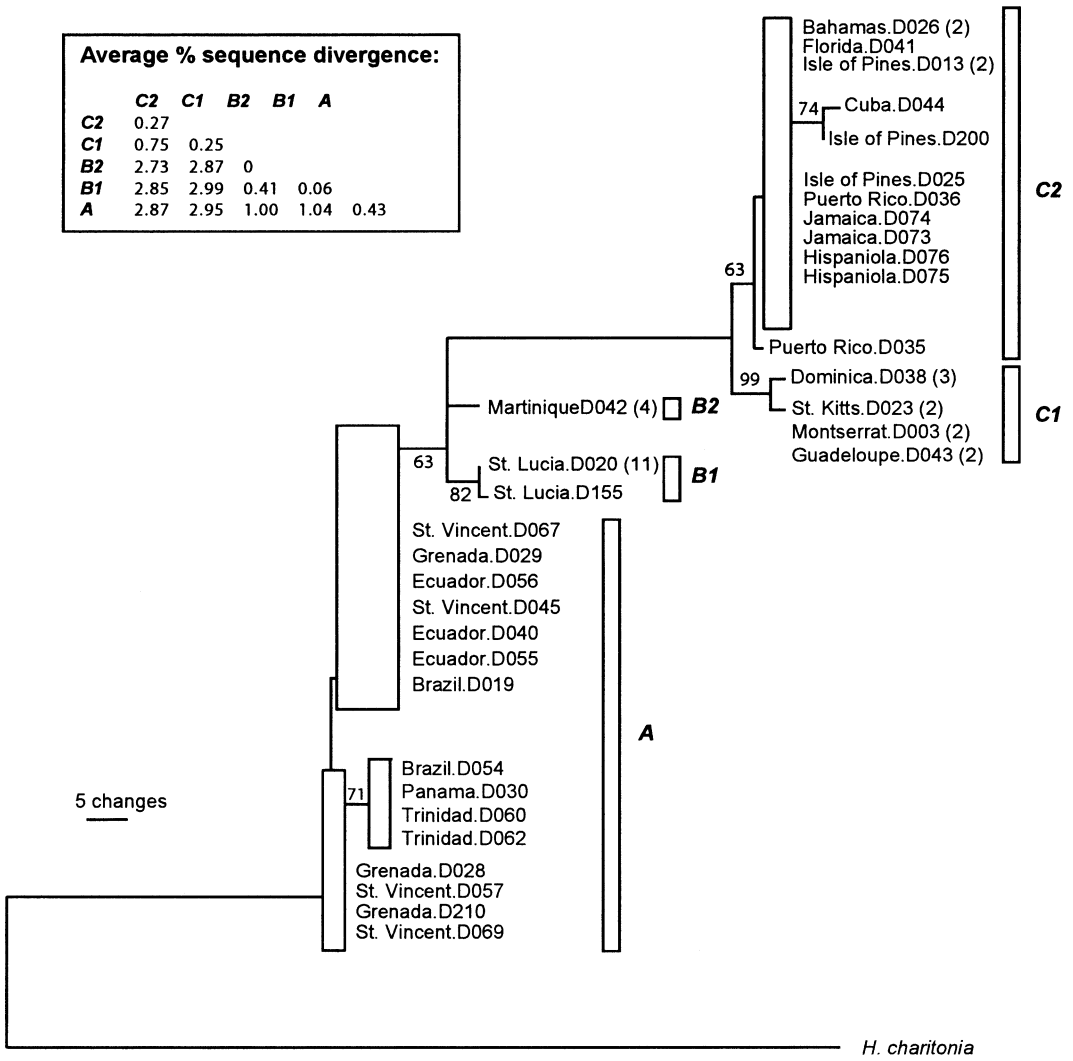

FIG. 6. Relationships among populations of *Dryas iulia* inferred from sequences of 1600 base pairs of mitochondrial COI and COII. Maximum likelihood phylogram based on 1000 puzzling steps (figures represent percent support for nodes). Haplotypes are coded Dxxx; multiple individuals from the same location with the same haplotype are indicated with the number of individuals in parenthesis. A box at a tip indicates that there was little support for relationships among those individuals; it represents a number of different haplotypes that form an unresolved clade. The width of a box indicates the level of diversity among its constituent haplotypes.

able explanation, therefore, is that *H. charithonia* only recently colonized Montserrat from Puerto Rico and has had insufficient time to spread further south.

Although *H. charithonia* might be a new arrival to most of the Antilles, it appears to have colonized Jamaica much earlier. Whereas Greater Antillean populations of *D. iulia* have little phylogenetic structure, the Jamaican subspecies *H. c. simulator* is very distinct from its neighbors. According to a molecular clock estimate for insect COII (Brower 1994b), *H. c. simulator*'s 2.4% average sequence divergence suggests approximately one million years of evolutionary independence, confirming Smith et al.'s (1994) view that *H. c. simulator* is one of *H. charithonia*'s more convincingly distinct subspecies. The three Jamaican individuals sequenced had identical mtDNA haplotypes and our PCR-RFLP assay suggested that this haplotype is fixed on Jamaica and does not occur elsewhere. Gene flow at mitochondrial loci thus appears to be severely limited between Jamaica and the other islands. Interestingly, however, the allozyme analysis did not pick out Jamaican *H. charithonia* as being particularly distinct. This might reflect some gene flow mediated by dispersing male butterflies, the greater impact of founder events and genetic drift on maternally inherited, haploid mtDNA (with its consequent smaller effective population size), and/or the lower detectable mutation rate of allozyme loci.

The geographic distribution of *H. charithonia* and its sister-group relationship to *H. peruviana* suggests that *H. charithonia* evolved on the mainland, probably in South America. A good disperser over land but a poor one over water, continental *H. charithonia* remained genetically homogenous (as did continental *D. iulia*) but failed to colonize the West Indies until the early Pleistocene. Then, approximately one million years ago a population became established on Jamaica and either did not spread to other islands or left no mtDNA trace. *Heliconius charithonia* probably did not reach the rest of the West Indies until the late Pleistocene, when a second colo-

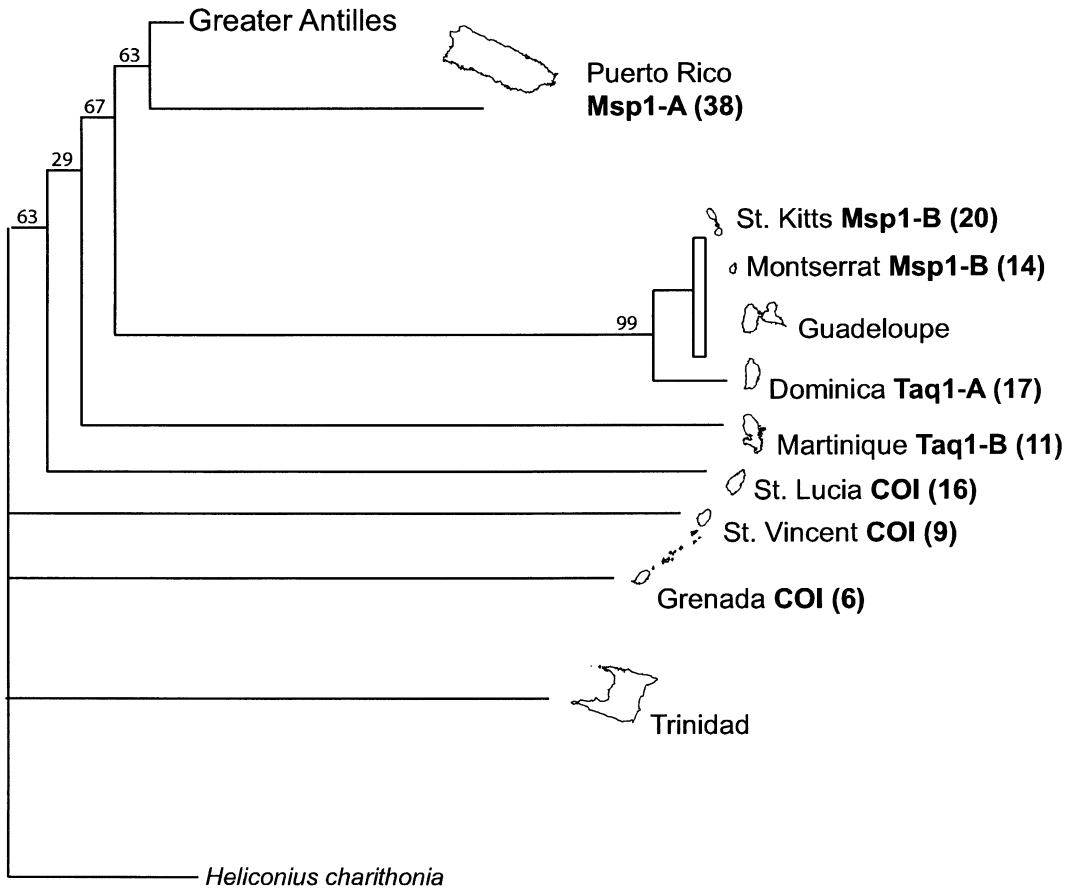

FIG. 7. Maximum likelihood cladogram for *Dryas iulia* with nodes supported by < 60% bootstraps collapsed (except Martinique), demonstrating how the tree is corroborated by geography.

nization occurred from the mainland some 110,000 years ago. At that time, Caribbean sea levels were 71 m lower than today (Steinen et al. 1973), presumably increasing the likelihood of colonization. If inter-island dispersal is rare, then *H. charithonia* could have colonized the West Indies rapidly on a geological timescale while insufficient migrants reached Jamaica to disturb its splendid isolation. Whether the second colonization was more widespread due to the genetic properties of the mainland population at that time relative to *H. c. simulator*, or whether it resulted solely from more favorable geological and environmental conditions, remains an interesting question.

Dryas iulia might also be a somewhat recent arrival to the Greater Antilles, although it seems longer established than *H. charithonia*. Allozymes and mtDNA showed very little subdivision among Greater Antillean *D. iulia*, but populations in Puerto Rico and the northern Lesser Antilles have been genetically separated for perhaps 300,000 years according to the Brower (1994b) mtDNA clock. This contrasts the pattern observed for *H. charithonia* in which recent colonization is indicated because of the close genetic relationship between populations on Montserrat and St. Kitts and those on Puerto Rico.

Heliconius charithonia and *D. iulia* both demonstrate a major biogeographic break in the Lesser Antilles, albeit at different points in the chain. For *H. charithonia*, this is the limit of its range, between Montserrat and Guadeloupe. For *D. iulia*, the disjunction occurs slightly further south, between Martinique and Dominica. Just as it is remarkable that *H. charithonia* is not found south of Montserrat, the level of sequence divergence (average of 2.87%) between populations of *D. iulia* in Martinique and Dominica is surprising, given the relatively narrow ocean gap. Nor is the genetic disjunction in *D. iulia* merely a chance sampling effect at a single locus, as evident through comparison of the mtDNA and allozyme data. Based on allele frequencies alone, one might think that *D. iulia* colonized the Lesser Antilles in a pincer movement from the Greater Antilles (north) and Trinidad (south). The phylogenetic analysis of mtDNA sequences, however, suggests otherwise: all West Indian *D. iulia* plausibly derive from a South American origin. The mtDNA tree clearly shows a pattern of nestedness that is consistent with a single, southern source of West Indian *D. iulia* (Fig. 7). Such a scenario is also quite plausible given the allozyme data. It remains possible that the mtDNA tree is not well rooted (the outgroup is *H. charithonia*), and that the species evolved in the Greater Antilles and spread to the Lesser Antilles and South America. Unfortunately, there are no congeneric species of *Dryas iulia* that might provide a more robust outgroup. A South American origin for the Antillean *D. iulia* population is also supported by the high mtDNA diversity characterizing the St. Vincent and Grenada populations (no two haplotypes were

alike), suggesting that expansion occurred from the mainland northwards into the islands.

Wherever *D. iulia* originated, it is clear from both the mtDNA PCR-RFLPs and the allozymes that there is little gene flow between Dominica, Martinique, St. Lucia, and St. Vincent. The levels of genetic divergence between populations of *D. iulia* on Dominica and Martinique are such that these might almost be different species. Further work is needed to determine whether the population structure has an entirely neutral explanation or results from additional reproductive and/or ecological factors. It is interesting to note that the divergence of *D. iulia* between Dominica and Martinique is of similar magnitude to that of *H. c. simulator* from the rest of *H. charithonia*. There is a possibility, therefore, that conditions just over a million years ago favored colonization, and that populations of *H. charithonia* and *D. iulia* became established in the islands at about the same time, though in different locations (perhaps reflecting their different continental distributions).

Given the differences that occur between two closely related butterflies, congruence among more distant taxonomic groups would seem unlikely. However, the patterns observed in *H. charithonia* and *D. iulia* are reminiscent of Caribbean birds, notably the bananaquit, *Coereba flaveola* (Seutin et al. 1994; Bermingham et al. 1996). The bananaquit is widely distributed in the neotropics and has several subspecies in the West Indies. As in *H. charithonia*, continental populations of bananaquit fall within the range of diversity found in the islands, and phylogenetic analysis revealed a highly divergent bananaquit lineage in Jamaica. In the northern Lesser Antilles, the similarities are with *D. iulia*. In the bananaquit, a single mtDNA haplotype dominates populations from St. Lucia north to St. Croix, and bananaquit mtDNA diversity decreases from the central Lesser Antilles northwards. Similarly, *D. iulia* has a single mtDNA haplotype occurring throughout St. Kitts, Montserrat, and Guadeloupe with a very similar but nevertheless distinct haplotype in Dominica to the south. These patterns suggest a range expansion in bananaquits and *D. iulia* that originated in the central Lesser Antilles and spread rapidly northwards. Populations of both bananaquits and *D. iulia* in the central Lesser Antilles are distinct from their neighbors to the south. In fact, there is a major genetic break or the end of a distribution between St. Lucia and St. Vincent in several bird species (Bermingham et al. 1996).

Even more surprising than concordance among winged taxa are the qualitatively similar relationships found among *D. iulia* and West Indian populations of *Anolis* lizards. Species of *Anolis*, for example, are closely related on what is known as the ''north Lesser Antillean platform'' (Roughgarden 1995), a group of islands matching the northern Lesser Antillean clade of *D. iulia*. The amount of divergence among the lizard species is much greater, reflecting a more ancient isolation than among the conspecific butterfly populations. Although the similarity in cladistic patterns could be a coincidence, another explanation is that similar vicariant events affected the groups at different points in history; thus, they are expressed at different taxonomic levels. Changes in sea level have expanded and contracted the northern Lesser Antilles several times over the last five million years, an example of sequential vicariance. This might lead to ''soft'' vicariance, in which isolation occurs in qualitatively the same pattern but the barriers to dispersal occur at different times or with different severity depending on the species. Such an explanation was invoked to explain similarities in branching pattern but differences in branch lengths among species of fish in the southeastern United States (Bermingham and Avise 1986).

In the West Indies, anoles may have speciated during one of the earlier periods of isolation while the qualitatively similar patterns in *D. iulia* reflect high gene flow among these insular populations during more recent periods of lower sea levels (an event that may not have affected the already reproductively isolated lizard species). The central Lesser Antilles are surrounded by deep channels and are unlikely to have been greatly affected by such changes, but St. Vincent and Grenada would have been almost linked when the Grenadine bank was exposed, explaining the homogeneity of the southern Lesser Antilles. The differentiation of birds, butterflies, and lizards in the central Lesser Antilles may well reflect their consistent isolation relative to the periodic landbridges that almost united the islands to their north and south.

As the phylogeographic database on West Indian taxa grows, comparative statistical analyses will test these preliminary hypotheses, revealing what, if any, common biogeographic patterns exist. Such information provides important insights into the evolutionary process. Already, the data from a few species of birds and butterflies demonstrate that the evolution of intraspecific variation is certainly a dynamic process with a large stochastic element. Neutral demographic processes seem capable of explaining the data presented in this study, but we do not rule out the influence of selection. Indeed, one of us has suggested that lags in the evolutionary responses of host and parasite populations might explain the intraspecific dynamics of West Indian birds (Ricklefs and Bermingham 1999). While this is a plausible and interesting hypothesis, we have no information on insect diseases. It is also possible that reproductive and/or ecological factors reinforced the differentiation that originated among partially isolated populations. Until such evidence is forthcoming, however, the most parsimonious explanation for the intraspecific biogeography of *H. charithonia* and *D. iulia* is a history of demographic serendipity constrained by geological events.

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APPENDIX I.
Allele frequencies for *Heliconius charithonia.*

Locus	Cuba, w	Cuba, e	Isle of Pines	Florida	Key Largo	Jamaica	Hispaniola	Bahamas	Puerto Rico	Mona	Montserrat	St. Kitts	*Peruviana*
GPI-1													
(*N*)	24	24	24	24	4	24	21	5	23	24	23	20	2
A	0.08	0.02	0.15	0.00	0.00	0.02	0.05	0.00	0.00	0.00	0.00	0.00	0.00
B	0.25	0.40	0.40	0.15	0.25	0.25	0.48	0.10	0.80	0.88	0.00	0.48	0.00
C	0.42	0.40	0.31	0.81	0.25	0.56	0.48	0.70	0.20	0.13	1.00	0.53	1.00
D	0.25	0.19	0.15	0.04	0.50	0.17	0.00	0.20	0.00	0.00	0.00	0.00	0.00
IDH-1													
(*N*)	24	24	24	24	5	24	21	5	24	24	24	20	2
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
B	0.06	0.04	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.94	0.96	1.00	1.00	1.00	0.81	1.00	1.00	1.00	1.00	1.00	1.00	0.25
IDH-2													
(*N*)	24	19	19	23	5	21	20	5	24	24	23	20	2
A	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.42	0.26	0.34	0.28	0.40	0.33	0.65	0.60	1.00	0.88	1.00	1.00	0.00
C	0.58	0.74	0.66	0.72	0.60	0.64	0.35	0.40	0.00	0.08	0.00	0.00	1.00
D	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00
PGM-1													
(*N*)	24	23	24	24	5	24	20	5	24	24	24	20	2
A	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
B	0.02	0.02	0.02	0.29	0.10	0.00	0.00	0.50	0.00	0.02	0.00	0.03	0.00
C	0.75	0.67	0.77	0.67	0.60	0.19	0.88	0.40	0.96	0.94	1.00	0.98	0.00
D	0.15	0.28	0.08	0.04	0.30	0.69	0.13	0.10	0.02	0.02	0.00	0.00	1.00
E	0.08	0.02	0.02	0.00	0.00	0.13	0.00	0.00	0.02	0.00	0.00	0.00	0.00
MDH-1													
(*N*)	24	23	24	24	5	24	21	5	24	24	24	20	2
A	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
G6PD-1													
(*N*)	24	15	18	22	5	18	21	5	23	23	22	20	2
A	0.04	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.96	1.00	1.00	0.59	0.40	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.00	0.00	0.00	0.41	0.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GOT-1													
(*N*)	24	24	24	24	5	24	19	5	24	24	21	20	2
A	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.96	1.00	0.98	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	0.75
C	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
D	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GOT-2													
(*N*)	24	24	24	23	5	24	21	5	24	24	23	20	2
A	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	1.00
B	1.00	1.00	1.00	1.00	1.00	0.98	0.98	1.00	1.00	1.00	1.00	1.00	0.00
C	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
ME-1													
(*N*)	24	24	24	24	5	24	21	5	24	24	24	20	2
A	0.00	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.38	0.58	0.42	0.44	0.80	0.06	0.19	0.30	0.21	0.10	0.08	0.05	0.25
C	0.60	0.35	0.54	0.56	0.20	0.46	0.76	0.70	0.79	0.88	0.85	0.95	0.75
D	0.00	0.00	0.00	0.00	0.00	0.48	0.05	0.00	0.00	0.02	0.06	0.00	0.00
E	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ME-2													
(*N*)	20	20	15	24	5	18	17	5	20	24	24	1	1
A	0.03	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.98	1.00	0.97	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
G3PD-1													
(*N*)	24	23	24	23	5	24	21	5	24	24	24	20	2
A	1.00	1.00	1.00	1.00	1.00	0.81	1.00	1.00	1.00	0.96	1.00	1.00	1.00
B	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.04	0.00	0.00	0.00
ACO-2													
(*N*)	24	24	24	24	5	24	21	5	24	24	24	20	2
A	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.98	0.98	0.92	1.00	1.00	0.94	0.98	1.00	1.00	1.00	1.00	1.00	1.00

APPENDIX I.
Allele frequencies for *Heliconius charithonia.*

Locus	Cuba, w	Cuba, e	Isle of Pines	Florida	Key Largo	Jamaica	Hispaniola	Bahamas	Puerto Rico	Mona	Montserrat	St. Kitts	*Peruviana*
C	0.02	0.02	0.02	0.00	0.00	0.06	0.02	0.00	0.00	0.00	0.00	0.00	0.00
GR-1													
(*N*)	23	24	24	24	5	24	21	5	24	24	24	1	1
A	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.98	1.00	1.00	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ADH-1													
(*N*)	23	22	23	22	5	22	19	4	21	21	24	18	2
A	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
B	0.91	0.98	0.85	0.59	0.50	0.98	0.97	0.75	1.00	1.00	1.00	1.00	1.00
C	0.09	0.02	0.15	0.41	0.50	0.02	0.00	0.25	0.00	0.00	0.00	0.00	0.00
MPI-1													
(*N*)	24	23	23	24	5	22	21	5	22	23	23	20	1
A	0.02	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
B	0.65	0.72	0.85	0.04	0.00	0.75	0.91	0.90	0.39	0.33	0.09	0.00	0.00
C	0.29	0.24	0.13	0.96	1.00	0.25	0.05	0.10	0.00	0.04	0.04	0.10	0.00
D	0.04	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.61	0.63	0.87	0.90	0.00
6PGD-1													
(*N*)	19	16	17	21	5	11	18	5	19	16	15	12	2
A	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	1.00	1.00	0.94	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.00	0.00	0.03	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LA-1													
(*N*)	24	24	24	24	5	24	21	5	24	24	24	20	2
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
B	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.98	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
LA-2													
(*N*)	18	16	18	14	5	18	16	5	17	18	12	16	2
A	0.06	0.06	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
B	0.94	0.94	0.92	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75
LA-3													
(*N*)	24	24	24	24	5	24	21	4	24	24	24	20	2
A	1.00	0.98	0.98	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	0.25
B	0.00	0.02	0.02	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.75

APPENDIX II.
9Allele frequencies for *Dryas iulia*.

Locus	Florida	Bahamas	Cuba, w	Cuba, e	Isle of Pines	Hispaniola	Puerto Rico	Jamaica	St. Kitts	Montserrat	Guadeloupe	Dominica	Martinique	St. Lucia	St. Vincent	Grenada	Trinidad	Panama
GOT-1																		
(*N*)	1	20	17	12	20	14	18	20	19	22	6	24	11	16	9	6	7	19
A	1.00	1.00	1.00	0.96	0.98	0.96	0.97	0.95	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.00	0.00	0.05
B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
C	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.05	1.00	1.00	1.00	1.00	0.05	0.09	1.00	1.00	1.00	0.95
D	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.88	0.00	0.00	0.00	0.00
GOT-2																		
(*N*)	1	20	17	12	20	14	18	20	19	22	6	24	11	16	9	6	7	20
A	0.00	0.00	0.00	0.08	0.00	0.04	0.03	0.00	0.00	0.00	0.00	0.00	1.00	0.38	0.67	0.00	0.07	0.08
B	1.00	0.93	0.97	0.75	0.98	0.96	0.97	1.00	1.00	1.00	1.00	1.00	0.00	0.63	0.33	0.92	0.86	0.75
C	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
D	0.00	0.00	0.03	0.17	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.08
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.10
PGM-1																		
(*N*)	1	20	17	12	20	14	18	20	19	22	6	24	11	16	9	6	7	19
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.03
B	0.00	0.00	0.18	0.08	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	1.00	0.83	0.64	0.47
C	1.00	0.98	0.77	0.92	0.93	0.89	0.97	0.03	1.00	0.96	1.00	1.00	0.96	1.00	0.00	0.00	0.36	0.50
D	0.00	0.00	0.03	0.00	0.05	0.11	0.03	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HBDH-1																		
(*N*)	1	20	17	12	20	14	18	20	19	22	6	24	11	16	9	6	7	5
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00
B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.84	1.00	1.00	1.00	0.90
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.10
LA-1																		
(*N*)	1	20	15	12	16	14	17	17	19	22	4	24	11	16	9	6	7	17
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
B	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.07	0.00	0.02	1.00	0.97	0.89	1.00	0.93	0.77
						0.14												
C	0.00	0.00	0.07	0.00	0.09		0.03	0.06	0.87	0.93	1.00	0.98	0.00	0.03	0.11	0.00	0.07	0.18
D	1.00	1.00	0.93	1.00	0.88	0.79	0.97	0.94	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LA-3																		
(*N*)	1	20	10	12	17	11	18	16	19	22	6	22	11	16	9	6	7	17
A	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	0.50	0.98	0.85	0.75	0.85	0.91	1.00	0.97	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.50	0.03	0.15	0.25	0.15	0.00	0.00	0.03	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
ADH-1																		
(*N*)	1	20	17	12	19	12	16	19	13	21	6	24	8	12	3	6	1	10
A	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	1.00	1.00	0.94	0.96	0.92	1.00	1.00	0.92	1.00	1.00	1.00	1.00	0.00	0.00	0.00	0.08	0.50	0.45
C	0.00	0.00	0.03	0.00	0.03	0.00	0.00	0.05	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.92	0.50	0.50
D	0.00	0.00	0.03	0.04	0.03	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
GPI-1																		
(*N*)	1	20	16	12	20	14	18	18	19	22	6	24	11	16	9	6	7	18
A	0.00	0.15	0.00	0.00	0.00	0.07	0.14	0.06	0.08	0.00	0.00	0.00	0.23	0.03	0.00	0.00	0.20	0.08
B	1.00	0.85	0.94	1.00	0.88	0.93	0.86	0.89	0.90	1.00	1.00	1.00	0.77	0.63	0.89	1.00	0.36	0.47
C	0.00	0.00	0.03	0.00	0.08	0.00	0.00	0.06	0.03	0.00	0.00	0.00	0.00	0.34	0.11	0.00	0.43	0.39
D	0.00	0.00	0.03	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03

APPENDIX II.
Continued

Locus	Florida	Bahamas	Cuba, w	Cuba, e	Isle of Pines	Hispaniola	Puerto Rico	Jamaica	St. Kitts	Montserrat	Guadeloupe	Dominica	Martinique	St. Lucia	St. Vincent	Grenada	Trinidad	Panama
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
G6PD-1																		
(*N*)	1	20	16	12	17	14	17	20	19	22	6	24	11	16	9	6	7	17
A	0.00	0.00	0.00	0.00	0.03	0.07	0.03	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24
B	1.00	1.00	0.97	1.00	0.97	0.93	0.97	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.65
C	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.12
GR-1																		
(*N*)	1	19	17	11	20	14	17	20	19	22	6	24	11	16	9	6	7	20
A	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
B	1.00	1.00	1.00	0.96	0.98	0.96	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95
C	0.00	0.00	0.00	0.05	0.00	0.04	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
GAPDH-1																		
(*N*)	1	20	17	12	20	14	18	20	19	22	6	24	11	16	9	6	7	6
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.86	1.00
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00
G3PD-1																		
(*N*)	1	17	15	12	16	14	14	20	19	22	6	24	11	16	9	6	5	12
A	0.00	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
B	1.00	1.00	1.00	0.92	1.00	0.96	0.96	0.98	1.00	1.00	1.00	1.00	0.05	0.00	1.00	1.00	1.00	0.96
C	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.03	0.00	0.00	0.00	0.00	0.96	1.00	0.00	0.00	0.00	0.00
IDH-1																		
(*N*)	1	20	16	12	20	14	18	18	19	22	6	24	11	16	9	6	7	18
A	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
IDH-2																		
(*N*)	1	20	16	12	20	14	18	18	19	21	6	24	11	16	9	6	7	16
A	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
B	0.00	0.00	0.03	0.00	0.00	0.04	0.11	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C	0.50	0.28	0.31	0.13	0.33	0.96	0.89	0.06	0.97	1.00	1.00	0.98	0.00	0.00	0.00	0.00	0.00	0.13
D	0.50	0.73	0.66	0.88	0.68	0.00	0.00	0.92	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.92	1.00	0.88
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00
AK-1																		
(*N*)	1	19	16	12	19	14	18	18	19	22	6	24	11	16	9	6	7	17
A	0.00	0.00	0.00	0.00	0.00	0.07	0.06	0.03	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	1.00	1.00	1.00	1.00	1.00	0.93	0.94	0.97	0.87	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ENO-1																		
(*N*)	1	20	16	12	20	14	18	18	19	22	6	24	11	16	9	6	7	18
A	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	1.00	1.00	1.00	0.96	0.93	1.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
C	0.00	0.00	0.00	0.04	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SDH-1																		
(*N*)	1	20	12	8	14	9	18	13	19	22	6	22	11	16	9	4	7	14
A	0.00	0.05	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
B	1.00	0.95	0.96	1.00	0.96	0.89	1.00	1.00	1.00	0.98	1.00	1.00	0.00	0.00	0.00	0.88	0.14	0.68
C	0.00	0.00	0.04	0.00	0.04	0.06	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.13	0.86	0.29