Development and Characterization of Microsatellite Loci for Two Caribbean Heliconia (Heliconiaceae: H. bihai and H. caribaea)¹

Vinata Gowda²,³,⁴, David L. Erickson³, and W. John Kress³

¹Department of Biological Sciences, The George Washington University, 2023 G Street, NW, Washington D.C. 20052 USA; and ²Department of Botany, MRC 166, PO Box 37012 Smithsonian Institution, Washington D.C. 20013-7012 USA

• Premise of the study: Microsatellite loci were developed to characterize genetic variation and population subdivision in Heliconia bihai and H. caribaea from the Caribbean Islands.

• Methods and Results: A total of 13 new microsatellite markers were developed and characterized in the two Caribbean heliconias. Di-, tri-, and tetranucleotide repeats were identified with one to 17 alleles per locus, and the observed heterozygosity ranged from 0.13 to 0.87. Additionally, cross-species amplification was successful in eight out of 13 loci.

• Conclusions: The microsatellite loci developed have discriminatory potential to be used in genetic characterizations of Caribbean Heliconia. Both H. bihai and H. caribaea are known to have adaptive interactions with their hummingbird pollinators, and the characterized microsatellite markers will be used to study mating system, genetic structure, and phylogeographic patterns in Caribbean Heliconia.

Key words: Caribbean; coevolution; Heliconia bihai; Heliconia caribaea; Heliconiaceae; microsatellites.

Heliconia L. (lobster claw plant, Heliconiaceae; one genus/~200 spp.) are predominantly neotropical, herbaceous plants known for their adaptive interactions with hummingbirds (Stiles, 1975; Berry and Kress, 1991). Only two native species of heliconias occur on the islands of the eastern Caribbean: H. bihai (L.) L. and H. caribaea Lam. The two native heliconias are a primary and predictable source of nectar for hummingbirds, especially the Purple-throated Carib (Eulampis jugularis) (Temeles and Kress, 2003). Male Purple-throated Caribs, depending on the island, defend feeding and/or breeding territories of either H. caribaea or H. bihai, or both, whereas female Purple-throated Caribs trapline-forage at undefended plants or rarely defend feeding territories (Temeles and Kress, 2003). This contrast in feeding patterns between the two genders of Purple-throated Caribs and the two heliconias presents a unique opportunity to investigate the role of pollinator foraging patterns in shaping the genetic population structures of plants.

To date, the only study of mating system in Caribbean Heliconia has used amplified fragment length polymorphisms (AFLPs), which in turn recognized the need for highly variable, codominant markers such as microsatellites (Meléndez-Ackerman et al., 2005). Here, we characterize 13 new microsatellite loci developed for two species of Caribbean Heliconia (H. bihai and H. caribaea), assess the variability of the markers, and test their cross-species amplification success.

METHODS AND RESULTS

A genomic library was constructed from a single H. bihai (voucher: W.J.K. 03-6835, Appendix 1) and H. caribaea (voucher: W.J.K. 02-7142, Appendix 1) individual from the island of Dominica. Silica-dried tissues were used for construction, enrichment, and screening of microsatellite libraries by Genetic Identification Services (Chatsworth, California, USA, http://www.genetic-id-services.com/) using a magnetic bead–based enrichment procedure (Jones et al., 2002) from approximately 100 ng of genomic DNA. Four repeat motifs were targeted for library enrichment: in H. bihai (CA)n, (AACC)n, (ATG)n, and (CAG)n; in H. caribaea (CA)n, (AACC)n, (AGG)n, and (ATG)n. A total of 100 microsatellite loci were identified in 100 cloned sequences, and primers were designed for 35 H. bihai and 35 H. caribaea candidate loci using Primer3 version 0.4.0 (Rozen and Skaltsky, 2000). Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, Iowa, USA).

To screen for variable microsatellite markers, genomic DNA was extracted from 30 individuals (15 per population) representing two different populations of H. bihai and H. caribaea from the Caribbean island of Dominica (Appendix 1). Genomic DNA was isolated from mature silica-dried leaves or frozen leaf tissue using an Autogen (Holliston, Massachusetts, USA) DNA extraction robot. PCR conditions were then optimized for all 70 candidate microsatellite loci. PCR amplification of primer pairs was performed with an MJ Research thermal cycler (Waltham, Massachusetts, USA). The 20 μL PCR reaction mixture contained: 2 μL 10x buffer (Bioline, Taunton, Massachusetts, USA), 1 μL of 50 mM MgCl2, 1 μL of 5 μg of template DNA. PCR amplification was carried out for 30 cycles with an initial denaturing for 2 min at 95°C followed by 30 s at 93°C, 40 s of annealing at the optimized annealing temperature (Table 1), ending with 30 s of elongation at 72°C. PCR products were sized and screened for variable loci using the Agilent 2100 Bioanalyzer and DNA 500 LabChip kit (Agilent Technologies, Santa Clara, California, USA). From the 70 candidate primer pairs, a final set of 20 microsatellite loci per species were identified based on their PCR success. The final sets of variable loci were end-labeled at the 5’ end of the reverse primer with one fluorescent phosphoramidite (6-FAM or HEX, Table 1) for polymorphism detection.
Size homoplasy of a targeted locus was checked by identifying at least two homozygous individuals for at least three alleles and by sequencing the heterozygosity; $H_o = unbiased expected heterozygosity; N = sample size within each species; $N_e = number of alleles.

Note: + = successful cross-species amplification; – = failed cross-species amplification; $T_a = annealing temperature for amplification.

Microsatellite loci were genotyped for a total of 30 diploid individuals per species, sampled from two different populations on Dominica. Protocols for genomic DNA extraction, PCR amplification of microsatellites, and cycling program were as described above. The 20 μL PCR reaction mixture with labeled primers contained: 2 μL of 10× buffer (Bioline), 1 μL of 50 mM MgCl2, 0.75 μL of 5 μM unlabeled primer, 0.5 μL of 5 μM of labeled primer, 1 μL of 10 mM dNTPs, 0.02 μL Taq DNA polymerase (Bioline), and 30–40 ng/μL of template DNA. Genotyping was done on the ABI 3730xl DNA analyzer in 12 μL reaction volumes as follows: 1 μL ROX-labeled size standard (De Woody et al., 2004), 1 μL of 1:10 diluted PCR product, and 10 μL Hi-Di Formamide (Applied Biosystems). Fragments were sized using GENEMAPPER software version 4 (Applied Biosystems). Cross-species amplification of all the primers was also conducted between the two heliconias following the same protocol described above.

Results from the analyses of the microsatellite loci developed and tested for $H. biihai$ and $H. caribaea$ are shown in Tables 2 and 3. GenAlEx version 6.1 (Peakall and Smouse, 2006) was used to calculate observed ($H_o$) and expected heterozygosities ($H_e$) while linkage disequilibrium (LD) between loci was calculated in GENEPOP version 4.07 (Raymond and Rousset, 1995).
No evidence of LD was detected in most locus-pair comparisons except between the loci Hc_B203 and Hc_B206 (P < 0.05). Most loci conformed to Hardy–Weinberg equilibrium (HWE), except for Hb_C115, Hc_C7, and Hc_D6, which showed significant excess of homozygotes (P < 0.05). Hb_B110 was found to be monomorphic while the remaining homozygote-excess markers were due to the presence of a high number of private alleles. The observed deviation from HWE is not surprising given that both Heliconia species have a self-compatible breeding system (Gowda and Kress, unpublished). Size homoplasy was not observed in the 13 loci presented here. Eight out of 13 loci also showed successful cross-species amplification.

CONCLUSIONS

Of the 13 microsatellites reported here for the Caribbean heliconias, 12 were found to be sufficiently polymorphic for phylogeographic studies across the species’ distributional range within the Caribbean Islands. The microsatellite markers identified here in both species of Heliconia will also be useful in future studies focused on rates of self-pollination, paternity analysis, and intra- and interisland gene flow patterns.

LITERATURE CITED


APPENDIX 1. Geographic localities and herbarium voucher information for Heliconia bhai and H. caribaea populations used in this study. All voucher specimens are deposited at the US National Herbarium (US), Washington, D.C., USA. Information presented: herbarium voucher accession code, locality (latitude, longitude).

Heliconia bhai: W.J.K. 01-6835, Syndicate (15°30'53"N, 61°25'05"W); W.J.K. 01-6832, Fresh Water Lake (15°20'38"N, 61°18'37"W); W.J.K. 01-6840, Salisbury (15°27'30"N, 61°24'41"W).

Heliconia caribaea: W.J.K. 02-7142, Laudat (15°20'22"N, 61°19'58"W); W.J.K. 01-6843, Central Forest (15°27'N, 61°17"W); W.J.K. 02-7136, Layou (15°23'01"N, 61°23'32"W).