Tracing an Invasion: Phylogeography of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States Based on Mitochondrial DNA

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Ann. Entomol. Soc. Am. 101(5): 899–905 (2008)

ABSTRACT The adventive cactus moth *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), a widely used biological control agent for *Opuntia* Mill. cacti, was detected in Florida in 1989. Since then, it has spread along the Atlantic and Gulf Coasts of southeastern United States, threatening native *Opuntia* populations. We examined the phylogeography of 20 *C. cactorum* populations from Australia, South Africa, Hawaii, the Caribbean, Mexico, and the southeastern United States based on 769 bp of *cytochrome oxidase subunit 1*. Five distinct haplotypes were discovered, three of which occur in the United States. *Cactoblastis cactorum* in the United States falls into two distinct lineages: a western haplotype along the Gulf Coast and an eastern lineage with two haplotypes along the Atlantic Coast, with one of the eastern haplotypes identified as occurring at a single locality on the Gulf Coast. The two lineages have nontrivial genetic divergence (0.5%), and both are more closely related to populations outside the United States than they are to each other. This leads us to conclude that *C. cactorum* has been introduced to the United States at least twice. The isolated eastern haplotype on the Gulf Coast may indicate that *C. cactorum* has been introduced a third time, either from the Atlantic Coast or from outside the United States. Evidence from analysis of haplotypes and other information indicates that dispersal by commercial import action and human transport may be more important than flight ranges of ovipositing females for determining long range expansion of the species. Interestingly, the east–west pattern mirrors other coastal species distributions that have been interpreted as being due to Pleistocene vicariance.

KEY WORDS *Cactoblastis cactorum*, phylogeography, invasive species, introduction, dispersal

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae), is well known as a major success story for the biological control of invasive, alien plant species. The moth is native to southeastern South America where it feeds on a wide range of species of prickly pear cacti in the genus *Opuntia* (Heinrich 1939 1956, Mann 1969). It was introduced from Argentina to Australia in 1926, from Australia to South Africa in 1933, and from Australia to Hawaii in 1950, where it quickly became a very effective control agent against introduced *Opuntia* (Mann 1969, Moran and Zimmermann 1984, Zimmermann et al. 2000, Walton 2005). The introduction to Australia was especially successful, with an area larger than Great Britain being reclaimed from *Opuntia* infestations in <15 yr (Dodd 1940, Walton 2005). After these initial successes, *C. cactorum* was introduced in 1957 from South Africa to the Caribbean island of Nevis from where it dispersed naturally or was purposefully introduced to other Caribbean islands to control various *Opuntia* species that had become weeds (Pemberton 1995, Mahr 2001, Zimmermann et al. 2004, Pemberton and Liu 2007). This introduction to the Caribbean was controversial because an exotic species was introduced to control native species that were considered weeds (Moran and Zimmermann 1984, Pemberton 1995, Zimmermann et al. 2000, Mahr 2001, Pemberton and Liu 2007). The first record of *C. cactorum* for the American mainland reportedly was a single female collected in October 1989 by a lepidopterist, Terhune Dickel, on Bahia Honda Key, Florida (Habeck and Bennett 1990, Dickel 1991). Other authors, such as Simberloff (1992) and Stiling (2002), have attributed the first discovery of the species in the United States to Carol Lippincott on Big Pine Key at the same time as Dickel's collection. However, Lippincott detected *C. cactorum* after she found larvae in cactus on Little Pine Key in April 1990 and brought them to be reared by Dickel, who made the identification of the emergent adults. Dickel reported the discovery of this species to Dale Habeck in June 1990. Since this first detection, the cactus moth has spread quickly along both coasts of the southeast United States and is now found as far north as Charleston County, SC, and as far west as Petit Bois Island, MS,

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the latter being detected in January 2008 (USDA–APHIS 2008).

The occurrence of *C. cactorum* in the southeastern United States poses a serious problem not only because the moth threatens local *Opuntia* species, of which one is formally recognized as endangered (Zimmermann et al. 2000, Hight et al. 2002), but more so because the *Opuntia* flora along the U.S. coast of the Gulf of Mexico could provide a corridor for dispersal to the western United States and northwestern and central Mexico. This could result in severe ecological and socioeconomic consequences (Zimmermann et al. 2000, Mahr 2001, Hight et al. 2002, Solis et al. 2004), e.g., see Pemberton and Liu (2007) for an assessment of possible consequences based on the effect of *C. cactorum* on cacti on the islands Nevis and St. Kitts. In 2006, *C. cactorum* was discovered on the Mexican island Isla Mujeres of the mainland of Quintana Roo (NAPPO 2006).

The presence of *C. cactorum* in the southeastern United States raises some important questions: 1) Where did the moth originally enter the United States? 2) Was it introduced by humans or did it spread by natural dispersal? 3) Has it been introduced several times or just once? and 4) Is its rapid spread in the southeastern United States partially aided by humans?

Materials and Methods

Data Sampling. Most samples of *C. cactorum* were collected as larvae from infested *Opuntia* cacti. Two samples were made up of adults collected in 15-W blacklight traps. Regardless of collecting method, specimens were stored directly in ethanol to preserve genetic material. The ethanol concentration used for shipping and storage was generally 95–99.9%. However, in the case of the Australian specimens, such alcohol was unavailable and the specimens were preserved in vodka. Upon receipt of specimens, they were transferred to 99.9% ethanol.

In all, 80 specimens from 20 localities were included in the analysis. DNA voucher specimens are deposited in the E. H. Strickland Entomological Museum, University of Alberta, Edmonton, AB, Canada, and additional specimens from each series are deposited in the Mississippi Entomological Museum. We attempted to sample as many populations in the United States and the Caribbean as possible to obtain the full extent of variation in *C. cactorum* in this region. However, we were unable to get material from several Caribbean islands or the native South American range due to recently increased restrictions in international airline transport of dangerous goods (in this case, vials with alcohol-preserved specimens). Four species [*Zophodia grosulariella* (Hübner), *Melitara dentata* (Grote), *Alberada parabates* (Dyar), and *Cahela ponderosella* (Barnes and McDunnough)] from the group of cactus-feeding phycitines to which *C. cactorum* belongs (Heinrich 1939, Neunzig 1997, Simonsen 2008) were used to root the parsimony/neighbor joining analyses. A full list of *C. cactorum* specimens and localities included in the study is given in Table 1. The distribution and sampling sites of *C. cactorum* in southeastern United States are shown in Fig. 1.

Gene Sampling. DNA was extracted from body wall tissue (larvae), thorax musculature, or legs (adults) with the DNEasy extraction kit (QIAGEN Sciences, Germantown, MD). Using the gene *cytochrome oxidase subunit 1* (COI), 769 bp was sequenced for all specimens. The primer pair used was Jerry-Pat II (Simon et al. 1994). The polymerase chain reaction (PCR) cycling profile comprised an initial heating period of 94.0°C for 2 min follow by 35 cycles of 94.0°C for 30 s, 45.0°C for 30 s, and 72.0°C for 2 min. PCR products were purified using the PCR purification kit (QIAGEN), and the PCR primers also were used for direct sequencing. Sequencing was done with an AB Hitachi 3730 DNA Analyzer capillary sequencer using Big Dye. Consensus sequences from the two sequencing directions were constructed using Sequencer 4.1 and aligned by eye.

Phylogeographic Analyses. Basic parsimony and neighbor-joining trees were obtained in PAUP* 4.10b (Swofford 2002) by using default settings. Haplotype relationships were analyzed in MacClade 4.0 (Maddison and Maddison 2000). Automated nested clade analysis (Templeton 1998, 2004; Templeton et al. 1995) followed Panchal (2007). Label data were used to establish locality coordinates for the nested clade analysis. Global positioning system (GPS) coordinates printed on the labels were used directly. When no coordinates were present, the label data were used to establish GPS coordinates by using Google Earth 4.1 (Google 2007). This was generally unproblematic as most labels had elaborate locality descriptions. The South African stock was originally collected in the Karoo near Graaff Reinet, Nieu Bethesda, Cradock, and other locations, and combined into a single South African colony at the USDA laboratory in Tifton, GA (J. E. Carpenter, personal communication). We arbitrarily used the GPS coordinates for Cradock for the composite material from South Africa. These specimens are the only ones included from the African continent. One sample, from Puerto Rico was simply labeled "Culebra and Guanica." For specimens from this sample, we used the coordinates for the offshore island of Culebra.

The nested clade analysis was not performed on the full 769-bp data set, but only on the first 731 bp for each specimen because the last 38 bp in some specimens contained uncertain scorings such as ambiguous bases. These had no effect on parsimony-based analyses, but they were removed because they gave the appearance of greater heterogeneity in the nested clade analysis.

Results

Five different haplotypes of *C. cactorum* were identified (Table 1; Fig. 2), displaying a clear geographical pattern. Haplotype 1 was found only in a single South African specimen. Haplotype 2 ($n = 22$) was found in specimens from Hawaii, Puerto Rico mainland, South

Table 1. Distributions and haplotypes of the specimens included in the study

Country	Locality	GPS	Specimens
South Africa	Cradock/Graaff Reinet	*32.16953S 25.61682E	cc-043 (HP5), cc-044 (HP1), cc-135 (HP2), cc-136 (HP4), cc-137 (HP5)
Australia	Queensland, Bundaberg, Inns Park	*24.850000S 152.333333E	cc-049, cc-050, cc-122, to cc-124 (all HP4)
Dominican Rep.	Peravia, Bani	*18.280556N 70.331111W	cc-045, cc-046, cc-139 (all HP5)
Mexico	Quintana Roo, Isla Mujeres	21.204241N 86.713476W	cc-104, cc-106, to cc-113 (all HP5)
Puerto Rico	Guanica, Bosque Estatal de Guanica	*17.964722N 66.846389W	cc-060, cc-061, cc-119 to cc-121 (all HP2)
Puerto Rico	Guanica Dry Forest	17.964722N 66.846389W	cc-055, to cc-057, cc-128, to cc-130 (all HP2)
Puerto Rico	Culebra & Guanica	*18.303056N 65.300556W	cc-064, cc-065, cc-125, to cc-127 (all HP3)
USA	Hawaii, Hawaii Isl.	19.468052N 155.905888W	cc-079, cc-080 (both HP2)
USA	Alabama, Mobile Co., Little Dauphin Island	30.272324N 88.119764W	cc-097 (HP5), cc-051, cc-052, cc-070 (HP5)
USA	Alabama, Baldwin Co., Bon Secour NWR, Fort Morgan Unit	30.226667N 88.026389W	cc-051, cc-052, cc-070 (all HP5)
USA	Florida, Lee Co., Sanibel Island, J. N. Darling NWR	*26.454722N 82.129444W	cc-081, to cc-083 (HP5)
USA	Florida, Highlands Co., Sebring	27.466668N 81.449445W	cc-084 to cc-088, cc-100 to cc-103 (all HP5)
USA	Florida, Sarasota Co., Nokomis Beach	27.121242N 82.469591W	cc-075, cc-090, cc-091, cc-093 (all HP5)
USA	Florida, Okaloosa Co., Okaloosa Island	*30.397778N 86.597222W	cc-047, cc-048, cc-053, cc-054, cc-116, cc-118, cc-131, cc-133 (all HP5)
USA	Florida, Tallahasse	*30.450833N 84.308333W	cc-066 (HP5)
USA	Florida, Santa Rosa Co., Pensacola Beach	30.491389N 87.092778W	cc-062, cc-063 (HP2)
USA	Florida, Indian River Co., Pine Island	*27.669444N 27.669444W	cc-068 (HP3)
USA	Florida, Brevard Co., Archie Carr NWR	*27.955278N 80.506667W	cc-073 (HP2)
USA	Georgia, Glynn Co., Jekyll Island	*31.068056N 81.413611W	cc-057, cc-059, cc-077 (HP2), cc-076, cc-115 (HP3)
USA	Georgia, Chatham Co., Cockspur Island	32.030278N 80.898611W	cc-088 (HP2)
USA	South Carolina, Charleston Co., Isle of Palms	32.799444N 79.762500W	cc-024 (HP2)
USA	South Carolina, Charleston Co., Cape Romain NWR	32.927500N 79.573889W	cc-074 (HP2)

An asterisk (*) in front of the GPS coordinates indicate that the coordinates were found using Google Earth as described in the text.

Africa, the U.S. Atlantic Coast, and, notably, in specimens from Pensacola Beach on the Florida Gulf Coast. Haplotype 3 ($n = 8$) was found in specimens from the U.S. Atlantic Coast and Puerto Rico. Haplotype 4 ($n = 6$) was found in specimens from Australia and South Africa. Haplotype 5 ($n = 43$) was found in all Mexican specimens, all specimens from the Dominican Republic, the remaining specimens from the U.S. Gulf Coast, interior Florida, and two South African specimens. The largest difference between two haplotypes (HP 2 and HP 5) was four base pairs, roughly corresponding to 0.5% divergence.

The nested clade analysis (Fig. 2) grouped haplotypes 1 and 2 together in a one-step clade. This clade was then grouped with haplotype 3 in a two-step clade. Neither clade was statistically significant at the $P < 0.05$ level. Haplotype 4 and 5 were grouped together in a statistically significant two-step clade.

One sequence of each haplotype has been deposited in GenBank (accession no. EU670266–EU670345).

Discussion

General Pattern. *C. cactorum* in the southeastern United States displays a very clear east–west distributional pattern of haplotypes (Figs. 1 and 2). The haplotypes shared by all western samples (HP 5), apart from the specimens from Pensacola Beach, also was present in all specimens from Mexico, the Dominican Republic, and some specimens from South Africa. The HP 5 haplotype clustered with HP4, which was found in all Australian specimens and one specimen from South Africa. This statistically significant clade demonstrated that specimens from western Florida and Alabama are more closely related to populations outside the United States than to populations from eastern Florida, Georgia, and South Carolina (Fig. 2). The latter populations (HP 2 and 3) are less homogeneous, but still closely related. These populations cluster together with the western specimens from Pensacola Beach, all specimens from Hawaii and Puerto Rico, and two from South Africa, one of which has its own haplotype (HP 1). Although this clade is not statistically significant, it supports an east–west distribution pattern in the United States. Apart from the heterogeneous South African sample that had four haplotypes (perhaps relating to specimens originally collected at several localities), different haplotypes were found only in one locality, Jekyll Island, where both HP 2 and HP 3 were present. Eastern and western haplotypes were never found in the same localities in the United States.

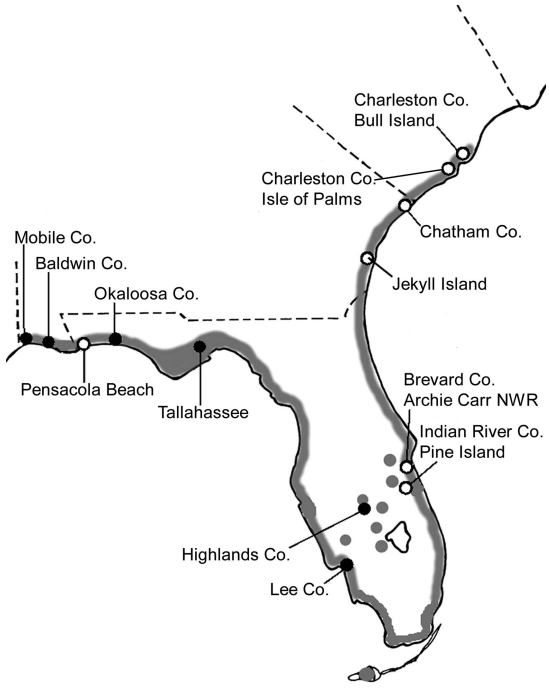

Fig. 1. Approximate distribution of *C. cactorum* in southeastern United States marked in gray (after Hight et al. 2002, Solis et al. (2004), and www.aphis.usda.gov/plant_health/plant_pest_info/cactoblastis) with sample sites for analyzed specimens. A closed circle represents the western haplotype (HP 5) whereas an open circle represents one of the eastern haplotypes (HP 2 and 3).

Comparable Studies on Invasive Species Based on Mitochondrial DNA. The study by Slade and Moritz (1998) on the marine toad (*Bufo marinus* L.), a species native to eastern South America, is similar to our study. *Bufo marinus* was introduced to Puerto Rico before 1844, to Hawaii in the 1920s, and Australia in the 1930s, and it has become a severe pest in all these areas (Slade and Moritz 1998). Based on 468 bp of the mitochondrial gene ND5, Slade and Moritz (1998) identified 14 haplotypes in the native range, but only one in the introduced areas.

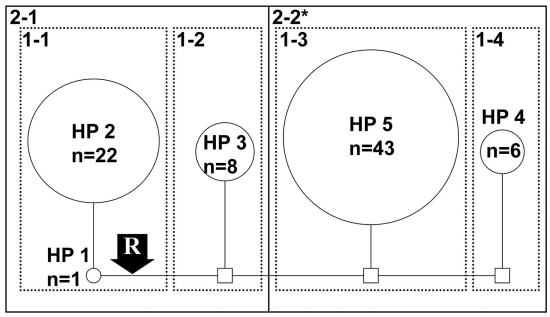

Fig. 2. Nested clade analysis of the five haplotypes. Clade 2-1 is the nonsignificant "eastern" clade and clade 2-2 the significant "western" clade. The R-arrow marks the root.

Scheffer and Grissell (2003) investigated the seed feeding wasp *Megastigmus transvaalensis* (Hussey) based on 800 bp of COI. *Megastigmus transvaalensis* is widespread in the tropics and subtropics where it feeds on *Rhus* L. in Africa and *Schinus* L. in South America, both widespread ornamental plants. Because *Rhus* was introduced to South America and *Schinus* to Africa, it was not clear where *M. transvaalensis* originated (Scheffer and Grissell 2003). These authors concluded that *M. transvaalensis* originated in Africa because they found 24 haplotypes in Africa, but only one in the rest of the world. They further concluded that *M. transvaalensis* was probably introduced from Kenya to Mauritius, where *Rhus* species are grown commercially, and then from Mauritius to North and South America and Hawaii on infested plants.

Laffin et al. (2005a,b) studied two weevil species, *Ceutorhynchus neglectus* Blatchley and *C. obstrictus* (Marsham), based on fragments of COI and the nuclear gene ITS1. *C. neglectus* is native to northern and western North America, whereas *C. obstrictus* is native to Europe and was introduced into North America where it is a pest of Brassicaceae (Laffin et al. 2005a). Surprisingly, they found higher variation in the introduced *C. obstrictus* compared with the native *C. neglectus.* They suggested that the low variation in *C. neglectus* might be due to a recent range expansion, whereas *C. obstrictus* probably was introduced more than once to North America because there seemed to be little gene flow between eastern and western populations.

Introductions of *C. cactorum* into United States. The single introduction of *B. maritimus* and the supposedly single introduction of *M. transvaalensis* resulted in far less genetic divergence than we have found in *C. cactorum* in the southeastern United States. The higher variation found by Laffin et al. (2005a) in the introduced *C. obstrictus* led to the conclusion that the species was introduced more than once. Similarly, we propose that *C. cactorum* probably has been introduced to United States at least twice if not three times. One introduction led to the eastern lineage, and an independent introduction led to the western lineage along the Gulf Coast. The exceptional sample of an eastern haplotype on the Gulf Coast at Pensacola Beach indicates either human assisted dispersal from the Atlantic Coast or an independent introduction from outside the United States. Infested nursery stock were intercepted at a national retail outlet store in Pensacola in 2000 (Floyd 2005), and it is possible that other infested cacti entered undetected before its initial detection at Pensacola Beach in 2003 (Hight et al. 2003). The similarity of haplotypes between the western group and specimens from the Dominican Republic indicates that this introduction could have come from infested *Opuntia* from Dominican cactus nurseries (Pemberton 1995, Zimmermann et al. 2000). This is supported by the fact that nurseries in both Florida and the Dominican Republic have had problems with *C. cactorum* at least since the early 1990s (Pemberton 1995). The higher genetic divergence in the eastern lineage (HP 2 and 3) indicates that either

more than one haplotype was present within a single introduction, e.g., infested cacti with egg sticks or larvae from two different females, or that two independent introductions occurred into the Atlantic Coast. Both scenarios suggest that the eastern lineage also was introduced by humans. Although this haplotype is present in Puerto Rico, its presence in the Dominican Republic cannot be discounted. During 1981–1993, *C. cactorum* was intercepted in 17 shipments of *Opuntia* cacti from Dominican Republic and Haiti by the USDA–APHIS–PPQ inspection station in Miami, even though only ≈2% of each shipment was examined (Pemberton 1995). More than 350,000 cacti plants were imported into Miami from Dominican Republic in 1986 alone (Pemberton 1995). These data demonstrate how easily the moth could have been introduced to United States. With this in mind, it is probably more relevant to ask whether *C. cactorum* may have been introduced much earlier than its first detection in 1989.

Dispersal of *C. cactorum* in Southeastern United States. After the initial detection of *C. cactorum* on Bahia Honda Key in 1989, surveys by Florida Division of Plant Industry inspectors and other collectors during 1990–1991 documented the presence of this species throughout the Florida Keys and the southern half of the Florida peninsula as far north as Brevard Co. on the east coast and Terra Ceia, Manatee Co., on the west coast (Dickel 1991; voucher specimens in McGuire Center for Lepidoptera). These data indicate that the species was widespread in southern Florida by 1990, in contrast to the suggested dispersal of 257 km/yr from 1989 to 1991 by Johnson and Stiling (1998). Johnson and Stiling (1998) conducted a survey of 10 sites in southern Florida during 1991–1993 and found a more conservative dispersal rate of only 38.6 km/yr. The rate of spread of the moth in Florida during 1989–1999 was estimated to be 50–75 km/yr (Hight et al. 2002, Stiling 2002). Because *C. cactorum* has three generations in the southeastern United States (Zimmermann et al. 2004), the potential dispersal distance of any single female would be 16–25 km. After its detection in Pensacola Beach in 2003, Bloem (2003) proposed that *C. cactorum* was advancing an average of 158 km/yr during 2000–2003. Based on these estimated rates of spread, the species was predicted to reach Texas by 2007 (Bloem 2003, 2005; Solis et al. 2004), recently updated to 2008 (USDA–APHIS 2008).

Zimmermann et al. (2004) questioned the hypothesis advanced by Johnson and Stiling (1998) that the cactus moth had dispersed naturally to the Florida Keys, which are 144 km from the closest point in Cuba, but 800 km from the southeastern area of Cuba where the cactus moth is concentrated. These authors further noted that the initial rates of dispersal in Florida were difficult to reconcile with those in Australia and South Africa. In South Africa, the unaided rate of dispersal of the cactus moth was ≈3–6 km in 2.5 yr (Pettey 1948). In Australia, the cactus moth spread unaided over 16–24 km in dense *Opuntia* infestations during 2.5 yr (Dodd 1940). Both Dodd (1940) and Pettey (1948) noted that moths disperse more widely as density of host plants decreases, and this may be a factor in higher rates of dispersal reported in Florida, which has discontinuous stands of cacti.

Personal communications have indicated that human transport of cacti can contribute to the dispersal of the cactus moth. In 2005, one of us (R.L.B.) received a phone call from a homeowner near Charleston, SC, who reported that cacti he had transplanted from the Florida Keys in 2001 had been killed during 2002 by large numbers of red and black caterpillars (a coloration unique to the cactus moth larvae). The first detection of cactus moth in South Carolina was in 2002 on Hunting, Edisto and Folly Islands, the latter near Charleston (Hight et al. 2002). It is unknown whether this homeowner's transplanted cacti were infested when they were transported from the Florida Keys or killed by the initial invasive wave of cactus moth into South Carolina. In 2006, one of us (R.L.B.) received an e-mail that stated succinctly "Is there a source for cactus moth eggs? I wish to introduce them on my central Texas ranch for prickly pear control." This communication illustrates the conflict between a species of biological control that would be valuable to ranchers/landowners who want to eliminate cacti as a weed, but detrimental to the socioeconomic environment of Mexico and the *Opuntia*-rich desert ecosystem.

The invasion of the southeastern United States by the cactus moth generally has been assumed to be due to rapid dispersal from a single point introduction, although some authors mentioned in the preceding discussion have questioned this idea. Although natural dispersal has occurred to account for its widespread occurrence in coastal areas of the Atlantic and Gulf coasts, our phylogeographic analysis documents more than one introduction into the southeastern United States and an invasion that cannot be attributed to natural dispersal alone. The interceptions of infested cacti from Caribbean islands indicate that human transport of infested cacti for landscaping purposes may be more important for long distance dispersal than natural dispersal.

Avise (2000), in his review of phylogeography, used the southeastern United States to illustrate case studies of genetic diversification with a clear geographic pattern. He showed differentiation in mitochondrial genes between the western (Gulf Coast) populations and the eastern (Atlantic) populations of a number of maritime or coastal species, ranging from vertebrates such as the seaside sparrow [*Ammodramus maritimus* (Wilson)] to arthropods such as the horse-shoe crab (*Limulus polyphemus* L.) and the tiger beetle (*Cicindela dorsalis* Say). He considered this east–west pattern to be the result of the complex geographical history of the region during the Pliocene and Pleistocene periods. Because *C. cactorum* has only been present in the southeastern United States for ≈30 yr, this cannot be the explanation for its current distribution. However, this is not the first instance where population genetics of an introduced organism in Florida has revealed the same east–west pattern found in

native organisms: Williams et al. (2005) found a similar pattern in the Brazilian peppertree (*Schinus terebinthifolius* Raddi), an introduced ornamental plant, based on microsatellite and chloroplast sequences, concluding that *S. terebinthifolius* had been introduced twice to Florida. This brings into question whether other east–west patterns found in Florida really are due to preglacial events, or they could be due to more recent similar twin invasions or introductions from the Caribbean.

Acknowledgments

Many people have helped us by collecting material or otherwise supporting the study, and we thank everyone warmly: S. D. Hight, J. Floyd, R. Taveras, W. Harrington, T. Hashimoto, T. D. Brackney, L. Majure, R. Westbrooks, and D. Herbison-Evans. Paul Goldstein and George Austin confirmed identifications and provided data for specimens of the cactus moth in the MacGuire Center for Lepidoptera, University of Florida. Members of F.A.H.S.'s lab at the University of Alberta (especially Marie Djernaes) and Molecular Biology Service Unit at the University of Alberta were of great assistance in the lab and provided constructive discussions. The study was supported financially by the Carlsberg Foundation, Copenhagen, Denmark (postdoc salary for T.J.S.); a contract from the U.S. Geological Survey, Biological Resources Discipline, Invasive Species Program, to the Geo-Resources Institute, Mississippi State University; U.S. Department of Agriculture, APHIS–PPQ, and NSERC (a Discovery Grant to F.A.H.S. that paid for the majority of the lab expenses and various other expenses).

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Received 14 January 2008; accepted 23 May 2008.