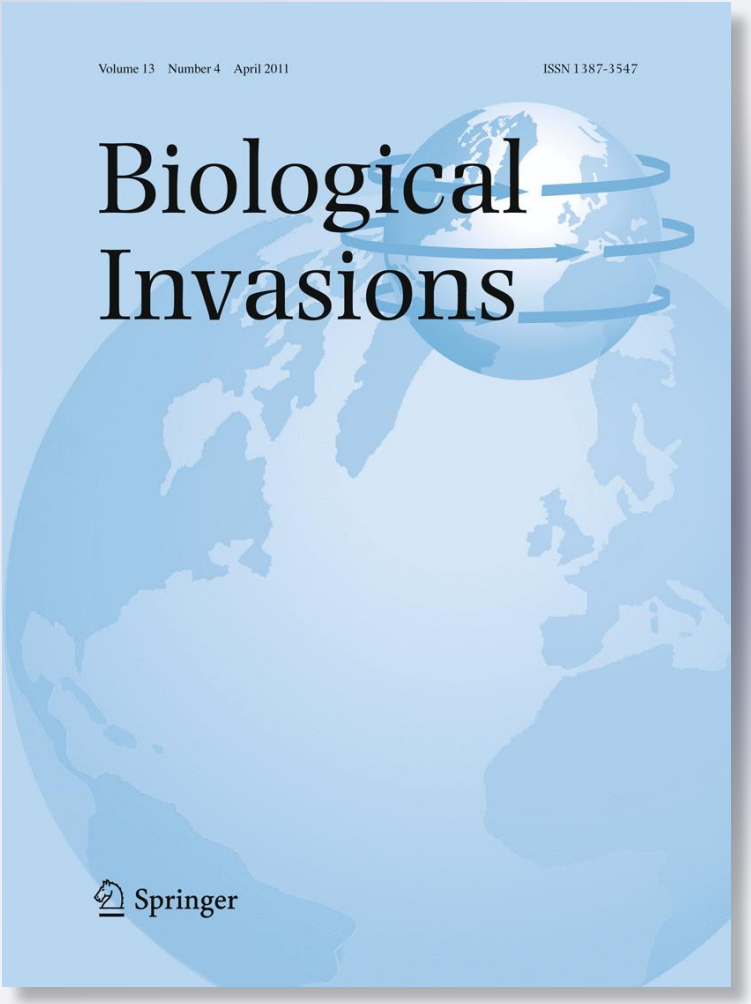
Geographic patterns of genetic diversity from the native range of Cactoblastis cactorum (Berg) support the documented history of invasion and multiple introductions for invasive populations

Biological Invasions

ISSN 1387-3547
Volume 13
Number 4

Biol Invasions (2011)
13:857-868
DOI 10.1007/
s10530-010-9874-9



Springer

Biol Invasions (2011) 13:857–868
DOI 10.1007/s10530-010-9874-9

ORIGINAL PAPER

Geographic patterns of genetic diversity from the native range of *Cactoblastis cactorum* (Berg) support the documented history of invasion and multiple introductions for invasive populations

Travis D. Marsico · Lisa E. Wallace · Gary N. Ervin · Christopher P. Brooks · Jessica E. McClure · Mark E. Welch

Received: 9 October 2009 / Accepted: 4 September 2010 / Published online: 18 September 2010

Abstract Spread of the invasive cactus-feeding moth *Cactoblastis cactorum* has been well documented since its export from Argentina to Australia as a biocontrol agent, and records suggest that all non-native populations are derived from a single collection in the moth's native range. The subsequent global spread of the moth has been complex, and previous research has suggested multiple introductions into North America. There exists the possibility of additional emigrations from the native range in nursery stock during the late twentieth century. Here, we present mitochondrial gene sequence data (*COI*) from South America (native range) and North America (invasive range) to test the hypothesis that the rapid invasive spread in North America is enhanced by unique genetic combinations from isolated portions of the native range. We found that haplotype richness in the native range of *C. cactorum* is high and that there was 90% lower richness in Florida than in Argentina. All Florida *C. cactorum* haplotypes are represented in a single, well-defined clade, which includes collections from the reported region of original export from Argentina. Thus, our data are consistent with the documented history suggesting a single exportation of *C. cactorum* from the eastern region of the native range. Additionally, the presence of geographic structure in three distinct haplotypes within the same clade across Florida supports the hypothesis of multiple introductions into Florida from a location outside the native range. Because the common haplotypes in Florida are also known to occur in the neighboring Caribbean Islands, the islands are a likely source for independent North American colonization events. Our data show that rapid and successful invasion within North America cannot be attributed to unique genetic combinations. This suggests that successful invasion of the southeastern US is more likely the product of a fortuitous introduction into favorable abiotic conditions and/or defense responses of specific *Opuntia* hosts, rapid adaptation, or a release from native enemies.

Keywords Argentina · Cactus moth · *Cactoblastis cactorum* · Genetic paradox · *Opuntia* · Phylogeography

T. D. Marsico · L. E. Wallace · G. N. Ervin · C. P. Brooks · J. E. McClure · M. E. Welch
Department of Biological Sciences, Mississippi State University, Mississippi State, MS, USA

T. D. Marsico (✉)
Department of Biological Sciences, Arkansas State University, PO Box 599, State University, AR 72467, USA
e-mail: tmarsico@astate.edu

Present Address:
J. E. McClure
College of Criminal Justice, Sam Houston State University, Huntsville, TX, USA

Introduction

Among the widely accepted tenets of evolutionary biology is that genetic diversity is prerequisite for species to adapt to changing selective pressures (Reznick et al. 1997; Lee 2002). Successful invasive species, however, often have reduced genetic diversity in their introduced ranges due to genetic bottlenecks associated with founder events (reviewed in Dlugosch and Parker 2008a; Puillandre et al. 2008, see also Rubinoff et al. 2010). Despite this, invasive populations with low genetic diversity are frequently successful in new environments and, at least in some cases, rapidly adapt to novel conditions (Holland 2000; Dlugosch and Parker 2008b; Suarez and Tsutsui 2008; Whitney and Gabler 2008). Successful invasion concomitant with low genetic diversity has become known as the "genetic paradox" (Frankham 2005). Increasingly, there is evidence that reduction in genetic diversity as measured by neutral markers does not necessarily result in a reduction of adaptive potential (Reed and Frankham 2001; Boman et al. 2008; Dlugosch and Parker 2008a, b), but there also is growing evidence that multiple introductions often enhance genetic diversity or that large initial introductions capture much of the native diversity through "gene pool capture" (Holland 2001; Novak and Mack 2005; Wares et al. 2005; Roman and Darling 2007). In addition, novel combinations of genetic diversity due to hybridization among distantly related populations in the non-native range may contribute to rapid evolution and spread (Kolbe et al. 2004; Novak 2007; Lavergne and Molofsky 2007). In cases where successful invaders do possess severely reduced genetic diversity, species may already possess characters that are necessary for successful establishment in the non-native environment or genotypes that promote plasticity (Meimberg et al. 2006; Boman et al. 2008). Finally, ecological mechanisms may facilitate the success of genetically depauperate invaders, including enemy release (Keane and Crawley 2002; Torchin et al. 2003; Torchin and Mitchell 2004) or naïve hosts (Roane et al. 1986; Fritts and Rodda 1998; Griffin 2000).

The South American cactus moth, *Cactoblastis cactorum* (Berg) (Pyralidae: Phycitinae), is a particularly interesting system for studying the genetic paradox because it has a well-documented history as a biocontrol agent. *Cactoblastis cactorum* is a specialist feeder on pricklypear cacti in the genera *Opuntia, Nopalea, and Consolea* (only the first of which is native to the moth's native range). The moth is native to regions of northern Argentina, Uruguay, Paraguay, and southern Brazil (Dodd 1940). In 1925, 2,750 eggs were taken from a single locality in northeastern Argentina to be used for the control of invasive prickly pear in Australia (Dodd 1940). Among the dominant species of cactus that the moth was meant to control was *Opuntia stricta* (Haworth) Haworth, a species that is native to the southeastern US and with which *C. cactorum* is most closely associated in North America (Pemberton and Liu 2007; Baker and Stiling 2009; Sauby 2009). It was estimated that as many as 3×10^9 *C. cactorum* were reared and released in Australia, resulting in reclamation of nearly 10 million hectares of *Opuntia*-infested land in fewer than 15 years (Dodd 1940). Thus, *C. cactorum* possesses a historical association with populations of its primary North American host that may have aided invasion in the absence of high genetic diversity.

In 1932, after substantial control of prickly pear in Australia, 18,000 eggs were successfully taken from Australia to South Africa to begin testing and breeding colonies for prickly pear control (Pettey 1947). *Cactoblastis cactorum* had lower success in reducing invasive *Opuntia* populations (primarily *Opuntia megacantha* Salm-Dyck and/or *Opuntia ficus-indica* (L.) Miller) in South Africa than in Australia (Annecke and Moran 1978; Hoffmann et al. 1998a, b; Foxcroft et al. 2007), where host plants were larger and "woodier" than those in Australia (Pettey 1947), where it may have been poorly adapted to the most abundant target hosts (Annecke and Moran 1978), and where the investment into releases of the moth were not as great as in Australia (Raghu and Walton 2007). Nevertheless, in November 1956 moths were sent from South Africa to a rearing station in Trinidad. After 6 months of captive breeding, 100 larvae and 300 eggs were shipped to Nevis and released. In the subsequent months a total of 5,200 *C. cactorum* individuals were released on the island of Nevis (Simmonds and Bennett 1966) to control native *Opuntia*, including *O. triacantha* (Willdenow) Sweet and *O. stricta*. From Nevis, infested *Opuntia* pads were taken to Antigua and Montserrat in 1960. In 1963, *C. cactorum* was reported in the US Virgin Islands, and in 1964 it

was reported on St. Kitts, an island neighboring Nevis, with additional islands in the Caribbean ultimately being colonized with no known human assistance.

By 1989 the moth had been detected in the Florida Keys, which may have been anticipated given the close proximity of these Caribbean islands to mainland North America and the extensive horticultural trade between Caribbean suppliers and US buyers (Habeck and Bennett 1990; Pemberton 1995; Simonsen et al. 2008). Since its detection in the United States, the moth has spread north to the South Carolina coast and west to the coast of Louisiana and has become a potential threat to native *Opuntia* populations across the region (Habeck and Bennett 1990; Dickel 1991; Hight et al. 2002; Simonsen et al. 2008; Payne 2009). Currently, *C. cactorum* is considered a pest species along the Gulf and Atlantic coasts of the US (www.aphis.usda.gov) and is recognized by many groups as an invasive species (Simonson et al. 2005). It has been demonstrated that *C. cactorum* can feed successfully on all available *Opuntia* host species in the southeastern U.S. (Johnson and Stiling 1996; Sauby 2009). Levels of *C. cactorum* damage to *Opuntia* individuals have commonly been severe, resulting in plant death, decrease in plant and population sizes, and reduction in reproductive output (Dodd 1940; Hoffmann et al. 1998b; Johnson and Stiling 1998).

A previous study of genetic structure of *C. cactorum* in Australia, South Africa, the Caribbean, and the southeastern US using cytochrome oxidase I (*COI*) documented the existence of at least five haplotypes, with three of those haplotypes found in the invaded North American range (Simonsen et al. 2008). Based on the geographic distribution of these haplotypes, Simonsen et al. (2008) suggested multiple separate introductions into Florida. Pemberton (1995) provided additional evidence that multiple introductions were likely using records of infested horticultural imports from the APHIS-Plant Protection and Quarantine inspection station in Miami, Florida. These records indicate potential import of *C. cactorum* from at least the Dominican Republic and Haiti over a 13 year period from 1981 through 1993 (Pemberton 1995). Since 1993, no additional imports of *C. cactorum* have been recorded at the Miami inspection station (J. Floyd, personal communication). It also is possible that *C. cactorum* entered Florida from sources outside the Caribbean because shipments of cacti, though not necessarily *Opuntia*, were imported through Miami at least in 1986 from Brazil and Argentina (Pemberton 1995), suggesting opportunities for introductions from additional parts of the moth's native range. Such introductions could have brought together previously isolated lineages of *C. cactorum*, providing potential for novel genetic combinations that could have facilitated invasion success. Due to the possibility that infested cacti could have been imported to the United States from the native range, a genetic investigation of native range samples is necessary to interpret relative genetic diversity in the invasive populations and to determine likely regional sources of invasive haplotypes.

This study used *COI* to investigate genetic diversity and phylogeography of *C. cactorum* in its native range in Argentina and compared the results to the main region of invasion in Florida, US. Because the literature suggests that all non-native populations are derived from a single collection in Concordia, Argentina (Fig. 1; Dodd 1927, 1940; Pettey 1947; Simmonds and Bennett 1966; Zimmerman et al. 2005), we expected that *C. cactorum* genetic diversity in the US would be substantially lower than in its native range. Also, we tested whether the rapid spread of *C. cactorum* in North America could have been facilitated by novel genetic admixture. Data with a signature of possible genetic admixture would show geographically isolated lineages in the Argentine native range, but disparate haplotypes in close proximity within the Floridian non-native range. A test of this hypothesis required broad sampling in the native range and in Florida in order to clarify the pattern of introduction into the invasive range. We determined that *C. cactorum* has reduced genetic diversity in its introduced North American range relative to its native Argentine range. Further, haplotypes of introduced populations are present in or are closely related to those from the original collection region in Argentina, and no haplotypes restricted to other regions of the native range are present in North America. Additionally, the data from Florida are highly consistent with three introductions, each coming from individuals with closely related haplotypes, and all likely originating in the neighboring Caribbean. Our results are congruent with the documented path of *C. cactorum* invasion, and they suggest that genetic admixture between genetically distinct lineages has not played a role in the successful invasion of North America.

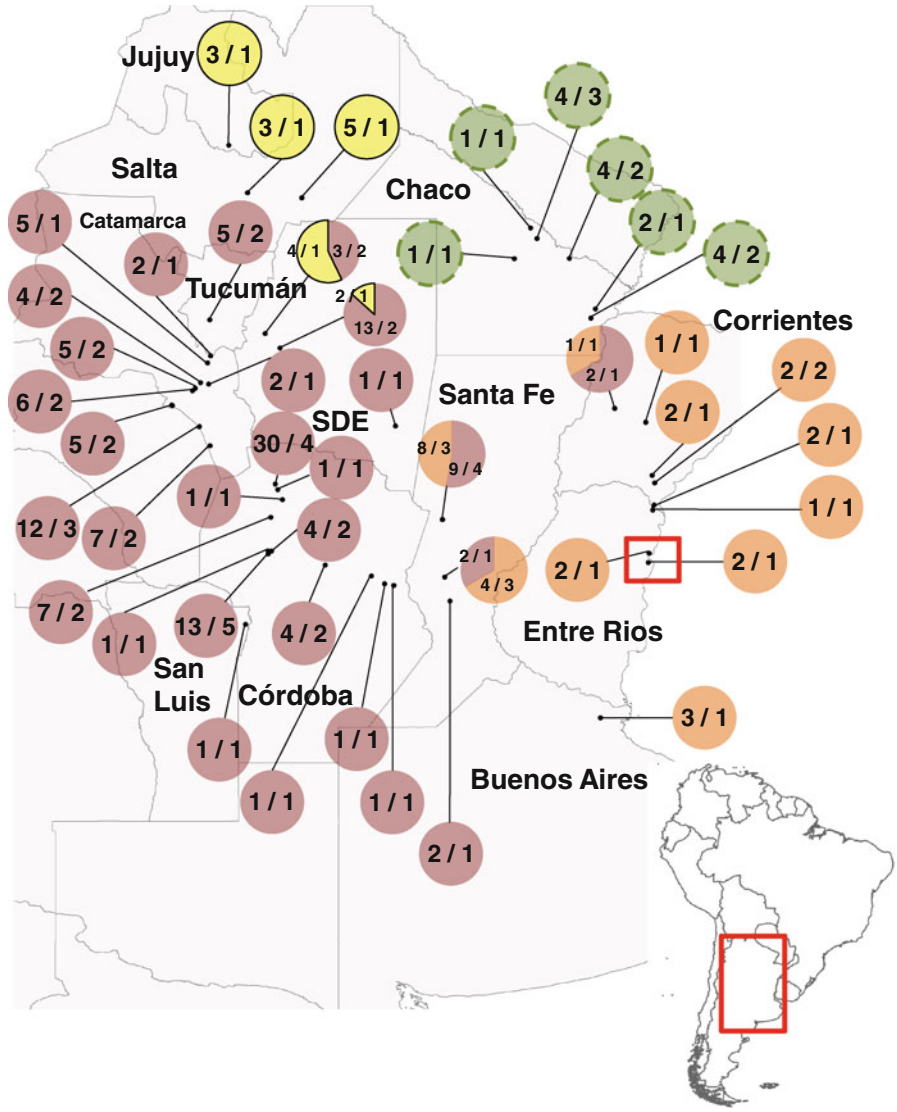


Fig. 1 Geographic distribution of clades of *C. cactorum* in its native range in Argentina. Pies are coded to match haplotype groupings in Fig. 3 (Argentina West *purple* [*dark*], Argentina Northwest *yellow* [*light*] surrounded by *solid black line*, Argentina Northeast *green* [*medium shading*] surrounded by *hatched line*, and Argentina East *orange* [*medium shading*]). *Numbers* within pies are the number of individuals sampled followed by the number of haplotypes found at each sampling location. Each province is identified by name; Santiago del Estero is abbreviated SDE. The (*red*) *square* around the two sites near Concordia in Entre Rios province are the locations nearest the original collections for exportation of *C. cactorum* to Australia in 1925. In addition, these sites are the only two sampled in this study from the native range that contained individuals with haplotypes CF and CL, the two most commonly found haplotypes in Florida

Materials and methods

Sample collection

Two hundred eleven *C. cactorum* samples (larvae and eggsticks) collected within the native range of northern Argentina in February, April, and December 2008, across 46 sites (Fig. 1) were used in this study. Samples were collected from seven host species: *Opuntia anacantha* Spegazzini, *O. bonaerensis* Spegazzini, *O. cardiosperma* Schumann, *O. elata* Salm-Dyck, *O. ficus-indica* (L.) Miller (including the feral, spiny *O. ficus-indica* forma *amyclaea* (Tenore) Schelle; neither is native to Argentina), *O. megapotamica*

Arechavaleta, and *O. sulphurea* G. Don. Within the North American invasive range of *C. cactorum*, 51 *C. cactorum* larvae were collected from 40 sites across Florida in March, May, July, and September, 2008 (Fig. 2). These individuals were collected from five host plant species: *Opuntia cochenillifera* (L.) Miller (non-native to the US), *O. ficus-indica* (non-native to the US), *O. humifusa*, *O. pusilla* (Haworth) Haworth, and *O. stricta*. In addition, *C. cactorum* data were used from research by Simonsen et al. (2008) to supplement our sampling. These data included 29 individuals from 8 sites in Florida, increasing our total to 80 individuals and 48 sites.

DNA extraction, PCR, and sequencing

Since the *COI* gene is maternally inherited and *C. cactorum* feed in groups of multiple individuals produced by a single mother, either a single larva or a single eggstick from one sampled plant was considered a unique sample. This approach limits the possibility of considering sibling larvae that share *COI* haplotypes as independent samples. DNA was extracted from ethanol-preserved samples using a PRISM 6100 Nucleic Acid PrepStation and proprietary reagents (Applied Biosystems Inc., Foster City, CA). Tissue was homogenized using a Retsch MM200 Ball Mill (Retsch, Newtown, PA). To extract DNA from the early (1st and 2nd) instars, whole larvae were utilized. For late instar larvae, ~50 mg sections were collected for homogenization and DNA extraction. For DNA samples obtained from eggsticks, whole or partial eggsticks were used.

A total of 875 nucleotides from the *COI* mitochondrial gene were amplified using the Jerry and PatII primers (Simon et al. 1994; Caterino and Sperling 1999; Jerry: 5′ CAACATTTATTTTGATT TTTTGG 3′; PatII: 5′ TCCATTACATATAATCTG CCATATTAG 3′). Amplifications were conducted in 50 µl reactions that contained 10–30 ng of template DNA, 2 units of *Taq* polymerase, 15 pmol of each

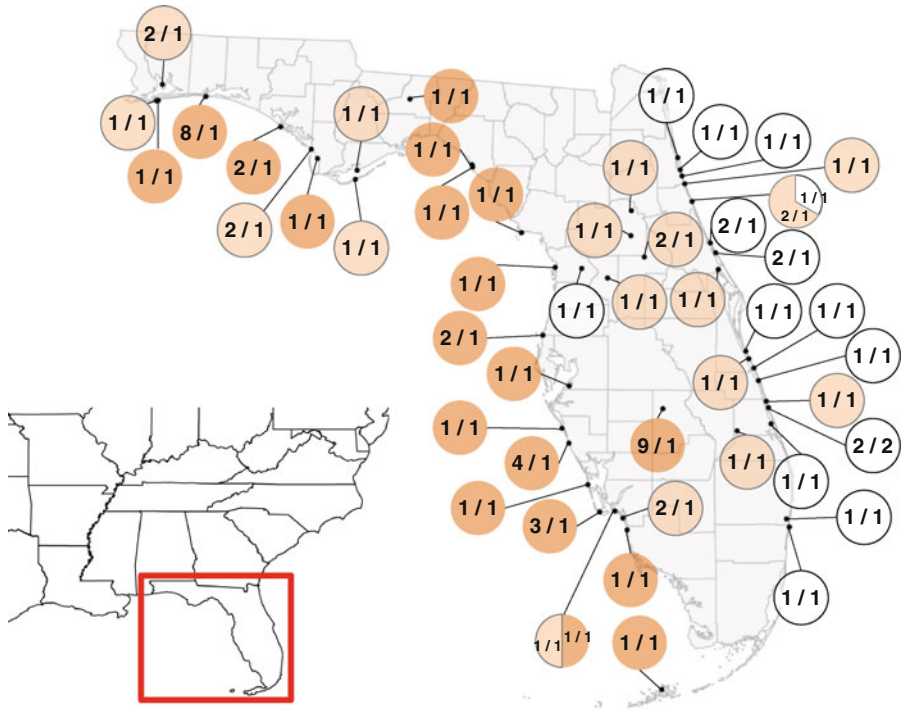


Fig. 2 Geographic distribution of *C. cactorum* in its invasive range in Florida. Shading (colors) for the *C. cactorum* invasive range map are designated following the phylogenetic subgroups within the Argentina East clade and are *dark* (*orange*) (Gulf Coast haplotype CF), *pale* (*light orange*) (widespread haplotype CL and single sample of haplotype CBO), and *white* (Atlantic Coast grouping, haplotypes CK and CBN). *Numbers* within pies are the number of individuals sampled followed by the number of haplotypes found at each sampling location

primer, 5 nmol of each dNTP, 3.6 µmol tricine, 6 µmol KCl, and 240 pmol $MgCl_2$. The thermocycling protocol was as follows: 95°C for 1 min followed by 35 cycles of 95°C for 15 s, 57°C for 15 s, and 72°C for 1 min. A final 7 min elongation step at 72°C completed the PCR run. PCR product was concentrated with a Savant DNA120 SpeedVac Concentrator (Thermo Fisher Scientific, Waltham, Massachusetts), and concentrated product was sent to the Arizona State University DNA Laboratory for sequencing on an ABI 3730 capillary sequencer using the same primers that were used for amplification and the Big Dye Terminator Kit v. 3.0 (Applied Biosystems). Sequences for all individuals were generated in the forward and reverse directions and assembled into consensus contigs using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, Michigan). Consensus sequences also were aligned in Sequencher 4.7. Unique sequences have been deposited in GenBank (accession numbers GU068087-GU068144).

Phylogenetic and phylogeographic analyses

We obtained summary data on nucleotide diversity (π) according to Nei (1987), and we determined differences between haplotypes using DnaSP version 5.10 (Librado and Rozas 2009). We used Bayesian phylogenetic analyses (Rannala and Yang 1996) to examine evolutionary relationships among unique mitochondrial haplotypes observed across *C. cactorum*. *Dioryctria abietella* Denis and Schiffermüller (GenBank Accession AJ868572) and *Melitara prodenialis* Walker (GenBank Accession GU068159) were used as outgroups to root the phylogeny. We included two *C. cactorum* haplotypes from Simonsen et al. (2008) collections in the phylogeny (Fig. 3) that were not represented in our Argentina or Florida sampling: one from Australia and South Africa (haplotype CE) and one from South Africa alone (haplotype CZ). Bayesian analyses were conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) using a GTR model with variable rates following a gamma distribution, selected as the best fitting model of sequence evolution using the Akaike Information Criterion (AIC) in ModelTest 3.7 (Posada and Crandall 1998). We ran MrBayes under the specified model with default flat priors and sampling every 100th generation. The analyses were stopped when the average standard deviation of split frequencies between the two independent runs was $\leq$0.01, which suggests convergence (Ronquist et al. 2005). In total, 2 million iterations were conducted. The first 25% of sampled trees were discarded as burn-in before computing posterior probabilities.

Differentiation between the invasive Florida and native Argentina population was analyzed using Analysis of Molecular Variance (AMOVA) accounting for pairwise mutational differences between haplotypes (Weir and Cockerham 1984; Excoffier et al. 1992; Weir 1996). In the AMOVA, all samples from Florida were analyzed as one population and compared with all Argentina samples using Arlequin 3.1 (Excoffier et al. 2005), and significance was determined with 10,000 permutations. Within Argentina and Florida, we conducted Mantel correlograms with the software PASSaGE1 (Rosenberg 2001) to investigate spatial autocorrelation among haplotypes. The genetic distance matrix was determined by pair-wise comparison of the mean base-pair divergence among sample sites in Arlequin 3.1 (Excoffier et al. 2005). Pairwise geographic distances among sample sites were determined using a GIS extension for ArcGIS 9.2 called Conefor inputs (www.jennessent.com), downloadable with Conefor Sensinode 2.2 (Pascual-Hortal and Saura 2006; Saura and Pascual-Hortal 2007).

Results

The aligned data set contained 791 nucleotides of the mitochondrial *COI* gene. A total of 57 *C. cactorum* haplotypes are now known, predominately from the native range in Argentina (52 of the 57) (Table 1; Fig. 3). Seven haplotypes (CE, CF, CK, CL, CZ, CBN, CBO) have been found in individuals outside South America, and only two of these (CF and CL) have been recorded to occur in the South American native range, both very near the location from which *C. cactorum* was transported to Australia in 1925 (Fig. 1; Entre Rios province). All seven of the haplotypes recorded from the non-native range cluster within the Argentina East clade (Fig. 3), the grouping that encompasses the geographic location of the original biological control sample collection in 1925 documented by Dodd (1940; Fig. 1). All five haplotypes found exclusively in the non-native range differ by a maximum of four nucleotide substitutions from CF or CL, which are the two haplotypes found

Table 1 Summary of sequencing data for *C. cactorum* for a 791 base region of the mitochondrial gene *COI*

	Number of individuals sequenced	Number of haplotypes	Number of polymorphic nucleotide sites	Nucleotide diversity (π)	Average number of nucleotide differences among haplotypes
C. cactorum in Argentina	211	52	54	0.00723	5.662
C. cactorum in Florida	80	5	7	0.00285	2.233

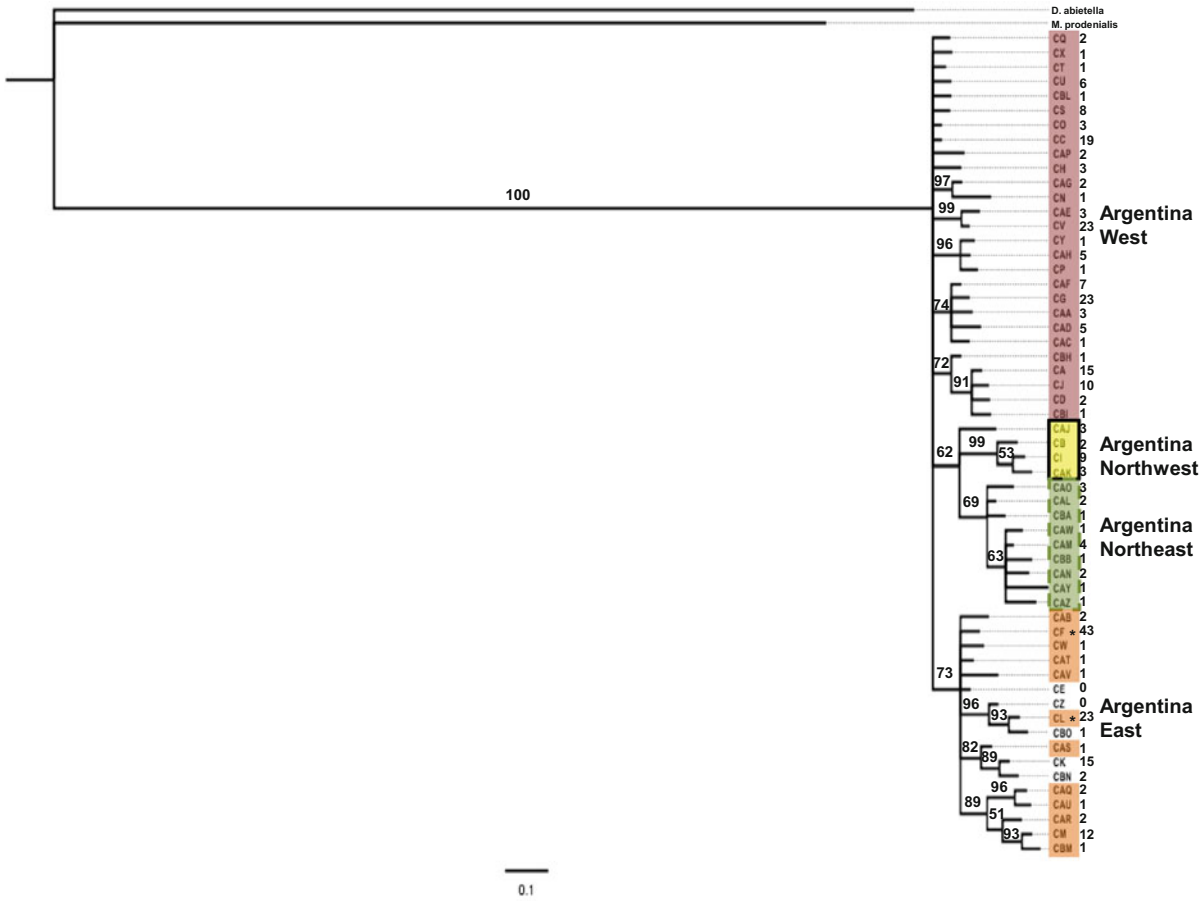


Fig. 3 Bayesian consensus phylogeny based on analysis conducted with MrBayes; phylogenetic depiction produced using FigTree version 1.2.2, available at http://tree.bio.ed.ac.uk/software/figtree/. *Dioryctria abietella* and *Melitara prodenialis* were used as outgroups. Clade support, shown as percentages on tree branches, represents posterior probabilities. *Cactoblastis cactorum* haplotypes begin with the letter "C". Clades are coded to roughly depict geographic regions where haplotypes are found for *C. cactorum* (Argentina West *purple* [*dark*], Argentina Northwest *yellow* [*light*] surrounded by *solid black line*, Argentina Northeast *green* [*medium shading*] surrounded by *hatched line*, and Argentina East orange [*medium shading*]). It is important to note that these names are given to the clades themselves and do not always reflect the strict geographic occurrence of a given haplotype. The non-native *C. cactorum* group (not colored) is contained within the Argentina East clade. Haplotypes CL and CF (marked with an *) are the only two *C. cactorum* haplotypes found both within Argentina and in the non-native range

in both the native and non-native ranges. Five *C. cactorum* haplotypes (CF, CK, CL, CBN, CBO) have been recorded in the invasive range of the southeastern US. AMOVA results showed that Argentina and Florida populations were significantly differentiated from each other ($F_{ST} = 0.39$; Table 2).

Table 2 AMOVA for *Cactoblastis cactorum COI* data that compares the partitioning of genetic variation between the invasive populations in Florida and the native populations in Argentina

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	*P*
Between Florida and Argentina	1	180.7	1.54	39	<0.0001
Within each region	289	682.7	2.36	61	
Total	290	863.4	3.90		

Significance of variance components assessed with 10,000 permutations

Table 3 Ad hoc AMOVA tests for *Cactoblastis cactorum COI* data comparing groupings within Argentina and Florida that correspond to geographic regions identified by phylogroups in Argentina and divergent haplotype frequencies in Florida

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	*P*
Argentina					
Among groups	3	175.6	1.27	39	<0.0001
Within groups	207	418.9	2.02	61	
Total	210	594.5	3.29		
Florida					
Among groups	2	30.4	0.55	42	<0.0001
Within groups	77	57.8	0.75	58	
Total	79	88.2	1.30		

The four groups in Argentina are East (samples from Buenos Aires, Corrientes, Entre Rios, and Santa Fe provinces), Northeast (Chaco province), Northwest (Jujuy, Salta, Santiago del Estero, and Tucumán provinces), and West (Catamarca, Córdoba, and San Luis); and the three groups in Florida are Atlantic Coast, Gulf Coast, and Panhandle regions

Significance of variance components assessed with 10,000 permutations

As ad hoc analyses, AMOVAs were used to quantify differentiation among geographic groupings within each sampled range accounting for pairwise mutational differences between haplotypes (see "Materials and methods" above). Argentina samples were grouped according to four geographic regions (Fig. 1; East, Northeast, Northwest, and West), and Florida samples were grouped according to three geographic regions (Fig. 2; Atlantic Coast, Gulf Coast, and Panhandle). In both Argentina and Florida we observed a structure among haplotypes that is correlated with geography (Table 3). A significant portion (39%) of the genetic variation in Argentina is explained by geographically distinct phylogroups (Table 3). In Florida, five haplotypes from the Argentina East clade cluster into three groups, two of which (CK/CBN and CL/CBO) are supported with posterior probabilities of greater than 88% (Fig. 3) due to only a single nucleotide substitution between the individuals of each pair. The three groups have distinct geographic patterns (Fig. 2). One group (haplotypes CK and CBN) is found along the Atlantic Coast, another (haplotype CF) is restricted to the Gulf Coast, and a third, widespread clade (haplotypes CL and CBO) is represented throughout Florida. A significant portion (42%) of the genetic variation in Florida is explained by geographically distinct groups with differing haplotype frequencies in Atlantic Coast, Gulf Coast, and Panhandle regions (Table 3).

Haplotypes with similar sequences were spatially autocorrelated in Argentina (Fig. 4A). Sites within 400 km show significantly positive spatial autocorrelation, and sites greater than 500 km apart show significantly negative spatial autocorrelation. The distribution of *C. cactorum* haplotypes in Florida is different. Alternate haplotypes are distributed lengthwise along each Floridian coast and have poor correspondence between genetic distance and geographic distance (Fig. 4B). That is, unlike in Argentina where haplotypes in specific clades are largely restricted to a single geographic region, groups in Florida are defined by distinct haplotype frequencies with two haplotypes being common in multiple geographic groups reflecting the rapid spread of this species in the state. Because of this poor correspondence between genetic distance and geographic

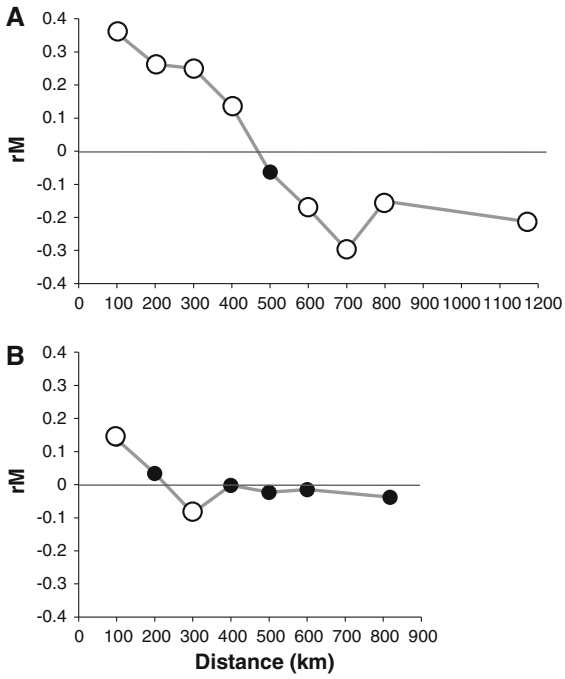


Fig. 4 Mantel correlograms of *C. cactorum* in its native range in Argentina (**A**) and its invasive range in Florida (**B**). The correlograms show the geographic distance classes at which the *COI* haplotypes of are significantly positively or negatively spatially autocorrelated (*open circle*) and distances classes in which the correlation is not different from zero (*closed circle*)

distance we also conducted an AMOVA in Arlequin 3.1 that treated all haplotypes as equally distinct, ignoring genetic distance between haplotypes. The results from this analysis were consistent with the prior AMOVA conducted for these groups and also were significant ($F_{ST} = 0.40$, $P < 0.0001$).

Discussion

Cactoblastis cactorum has ten-fold fewer haplotypes in Florida (invasive range) than were found in Argentina (native range) with less than a three-fold difference in number of samples and nearly the same number of sampling localities between the two regions. The haplotypes recovered from throughout the non-native range of *C. cactorum* (this study and Simonsen et al. 2008) are most closely related to those found near the original 1925 biological control collection site in Argentina (Dodd 1940). These results, therefore, are inconsistent with the possibility of separate introductions to the US from the western and northern portion of the *C. cactorum* native range. Instead, these results support the hypothesis that the historical biological control introduction pathway ultimately may be the only source of invasive *C. cactorum* in the US (Dodd 1940; Pettey 1947; Simmonds and Bennett 1966).

Even though genetic diversity in the invasive range is low and supports the documented colonization pathway, *C. cactorum* also has apparently undergone multiple introductions to Florida. However, the genetic similarities of populations founded independently in the US suggest that all share a similar history and common ancestry. Caribbean islands are the most likely sources because haplotypes found in Florida also have been found in the islands that exported *Opuntia* for the US horticulture industry (Pemberton 1995; Simonsen et al. 2008). Simonsen et al. (2008) suspected that *C. cactorum* in the southeastern US was introduced multiple times, and our data covering many more locations in Florida refine this inference. Simonsen et al. (2008) found three haplotypes in the southeastern U.S. (haplotype 2 in the Simonsen et al. data = haplotype CL in our data, haplotype 3 = CK, and haplotype 5 = CF). In their analyses, haplotypes CL and CK were found on the Atlantic Coast, and haplotype CF predominated on the Gulf of Mexico Coast. The only departure from this pattern observed in their data was two samples from Pensacola Beach (on the Gulf Coast) that carried the Atlantic Coast haplotype CL. Simonsen et al. (2008) inferred from this pattern that multiple introductions to Florida were likely. Our additional sampling in Florida increased the number of sampled locations six-fold, allowing for increased confidence in interpretation of the phylogeographic pattern. We argue that the presence of distinct Atlantic Coast haplotypes (CK and CBN), a Gulf Coast haplotype (CF), and a widespread haplotype (CL), particularly abundant in the interior of the peninsula and in the Florida panhandle, is most consistent with at least three independent introductions. AMOVA results indicate that 42% of the variation in Florida can be attributed to Atlantic Coast, Gulf Coast, and Panhandle geographic groupings. This geographic pattern is more consistent with progressive northward and westward dispersal on independent trajectories than with many, independent introductions because with many introductions of each haplotype we would not expect to see a distinct

geographic distribution of haplotypes. Our data also suggest that multiple introductions into Florida have not promoted novel genetic mixing from previously isolated lineages. On the contrary, closely related haplotypes have distinct geographic regions.

Given our data, novel genetic combinations in the invasive range do not provide a satisfactory explanation for the genetic paradox of *C. cactorum* invasion observed in the US. While we cannot rule out enemy release as a possibility for enhancing invasion in the US, the strong host preference of *C. cactorum* in the southeastern US and the Caribbean for *O. stricta* (Pemberton and Liu 2007; Baker and Stiling 2009; Sauby 2009) suggests that this host may be more suitable for introduced *C. cactorum* than other potential hosts. *Opuntia stricta* is largely restricted to coastal areas of Florida (Pinkava 2003; Sauby 2009), Alabama, and Mississippi, such that dispersal from the Atlantic coast to the Gulf coast is more difficult than movements along the coast. *Opuntia stricta* was the predominant host in Australia where *C. cactorum* had its greatest impact as a biocontrol agent (Dodd 1940), and it has been suggested that the limited success of prickly pear biological control in South Africa may have resulted from the lack of more suitable *Opuntia* host species (Annecke and Moran 1978). Because it appears that *C. cactorum* populations in North America originated through the documented colonization pathway, individuals in Florida would be descendents of moths that began an association with *O. stricta* approximately 200 generations before they colonized the US (Dodd 1940). The rapid *C. cactorum* population growth in Australia must have coincided with a new favorable chance association on a novel host or with selection for fitness on its new host, *O. stricta*. Furthermore, the invasion data are more consistent with host suitability as a mechanism of rapid spread than with rapid evolution after arrival in North America. If invasive *C. cactorum* populations could rapidly evolve greater fitness on novel hosts, we would expect recently encountered *O. humifusa* and *O. pusilla* to be favored host species. Instead, *C. cactorum* exhibits strong preference for the host species with which it has the longest association outside Argentina, versus other more recently encountered suitable hosts in Florida (Sauby 2009). Host naïveté could be an important factor in explaining the rapid spread of *C. cactorum* throughout its invasive range since the association with *O. stricta* was one sided. That is, *O. stricta* plants in North America had never experienced *C. cactorum* feeding until the late 1980s. Evidence is emerging that poor defenses by *O. stricta* and *O. humifusa* may have aided in the rapid spread of *C. cactorum* throughout Florida (Marsico et al., unpublished data). Still, because *O. humifusa* and *O. pusilla* have not emerged as preferred host species, host naïveté alone does not explain *C. cactorum* success. One potential explanation for the lack of host expansion onto *O. humifusa* is that the sole native cactophagous moth in Florida, *M. prodenialis*, exhibits significant association with *O. humifusa* (Sauby, 2009), possibly presenting a competitive barrier to *C. cactorum* expansion onto that host. Finally, little is known about predators, parasitoids, and pathogens of *C. cactorum* in its native and non-native ranges, making a full assessment of enemy release difficult. Available data have suggested that consumers may not be limiting populations in the native range of *C. cactorum* (Pemberton and Cordo 2001a; Logarzo et al. 2008), and generalist parasitoid species on *C. cactorum* have been documented in Florida (Pemberton and Cordo 2001b); however, these limited examples do not exclude enemy release as one viable mechanism contributing to *C. cactorum* spread in North America.

Acknowledgments This work was supported in part by grants from the U.S. Geological Survey Biological Resources Discipline (04HQAG0135 and 08HQAG0139) and U.S. Department of Agriculture (2007-55320-17847) to GNE. Additional funds were provided by the Mississippi State University Office of Research and Economic Development and the College of Arts and Sciences. We also thank Guillermo Logarzo and Laura Varone (USDA South American Biological Control Laboratory, Buenos Aires), James Carpenter (USDA-ARS Crop Protection and Management Research Unit, Tifton, GA), Stephen Hight (USDA-ARS Insect Behavior and Biocontrol Research Unit, Gainesville, FL), Kristen Sauby, and Anastasia Woodard for assistance with sample collection. We are grateful to Richard Brown (Mississippi Entomological Museum) who assisted in the identification of some samples. We thank three anonymous reviewers for their comments that improved the manuscript.

References

Annecke DP, Moran VC (1978) Critical reviews of biological pest control in South Africa. 2. The prickly pear, *Opuntia ficus-indica* (L.) Miller. J Entomol Soc S Afr 41:161–188

Baker AJ, Stiling P (2009) Comparing the effects of the exotic cactus-feeding moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) and the native cactus-feeding

moth, *Melitara prodenialis* (Walker) (Lepidoptera: Pyralidae) on two species of Florida *Opuntia*. Biol Invasions 11:619–624

Boman S, Grapputo A, Lindström L, Lyytinen A, Mappes J (2008) Quantitative genetic approach for assessing invasiveness: geographic and genetic variation in life-history traits. Biol Invasions 10:1135–1145

Caterino MS, Sperling FAH (1999) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. Mol Phylogenet Evol 11:122–137

Dickel TS (1991) *Cactoblastis cactorum* in Florida (Lepidoptera: Pyralidae: Phycitinae). Trop Lepid 2:117–118

Dlugosch KM, Parker IM (2008a) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449

Dlugosch KM, Parker IM (2008b) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecol Lett 11:701–709

Dodd AP (1927) The biological control of prickly pear in Australia. Commonwealth Council for Scientific and Industrial Research, Bulletin no. 34, Melbourne, Australia

Dodd AP (1940) The biological campaign against prickly-pear. Commonwealth Prickly Pear Board, Brisbane

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Foxcroft LC, Hoffmann JH, Viljoen JJ, Kotze JJ (2007) Factors influencing the distribution of *Cactoblastis cactorum*, a biological control agent of *Opuntia stricta* in Kruger National Park, South Africa. S Afr J Bot 73:113–117

Frankham R (2005) Resolving the genetic paradox in invasive species. Heredity 94:385

Fritts TH, Rodda GH (1998) The role of introduced species in the degradation of island ecosystems: a case history of Guam. Annu Rev Ecol Syst 29:113–140

Griffin GJ (2000) Blight control and restoration of the American chestnut. J For 98:22–27

Habeck DH, Bennett FD (1990) *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), a Phycitine new to Florida. Entomology Circular No. 333, Florida Department of Agriculture and Consumer Services, Division of Plant Industry

Hight SD, Carpenter JE, Bloem KA, Bloem S, Pemberton RW, Stiling P (2002) Expanding geographical range of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. Fla Entomol 85:527–529

Hoffmann JH, Moran VC, Zeller DA (1998a) Evaluation of *Cactoblastis cactorum* (Lepidoptera: Phycitidae) as a biological control agent of *Opuntia stricta* (Cactaceae) in the Kruger National Park, South Africa. Biol Control 12:20–24

Hoffmann JH, Moran VC, Zeller DA (1998b) Long-term population studies and the development of an integrated management programme for control of *Opuntia stricta* in Kruger National Park, South Africa. J Appl Ecol 35:156–160

Holland BS (2000) Genetics of marine bioinvasions. Hydrobiologia 420:63–71

Holland BS (2001) Invasion without a bottleneck: microsatellite variation in natural and invasive populations of the brown mussel, *Perna perna* (L.). Mar Biotechnol 3:407–415

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Johnson DM, Stiling PD (1996) Host specificity of *Cactoblastis cactorum* (Lepidoptera: Pyralidae), an exotic *Opuntia*-feeding moth, in Florida. Environ Entomol 25:743–748

Johnson DM, Stiling PD (1998) Distribution and dispersal of *Cactoblastis cactorum* (Lepidoptera: Pyralidae), an exotic *Opuntia*-feeding moth, in Florida. Fla Entomol 81:12–22

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. Trends Ecol Evol 17:164–170

Kolbe JJ, Glor RE, Schettino LR, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181

Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci USA 104:3883–3888

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Logarzo G, Varone L, Briano J (2008) Cactus moth. 2008 Annual Report. South American Biological Control Laboratory. USDA, ARS, Buenos Aires, Argentina

Meimberg H, Hammond JI, Jorgensen CM, Park TW, Gerlach JD, Rice KJ, McKay JK (2006) Molecular evidence for an extreme genetic bottleneck during introduction of an invading grass to California. Biol Invasions 8:1355–1366

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Novak SJ (2007) The role of evolution in the invasion process. Proc Natl Acad Sci USA 104:3671–3672

Novak SJ, Mack RN (2005) Genetic bottlenecks in alien species invasions. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species invasions: insights into ecology, evolution, and biogeography. Sinauer, Sunderland, pp 201–228

Pascual-Hortal L, Saura S (2006) Comparison and development of new graph-based landscape connectivity indices: towards the prioritization of habitat patches and corridors for conservation. Landsc Ecol 21:959–967

Payne JH (2009) US Department of Agriculture—Animal and Plant Health Inspections Services report DA-2009-25. Available via http://www.aphis.usda.gov/plant_health/plant_pest_info/cactoblastis/reports.shtml. Accessed 20 July 2009

Pemberton RW (1995) *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States: an immigrant biological control agent or an introduction of the nursery trade? Am Entomol 41:230–232

Pemberton RW, Cordo HA (2001a) *Nosema* (Microsporidia: Nosematidae) species as potential biological control agents of *Cactoblastis cactorum* (Lepidoptera: Pyralidae):

surveys for the microsporidia in Argentina and South Africa. Fla Entomol 84:527–530

Pemberton RW, Cordo HA (2001b) Potential and risks of biological control of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in North America. Fla Entomol 84:513–526

Pemberton RW, Liu H (2007) Control and persistence of native *Opuntia* on Nevis and St. Kitts 50 years after the introduction of *Cactoblastis cactorum*. Biol Control 41:272–282

Pettey FW (1947) The biological control of prickly pears in South Africa. Department of Agriculture, Science Bulletin 271. Entomology Series No. 22, Pretoria

Pinkava DJ (2003) *Opuntia*. In: Flora of North America Editorial Committee (ed) Flora of North America north of Mexico, vol 4. Oxford University Press, New York, pp 123–148

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–819

Puillandre N, Dupas S, Dangles O, Zeddam J-L, Capdevielle-Dulac C, Barbin K, Torres-Leguizamon M, Silvain J-F (2008) Genetic bottleneck in invasive species: the potato tuber moth adds to the list. Biol Invasions 10:319–333

Raghu S, Walton C (2007) Understanding the ghost of *Cactoblastis* past: historical clarifications on a poster child of classical biological control. Bioscience 57:699–705

Rannala B, Yang ZH (1996) Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J Mol Evol 43:304–311

Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103

Reznick DN, Shaw FH, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934–1937

Roane MK, Griffin GJ, Elkins JR (1986) Chestnut blight, other *Endothia* diseases and the genus *Endothia*. APS Press, St. Paul

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574

Ronquist F, Heulsenbeck JP, van der Mark P (2005) MrBayes 3.1 Manual. Available via http://mrbayes.csit.fsu.edu/index.php. Accessed 2 October 2009

Rosenberg MS (2001) PASSAGE. Pattern analysis, spatial statistics, and geographic exegesis. Version 1.1. Department of Biology, Arizona State University, Tempe, AZ

Rubinoff D, Holland BS, Shibata A, Messing RH, Wright MG (2010) Rapid invasion despite lack of genetic variation in the erythrina gall wasp (*Quadrastichus erythrinae* Kim). Pac Sci 64:23–31

Sauby KE (2009) The ecology of *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) in Florida. Master's thesis, Mississippi State University, Mississippi State, MS

Saura S, Pascual-Hortal L (2007) A new habitat availability index to integrate connectivity in landscape conservation planning: comparison with existing indices and application to a case study. Landsc Urban Plan 83:91–103

Simmonds FJ, Bennett FD (1966) Biological control of *Opuntia* spp. by *Cactoblastis cactorum* in the Leeward Islands (West Indies). Entomophaga 11:183–189

Simon C, Frati F, Breckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc Am 87:651–701

Simonsen TJ, Brown RL, Sperling FAH (2008) Tracing an invasion: phylogeography of *Cactoblastis cactorum* (Lepidoptera: Pyralidae) in the United States based on mitochondrial DNA. Ann Entomol Soc Am 101:899–905

Simonson SE, Stohlgren TJ, Tyler L, Gregg WP, Muir R, Garrett LJ (2005) Preliminary assessment of the potential impacts and risks of the invasive cactus moth, *Cactoblastis cactorum* Berg, in the U.S. and Mexico. Final Report to the International Atomic Energy Agency, IAEA

Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. Mol Ecol 17:351–360

Torchin ME, Mitchell CE (2004) Parasites, pathogens, and invasions by plants and animals. Front Ecol Environ 2:183–190

Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. Nature 421:628–630

Wares JP, Hughes AR, Grosberg RK (2005) Mechanisms that drive evolutionary change. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species invasions: insights into ecology, evolution, and biogeography. Sinauer, Sunderland, pp 229–257

Weir BS (1996) Genetic data analysis II: methods for discrete population genetic data. Sinauer, Sunderland

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. Divers Distrib 14:569–580

Zimmerman HG, Sandi y Cuen MP, Rivera AB (2005) The status of Cactoblastis cactorum (Lepidoptera: Pyralidae) in the Caribbean and the likelihood of its spread to Mexico. Report to the IAEA—TC Project MEX/5/029