

A Case of Recent Long-Distance Dispersal in the *Piriqueta caroliniana* Complex

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We investigated restriction fragment length polymorphism (RFLP) variation in the chloroplast DNA regions *trnS* and *trnC* in 53 populations of the herbaceous perennial *Piriqueta caroliniana* complex, encompassing its known North American range. The goals of this study were to expand the geographic range of a previous study and to determine the origin of an apparently anomalous population, which possessed a phenotype that was incongruous with other populations in the region. The phylogeography of these populations was investigated with nested clade analysis of a one-step network phylogeny that was based on the RFLP variation of the chloroplast genome. We found evidence of restricted gene flow and past fragmentation, which is consistent with a previous study on the phylogeography of this species complex. The morphological and genetic profile of the one anomalous population indicates that it recently originated from south Florida. Given the urban location of this geographically disjunct population, it is probable that this is an example of a recent human-mediated long-distance dispersal event.

Long-distance dispersal can be important in determining the rates of migration and distributional patterns of species (Cain et al. 2000). However, it is exceedingly difficult to obtain accurate estimates of dispersal patterns using direct observation because long-distance dispersal events are often missed (Ouborg et al. 1999). The stochasticity and rarity of successful colonizations that are displaced from the geographic range of a species renders them intrinsically difficult to trace back to their origin. Moreover, disjunct populations that might be interpreted as originating via recent long-distance dispersal may have a longer history of separation and an independent origin relative to populations in the primary range of a species (Avice 1994; Cruzan and Templeton 2000). Methods based on genetic marker variation can detect long-distance dispersal and discriminate between alternative hypotheses for the origin of disjunct populations (Avice et al. 1987; Taberlet et al. 1998;

Templeton 1998). In the present study, we apply genetic marker assays to assess competing hypotheses on the origin of a geographically disjunct population of *Piriqueta caroliniana* (Turneraceae).

The *P. caroliniana* complex is a widely distributed group of herbaceous perennials found throughout South America, the Caribbean, and the southeastern United States. In North America, its range is entirely consistent with the Floridian biome of Remington (1968), which includes the eastern seaboard of Georgia and the panhandle of Florida. This study focuses on three North American morphotypes of this complex that have been distinguished based on morphological and genetic characteristics [see Table 1 in Maskas and Cruzan (2000)]. The *caroliniana* (C) morphotype [= *P. caroliniana* Urban: Small (1933); = *P. caroliniana* var. *tomentosa*: Long and Lakela (1971)] has long, stiff hairs on its stem and broad leaves, and has a decumbent growth form (stems to more than 30 cm). The *viridis* (V) morphotype [= *P. viridis* Small: Small (1933); = *P. caroliniana* var. *glabra*: Long and Lakela (1971)] has narrow, glabrous leaves and glabrous/erect stems that are often more than 30 cm tall. The *tomentosa* (T) morphotype [= *P. tomentosa* Small: Small (1933)] is a diminutive plant (generally less than 20 cm) with multiple short stems and small oval leaves that are covered with short, stellate pubescences. These plants have pale yellow flowers with five petals and are insect pollinated. Seeds appear to be largely gravity dispersed, however, the presence of an eliasome suggests ants may also play a role in dispersal. The three morphotypes studied here appear to have similar floral morphologies and will readily cross to form viable F₁ and later generation hybrids (Ornduff 1970; Wang and Cruzan 1998; Cruzan MB, unpublished data).

In addition to the morphological characters, these three morphotypes differ in their ecological preferences and geographical ranges. The C morphotype is associated with the quartz sand soil that is prevalent in xeric sandhill and turkey oak scrub habitats in northern Florida and southern

Georgia (Figure 1a). The V morphotype is associated with mesic and periodically flooded limestone sand soils of the slash pine/palmetto flatwoods and the short hydroperiod, sparse sawgrass marshes of southern Florida and the Everglades. Between the allopatric regions of the V and C morphotypes lies a broad hybrid zone (H populations; Figure 1a) that appears to have expanded north in recent history (Martin and Cruzan 1999). The T morphotype is restricted to arid hardrock limestone soils that are characteristic of the Atlantic Coastal Ridge, which extends from Miami into Everglades National Park. The T and V morphotypes are known to interbreed to produce hybrid zones along ecotones between dry pine and marsh habitats in the Everglades (Cruzan MB, unpublished data).

There are two characters that suggest that plants in the *P. caroliniana* complex should be poor colonizers. First, their distylous self-incompatibility system renders them obligate outcrossers (Ornduff and Perry 1964; Wang and Cruzan 1998), so at least one plant of each style morph would have to be present to establish a new population (Baker 1955). Second, the primary mode of seed dispersal is by gravity from dry, dehiscent capsules, with the possibility of secondary dispersal via ants. Since there are no specific adaptations that promote long-distance dispersal, we would expect that movement of seeds over larger distances would be uncommon. However, in spite of these impediments, *Piriqueta* seems readily capable of establishing populations following long-distance dispersal, as shown by the multiple colonization events on the Florida peninsula from the Bahamas that were inferred by Maskas and Cruzan (2000). Since these plants are most commonly found in disturbed habitats such as roadsides or vacant lots, it is likely that human-mediated dispersal has become more important in the last century.

Several specimens from a unique population (FL47) located in the western extreme of the Florida panhandle near Pensacola have been deposited in the herbarium at the University of Florida, Gainesville, over the last 10 years (Jones D, personal communication). Existence of both style morphs and plants producing fruits and seeds in this population were confirmed by a visit to the site. This population appears to be anomalous because the plants bore a strong phenotypic resemblance to V plants from south Florida (i.e., erect habit with narrow, glabrous leaves) and did not have the wide leaves and dense trichomes that are characteristic of the C morphotype, the geographic range of which is closest to the location of this disjunct population. While this population may represent a case of recent long-distance dispersal from south Florida, its location outside of the primary range of this species suggests that it could have more ancient origins. Two separate Pleistocene refugia were detected in a previous study [see Table 1 in Maskas and Cruzan (2000)], and it is possible that this disjunct population was derived from a third refuge that was located further west along the Gulf Coast.

Here we describe analyses designed to determine the origin of this anomalous population, which is located several hundred kilometers north of the previously believed

northern limit of the V morphotype. We use nested clade analysis and observations of haplotype distributions to discriminate between two alternative hypotheses for the origin of this population:

That it has arrived in this area via long-distance dispersal from a southern V population and so will share haplotypes with populations in south Florida, or
That it represents a remnant of a western refuge of *P. caroliniana*, as yet unsampled. In this case we would expect that it would be characterized by unique haplotypes that are not represented in previously sampled populations.

It was also our goal to expand the previously sampled North American range of this complex from Maskas and Cruzan (2000) to determine whether the distribution of haplotypes along the eastern seaboard was congruent with previously described geographic patterns found in central Georgia and northern Florida.

Methods

Sampling and DNA Isolation

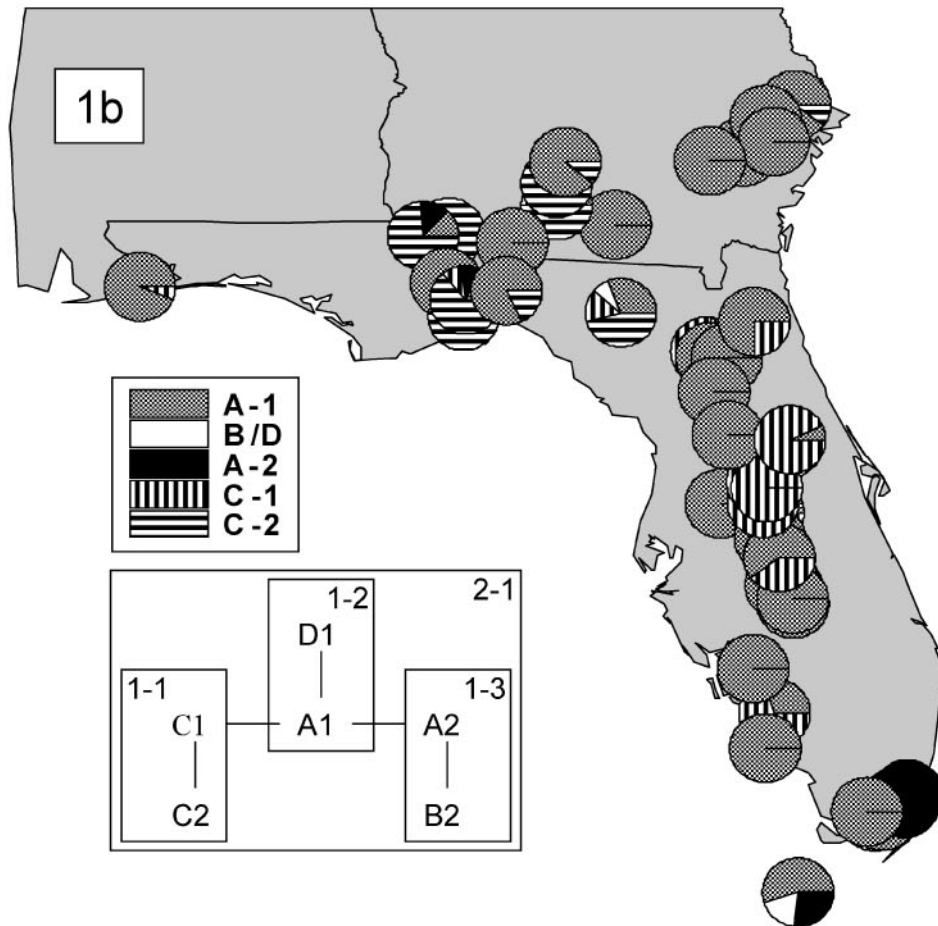
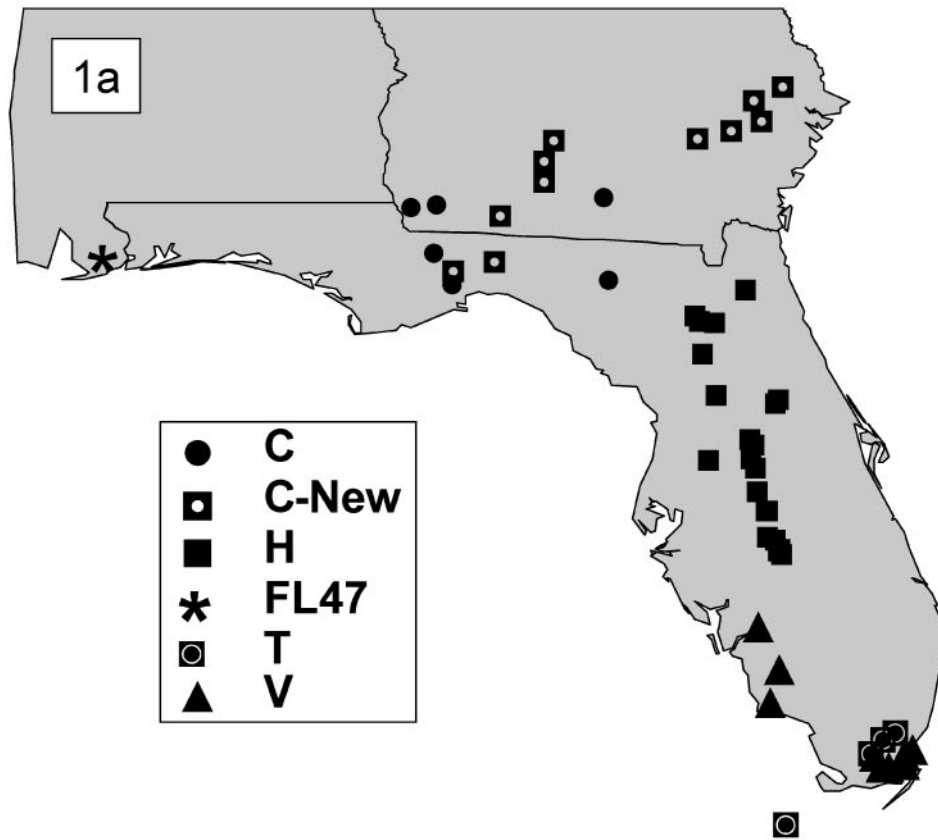
The 53 *Piriqueta* populations included here represent the known North American range of *Piriqueta*, from the Florida Keys in the south to the Atlantic coast of Georgia in the north, and west to the Florida–Alabama border (Figure 1a). Data from 41 populations analyzed were included in previous analyses (Maskas and Cruzan 2000). Twelve populations in the northern portion of the range from the eastern seaboard of Georgia to the western end of the Florida panhandle are unique to this study (Figure 1a). Leaf tissue was sampled from 10 to 20 widely spaced individuals in each population, or from all individuals if fewer than 10 plants were present. Leaf material was stored in silica gel until DNA extraction. DNA was extracted and purified from these leaves based on the method of Edwards et al. (1991) as modified by Martin and Cruzan (1999).

Restriction Fragment Length Polymorphism (RFLP) Analysis

Chloroplast DNA restriction site variation was examined in two intergenic regions, corresponding to *trnS* and *trnC* of Maskas and Cruzan (2000): *trnS*: *trnS*[GGA]-*trnT*[UGU]; *trnC*: *trnC*[GCA]-*trnD*[GUC] (Demesure et al. 1995). PCR products were amplified cut with the four-base recognition enzymes as in Maskas and Cruzan (2000): *trnS* was cut with *Hinf*I, *trnC* with *Rsa*I. RFLP profiles were visualized under ultraviolet (UV) light on 2% agarose gels containing ethidium bromide. All uncommon or unexpected banding profiles were checked by repeating the RFLP procedure for those individuals or all the individuals from their populations.

Analysis

Individuals were assigned to haplotypes based on the RFLP profiles. Relationships among haplotypes were estimated



based on principles of coalescent theory (Crandall and Templeton 1993), and a nested haplotype tree was constructed using the method of Templeton et al. (1992). The geographic associations present among individuals were tested using nested clade analysis as implemented by GeoDis (Posada et al. 2000). Significant geographic associations for genotypes and clades, either dispersion (D_c) of individual clades or displacement (D_n) from their higher-level clade, were used to assess nonrandom geographic associations using the methods of Templeton et al. (1995) and Templeton (1998).

Results and Discussion

This study of the phylogeography of the *P. caroliniana* complex adds to the work of Maskas and Cruzan (2000) by confirming the continuity of RFLP haplotype distributions across the eastern seaboard of Georgia, and by resolving the origin of the aberrant western Florida panhandle population, FL47. Restriction digestion of the *trnS* and *trnC* regions produced two distinct banding patterns each [corresponding to types A/C and 1/2 from Maskas and Cruzan (2000)], resulting in a total of four haplotypes. The previously sampled populations included two additional *trnS* RFLP banding patterns (B and D), for a total of six haplotypes used in these analyses (Figure 1b). No unique or new RFLP haplotypes were found in the 13 populations sampled in this study. The heteroduplex analyses of sequence variation conducted by Maskas and Cruzan (2000) were not included in this study since all of the haplotypes observed using that method had restricted distributions and did not contribute to the interpretation of broader geographic patterns.

Distributions of RFLP haplotypes are consistent with those found by Maskas and Cruzan (2000): both the banding patterns for each cpDNA region were found throughout the area sampled by these 13 populations (restricted to the northern extent of the range of *P. caroliniana*). Banding pattern A from the *trnS* region is more common than C and is monotypic or the dominant haplotype in eight populations in Georgia and two populations in Florida. The C haplotype is monotypic or dominant in two populations in Georgia and one population in Florida. The distribution of types for *trnC* is almost identical to *trnS*, with banding pattern 1 monotypic or dominant in the same eight Georgia and two Florida populations as A, and banding pattern 2 is monotypic or dominant in two Georgia and one Florida population. The new popula-

tions sampled along the eastern seaboard of Georgia contained only A1 or C2 haplotypes and had similar haplotype distributions as the rest of Georgia and north Florida.

The haplotype tree constructed for the data (Figure 1b) follows the basic topology of RFLP haplotypes previously described (Maskas and Cruzan 2000) and meets the 95% confidence criteria to justify the application of maximum parsimony to these data (Templeton et al. 1992). Based on geographic distributions, frequencies within populations, and considerations from coalescence theory [as discussed in Crandall and Templeton (1993) and Maskas and Cruzan (2000)], haplotypes D1, C1, and A2 are most likely to have been derived from A1, C2 from C1, and B2 from A2. Individuals with the A1 haplotype are most common and are found throughout the range studied (Figure 1b). This suggests that A1 is the most ancestral haplotype (Donnelly and Tavaré 1995). With the notable exception of population FL47, individuals with the C1 haplotype were found primarily in populations in central and south Florida, whereas C2 individuals were only found in populations in Georgia and north Florida. The distribution of the A2 haplotype is surprising, with two individuals exhibiting this haplotype in the northern area of the range and the remainder in the south, leaving a large gap in-between. As previously noted by Maskas and Cruzan (2000), it is most likely that the A2 haplotype has independently arisen twice. The two northern populations where it occurs are both dominated by the C2 haplotype, while in the south it is associated with the T morphotype and is found in populations dominated by A1. Derivation of A2 from C2 would require a reinsertion of a large deletion in *trnS*, while derivation of A2 from A1 is more likely, as it requires only a single deletion (Maskas and Cruzan 2000). However, speculation on the origin of northern A2 haplotypes is premature until sequencing confirms their homology.

When the geographic distributions of haplotypes in the nested haplotype tree (Figure 1b) were analyzed by GeoDis, some associations were found to be significantly different from random (Table 1). Of the four haplotypes sampled from more than one population, only the C1 and C2, and all of the higher-order clades (1.1, 1.2, 1.3) displayed significantly nonrandom geographic distributions. The historical processes that have led to the contemporary distribution of these North American populations of *P. caroliniana* can be inferred by comparing the pattern of significant D_c and D_n

←

Figure 1. Distribution of morphotypes (a) and four most common cpDNA haplotypes (b) for plants in the *P. caroliniana* complex in southeastern North America. The *caroliniana* (C) and *viridis* (V) morphotypes have hybridized to produce a broad hybrid zone (H populations) (Martin and Cruzan 1999). The distributions of the *tomentosa* (T) and V morphotypes overlap in southeastern Florida (Maskas and Cruzan 2000). The star in (a) indicates the location of FL47, the aberrant population with a *viridis* phenotype. The C population samples that are unique to this study are labeled as “C-NEW.” The RFLP nested haplotype tree in (b) includes all six haplotypes connected by lines that indicate single RFLP differences. Boxes delineate the arrangement of nested clades analyzed in Table 1. Shading in each circle on the distribution map in (b) represents the frequency of each cpDNA haplotype in each of the populations.

Table 1. Geographic associations based on the nested haplotype tree (Figure 1b) as calculated by GeoDis (Posada et al. 2000)

Clade	Clade/haplotype	Position	D_c	P	D_n	P
1.1	C1	Interior	92.35	↓ <.0001	153.59	↓ <.0001
	C2	Tip	76.71	↓ <.0001	228.81	↑ <.0001
	Interior-tip	—	15.64	>.05	-75.22	↓ <.0001
1.2	A1	Interior	251.36	>.05	251.38	>.05
	D1	Tip	0	>.05	179.00	>.05
	Interior-tip	—	251.36	>.05	72.38	>.05
1.3	A2	Interior	71.98	>.05	71.24	>.05
	B2	Tip	0	>.05	126.83	>.05
	Interior-tip	—	71.98	>.05	-55.60	>.05
2.1	1.1	Interior	251.21	↑ .012	251.25	↑ .015
	1.2	Tip	73.42	↓ <.0001	386.25	↑ <.0001
	1.3	Tip	178.87	↓ <.0001	176.25	↓ <.0001
	Interior-tip	—	125.06	↑ <.0001	-30.00	↓ .012

Significant probabilities of larger (↑) or smaller (↓) values are given; probabilities are calculated from the distribution of 1000 permutations of the data.

values for each haplotype or clade, as interpreted with the key provided by Templeton et al. (1995). The overriding pattern identified in these analyses is restricted gene flow and dispersal, with long-distance dispersal over areas where the *P. caroliniana* complex is absent. The largely non-overlapping distribution of the two C haplotypes (Figure 1b) led to an inference of past fragmentation, probably as a consequence of multiple Pleistocene refugia (Maskas and Cruzan 2000).

Based on the geographic distributions and phylogenetic relationships among cpDNA haplotypes, phylogeographic inferences are largely in agreement with the previous work of Maskas and Cruzan (2000). Coalescence theory predicts that more recently derived haplotypes will have more restricted ranges due to the reduced amount of time for dispersal compared to older haplotypes (Hudson 1990). Such restricted gene flow largely describes the pattern found in these populations of *Piriqueta*. Maskas and Cruzan's (2000) inference of past fragmentation for the C haplotypes has been strengthened by the more intensive sampling of mainland *Piriqueta* populations in the present study. While it is important to recognize that there may be alternative interpretations for these observed geographic associations (Knowles and Maddison 2002), the patterns inferred in this study are corroborated by regional biogeographic patterns (Webb 1990) and by studies on other plant and animal species in southeastern North America (Avice 1994; Sewell et al. 1996; Walker and Avice 1998).

Origin of the Disjunct Population

Of the 16 individuals analyzed from the disjunct population FL47, 15 were A1 haplotype and 1 exhibited the C1 haplotype. No novel haplotypes were detected as would be likely if it had been derived from a novel Pleistocene refuge. Based on the location of this population outside the range of the *P. caroliniana* complex, the morphological similarity with the V morphotype, and the results of the nested clade

analysis, it is most likely that it was derived via recent long-distance dispersal (Figure 1b).

The presence of a C1 haplotype in the disjunct population suggests that the first individuals of this population may have arrived at this location via long-distance dispersal from *viridis* populations in south Florida. This hypothesis is corroborated by the observation that both the A1 and C1 haplotypes occur together in several south Florida populations of plants that are phenotypically similar to this putative colonial population. It is also important to note that more than one cpDNA haplotype was detected in this population, which is consistent with the requirement of colonization by more than one propagule for sexual reproduction in this self-incompatible herb.

Observations made during a site visit and the large number of collections from this site made over the last 10 years indicates that these transplanted V genotypes are thriving in their new location, which is much further north than its native range. This suggests that climate may not be limiting the northward migration of the V genotype. However, it is important to note the location of the FL47 is in a churchyard that may be subjected to regular irrigation during drier periods. Its ultimate success in this region will depend on its ability to establish populations in local natural habitats.

Although *Piriqueta* is not a typical invasive, it does possess some of the characteristics that have been identified as important for successful invasion [as summarized by Rejmánek and Richardson (1996)]. For example, it has several characteristics that would promote the establishment of new populations, such as a relatively short minimum generation time, the ability to reproduce vegetatively, and nonspecific pollination and seed-dispersal mechanisms. In addition, the perennial life history gives a wider window of opportunity for successful pollination to occur (Pannell and Barrett 1998), which would increase the opportunities for the establishment of populations through temporally staggered dispersal to the same site. The introduction of V genotypes to the northern panhandle is so recent that there is so far only

one documented population. If these plants succeed in spreading, introgression with the C morphotype may occur even more rapidly with gene flow from both the west and south into the C allopatric range.

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References

- Avise J, Arnold J, Ball R, Eldredge B, Lamb T, Neigel J, Reeb C, and Saunders N, 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst* 18:489–522.
- Avise JC, 1994. Molecular markers, natural history, and evolution. New York: Chapman & Hall.
- Baker HG, 1955. Self-compatibility and establishment after “long-distance” dispersal. *Evolution* 9:347–348.
- Cain ML, Milligan BG, and Strand AE, 2000. Long-distance seed dispersal in plant populations. *Am J Bot* 87:1217–1227.
- Crandall K and Templeton A, 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134:959–969.
- Cruzan MB and Templeton AR, 2000. Paleocology and coalescence: phylogeographic analysis of hypotheses from the fossil record. *Trends Ecol Evol* 15:491–496.
- Demesure B, Sodji N, and Petit R, 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol Ecol* 4:129–131.
- Donnelly P and Tavaré S, 1995. Coalescents and genealogical structure under neutrality. *Annu Rev Genet* 29:401–421.
- Doyle JJ and Doyle JL, 1988. Natural interspecific hybridization in eastern North America *Claytonia*. *Am J Bot* 75:1238–1246.
- Edwards K, Johnstone C, and Thompson C, 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res* 19:1349.
- Hudson RR, 1990. Gene genealogies and the coalescent process. *Oxf Surv Evol Biol* 7:1–44.
- Knowles LL and Maddison WP, 2002. Statistical phylogeography. *Mol Ecol* 11:2623–2635.
- Long RW and Lakela O, 1971. A flora of tropical Florida. Coral Gables: University of Miami Press.
- Martin LJ and Cruzan MB, 1999. Patterns of hybridization in the *Piriqueta caroliniana* complex in central Florida: evidence for an expanding hybrid zone. *Evolution* 53:1037–1049.
- Maskas SD and Cruzan MB, 2000. Patterns of intraspecific diversification in the *Piriqueta caroliniana* complex in eastern North America and the Bahamas. *Evolution* 54:815–827.
- Ornduff R, 1970. Relationships in the *Piriqueta caroliniana*–*P. cistoides* complex (Turneraceae). *J Arnold Arbor* 51:492–498.
- Ornduff R and Perry J, 1964. Reproductive biology of *Piriqueta caroliniana* (Turneraceae). *Rhodora* 66:100–109.
- Ouborg NJ, Piquot Y, and Van Groenendael JM, 1999. Population genetics, molecular markers and the study of dispersal in plants. *J Ecol* 87:551–568.
- Pannell JR and Barrett SCH, 1998. Baker’s law revisited: reproductive assurance in a metapopulation. *Evolution* 52:657–668.
- Posada D, Crandall KA, and Templeton AR, 2000. A program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. *Mol Ecol* 9:487.
- Rejmánek M and Richardson DM, 1996. What attributes make some plant species more invasive. *Ecology* 77:1655–1661.
- Remington CL, 1968. Suture zones of hybrid interaction between recently joined biotas. *Evol Biol* 2:321–328.
- Sewell MM, Parks CR, and Chase MW, 1996. Intraspecific chloroplast DNA variation and biogeography of North American *Liriodendron* L. (Magnoliaceae). *Evolution* 50:1147–1154.
- Small JK, 1933. Manual of the Southeastern Flora. Chapel Hill: University of North Carolina Press.
- Taberlet P, Fumagalli L, Wust-Saucy A-G, and Cossons J-F, 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Mol Ecol* 7:453–464.
- Templeton AR, 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Mol Ecol* 7:381–397.
- Templeton AR, Crandall KA, and Sing CF, 1992. A cladistic analysis of phenotypic associations and haplotypes inferred from restriction endonuclease mapping and sequence data. III. Cladogram estimation. *Genetics* 132:619–633.
- Templeton AR, Routman E, and Phillips CA, 1995. Separating population structure from population history: a cladistic analysis of geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140:767–782.
- Walker D and Avise JC, 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. *Annu Rev Ecol Syst* 29:23–58.
- Wang J and Cruzan MB, 1998. Interspecific mating in the *Piriqueta caroliniana* (Turneraceae) complex: effects of pollen load size and composition. *Am J Bot* 85:1172–1179.
- Webb SD, 1990. Historical biogeography. In: *Ecosystems of Florida* (Myers RL and Ewel JJ, eds). Orlando: University of Central Florida Press; 70–100.

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