

Patterns of introgression across an expanding hybrid zone: analysing historical patterns of gene flow using nonequilibrium approaches

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Summary

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- Previous studies suggest that the hybrid zone between two taxa in the *Piriqueta caroliniana* complex in central Florida (south-eastern North America) has recently expanded with hybrids replacing parental genotypes across a broad region of the Florida peninsula.

- Here I use patterns of genetic disequilibria and levels of differentiation among populations to infer historical patterns of introgression across this broad hybrid zone.

- There were strong positive associations among taxon-specific alleles at the southern extent of hybridization, but disequilibria values were close to zero across the central and northern portions of the hybrid zone. Levels of among-population differentiation in the central portion of the hybrid zone were relatively low, and increased towards the northern extent of hybridization.

- The high levels of disequilibria are coincident with the sharp clines at the southern end of the hybrid zone, suggesting that there is a tension zone in this region that is maintained by selection against hybrids and dispersal from parental regions. The levels of disequilibria within populations and patterns of differentiation among populations are consistent with historical introgression and northward expansion of this hybrid zone, which may have slowed or ceased in recent generations.

Key words: dispersal, Florida, genetic disequilibria, introgression, *Piriqueta caroliniana*, population differentiation, tension zone, Turneraceae.

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Introduction

The exchange of genetic material among taxa by hybridization has been a potent evolutionary force in a variety of plant and animal groups (Stebbins, 1959; Knobloch, 1972; Harrison, 1990; Arnold, 1997; Dowling & Secor, 1997). It has been hypothesized that gene flow between recently diverged lineages (i.e. introgression; Anderson, 1949) represents an important mechanism for the acquisition of novel traits (Anderson, 1949; Stebbins, 1959; Grant, 1963; Rieseberg *et al.*, 2003). Opportunities to study extensive introgression and the spread of traits across a species' range are rare (Grant & Grant, 1996; Martin & Cruzan, 1999). It is theoretically possible for neutral or adaptive alleles to penetrate a tension zone (i.e. a region of sharp clines between two taxa that is maintained by dispersal and selection

against hybrid genotypes: Key, 1968; Barton, 1979) and infiltrate the range of another taxon through repeated backcrossing (Barton & Gale, 1993). These events may be relatively common, but might be difficult to detect because (1) the phenotypic expression of novel alleles may be subtle and (2) intermediate stages of introgression would be ephemeral if selection were strong or if dispersal rates were high (Barton, 1979; Gavrilets & Cruzan, 1998). There are a number of documented broad hybrid zones (e.g. where the width of the region of hybridization greatly exceeds dispersal: Avise & Smith, 1974; Hafner *et al.*, 1983; de Pamphilis & Wyatt, 1990; Martin & Cruzan, 1999). These cases of extensive introgression deserve special attention because of the opportunities they may offer for investigations into processes of adaptive evolution and trait acquisition via hybridization. By contrast, narrow hybrid

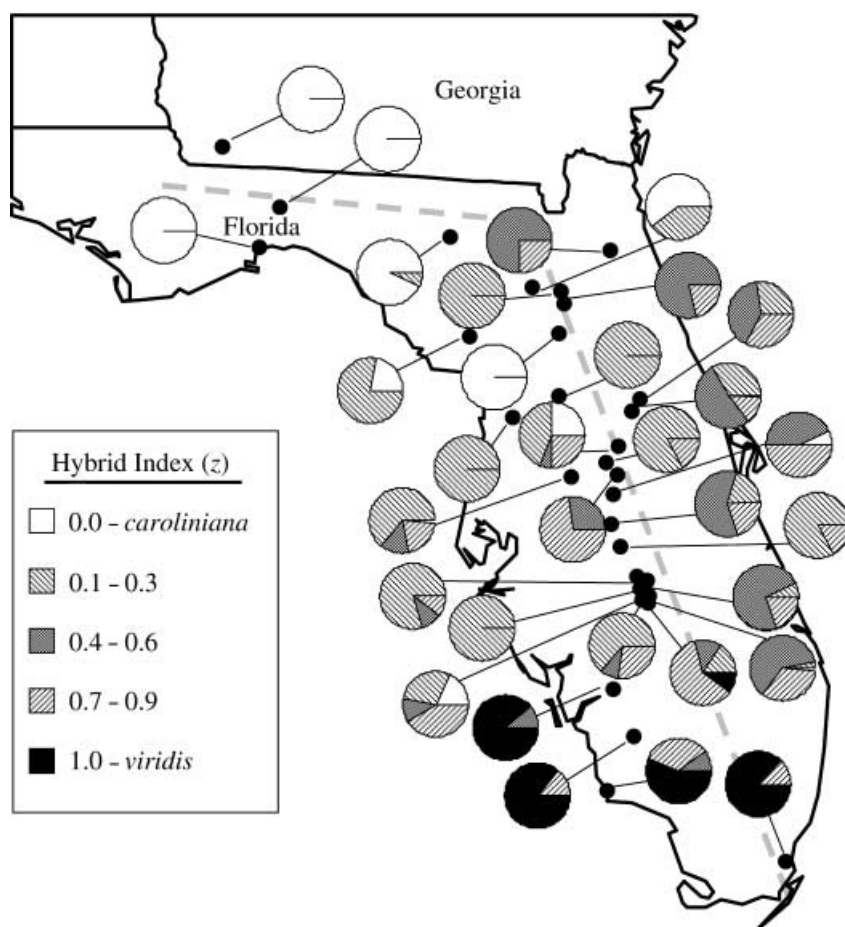


Fig. 1 Distribution of populations and hybrid indices (z-score) for a hybrid zone between the *caroliniana* and *viridis* morphotypes in the *Piriqueta caroliniana* complex in central Florida. The light grey dashed line indicates the position of the transect used to determine the position of each population.

zones typically represent regions of strong selection against hybrids and little or no introgression past sharp clines (Key, 1968; Barton, 1979; Szymura & Barton, 1986; Barton & Gale, 1993; Barton & Shpak, 2000). Analysis of the extent and rate of introgression in broad hybrid zones may provide insights into the processes responsible for the spread of novel alleles within and among populations.

Here I examine patterns of introgression across a broad hybrid zone between two taxa in the *Piriqueta caroliniana* Urban (Turneraceae) complex that extends over much of the Florida peninsula (approx. 300 km) in south-eastern North America (Fig. 1). Previous analyses of this hybrid zone found the highest levels of morphological variation in populations in the northern region of hybridization (Martin & Cruzan, 1999). This high level of morphological variation contrasted with low levels of diagnostic marker diversity (i.e. effective allele number) within populations and strong allele frequency differences among populations. Martin & Cruzan (1999) reasoned that the higher level of morphological variation within northern hybrid populations was indicative of a higher frequency of early generation hybrids, suggesting that hybrid populations in the southern portion of this broad hybrid zone were older. This gradient in the within-population levels of

morphological variation, combined with observed lower levels of genetic marker diversity within northern hybrid populations (i.e. as a result of repeated dispersal followed by population bottlenecks) is consistent with the hypothesis that this hybrid zone has recently undergone a northward expansion (Martin & Cruzan, 1999). Recent population bottlenecks were evident in these northern hybrid populations from the pattern of adjacent populations being nearly fixed for alternative diagnostic alleles. A phylogeographic analysis of the distribution of chloroplast DNA genotypes indicated that one of these taxa (*caroliniana*, see 'The *P. caroliniana* complex' later in this section) had arrived in south-eastern North America before the last Pleistocene glaciation, while the second (*viridis*) had dispersed to southern Florida much more recently (Maskas & Cruzan, 2000). This interpretation of the distribution of chloroplast haplotypes was corroborated by ancillary information on the biogeography and vegetation history of Florida. Interpretation of the chloroplast DNA data in the context of the palaeoecological data suggested that the hybrid zone was formed within the last 7000 yr (Maskas & Cruzan, 2000). The hybrid zone described by Martin & Cruzan (1999) provides an opportunity to assess patterns of introgression that may contribute to the spread of novel traits within a species.

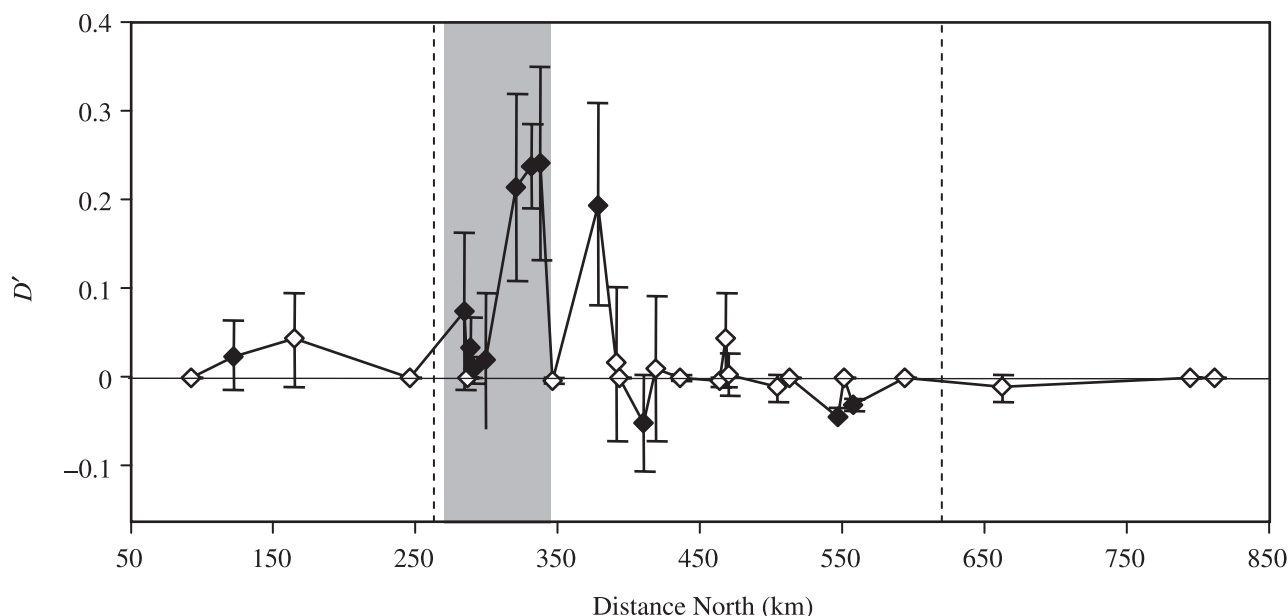


Fig. 2 Mean levels of gametic disequilibrium (D') for all possible pairs of all diagnostic markers combined calculated using methods suitable for dominant markers (Hill, 1974). The shaded region indicates the position of sharp clines for *caroliniana* markers (see Fig. 3) and the vertical dashed lines indicate the geographic extent of the hybrid zone. Filled circles represent populations with D' values that were significantly different from zero. The horizontal axis represents the position of each population along the transect indicated in Fig. 1.

Assessing levels of introgression (gene flow) can be problematic because traditional inference procedures for the frequencies of allele movement assume equilibrium conditions that are probably unrealistic in most cases (Sork *et al.*, 1999; Whitlock & McCauley, 1999). Estimation of gene flow from levels of population differentiation (F_{ST}) are unreliable because historical events (migration, colonization, and extinction) and fluctuations in population size may prevent metapopulations (used here in the broad sense; Hanski & Gaggiotti, 2004; Ouborg & Erickson, 2004) from achieving a balance between gene flow and genetic drift. This is particularly true for hybrid zones, where recent migration of foreign genomes into populations disrupts native allele frequencies. Introgression, along with stochastic dispersal processes or environmental heterogeneity, will produce a genetic mosaic of populations where levels of differentiation are inflated, which would underestimate gene flow if traditional F_{ST} methods are used. While introgression is likely to produce metapopulation conditions that render equilibrium approaches to gene flow untenable, it creates characteristic changes in population genetic structure that may be used to estimate the time since introgression last occurred.

Assessing historical patterns of introgression

I infer historical patterns of introgression across the *Piriqueta* hybrid zone using two nonequilibrium methods for assessing the historical and contemporary effects of gene flow. First, I used levels of gametic disequilibrium (associations among alleles at separate loci) among (nearly) diagnostic marker alleles

(Martin & Cruzan, 1999) to detect recent gene flow. High levels of positive disequilibrium in hybrid populations would be indicative of gene flow from parental regions (Barton & Gale, 1993). Coincidence between a region of positive disequilibrium and the position of the sharp clines described by Martin and Cruzan (1999) (Fig. 2) would support the hypothesis for the presence of a tension zone (i.e. a region of selection against certain hybrid genotypes that are continuously regenerated via dispersal from parental regions: Barton & Hewitt, 1985; Barton & Gale, 1993). In this model, the complement of markers carried by newly arrived migrants produces a positive association among taxon-specific alleles. The level of disequilibrium between diagnostic alleles at separate unlinked loci will be reduced by 50% each generation and can only be maintained by recurring gene flow. Thus, high levels of gametic disequilibrium are indicative of gene flow that has occurred within the last few generations, a condition necessary for the maintenance of a tension zone.

In the second method for the analysis of historical patterns of introgression, I examined levels of population differentiation for diagnostic and shared genetic markers in hybrid metapopulations. This analysis is based on the prediction that groups of populations derived by admixture of two differentiated taxa will initially display high levels of among-population differentiation (F_{ST}), and may require some time before interpopulation migration can reduce the level of differentiation to approach equilibrium conditions (Robertson, 1975; Crow & Aoki, 1984; Whitlock, 1992). The model described by Crow & Aoki (1984) predicts changes in variation among populations under a specific set of conditions

(i.e. an island model where migrants are drawn from other populations at random), but the expectation that an isolated group of genetically differentiated populations will become more homogeneous over time as a result of gene flow is general and will hold true under a variety of models and population structures (Crow & Aoki, 1984; Hedrick, 2004). Genetically heterogeneous metapopulations would result any time there is contact between groups of populations that are characterized by different allele frequencies. Therefore, regions of historical introgression may be marked by high levels of among population differentiation (Latta & Mitton, 1999). While the analysis of among population genetic differentiation has the potential to provide information on historical patterns of gene flow, there have not been previous attempts to characterize patterns of introgression between hybridizing taxa with this method.

Analysis of patterns of genetic disequilibria and differentiation in the same hybrid zone could provide information on different temporal scales of introgression. With low rates of dispersal and sufficient isolation from parental regions, metapopulations in hybrid zones would be expected to approach equilibrium in allele frequencies relatively slowly compared with the loss of within-population gametic disequilibria (Crow & Aoki, 1984; Whitlock, 1992; Hedrick, 2004). These two methods complement each other, as the level of disequilibria indicates very recent gene flow (less than 10 generations) and the variance in allele frequencies among populations can provide an assessment of historical patterns of introgression over a much longer time-frame (tens to hundreds of generations). The combination of these methods can provide a relatively thorough analysis of the historical patterns of introgression and the dynamics of hybrid zone formation and development.

The *P. caroliniana* complex

Here I examine patterns of introgression across a hybrid zone between two taxa in the *P. caroliniana* complex in central Florida. The Florida taxa in this group have historically been separated into species (Small, 1933) or varieties (Long & Lakela, 1971), but most recently have been grouped into a subspecies of *Piriqueta cistoides*, which is widespread throughout the Caribbean and South America (Arbo, 1995). The present study focuses on two taxa in this complex that form a broad hybrid zone across the Florida peninsula (Fig. 1; Martin & Cruzan, 1999). Populations of the two parental taxa that appear to be responsible for this broad region of hybridization are still present in parental regions north of Gainesville and south of Lake Okeechobee, FL, USA. The *caroliniana* morphotype (= *Piriqueta caroliniana* Urban (Small, 1933), = *P. caroliniana* var. *tomentosa* (Long & Lakela, 1971)) occurs in xeric open sites in sandhill scrub communities in north Florida and south Georgia (Maskas & Cruzan, 2000). Plants of this taxon have a decumbent growth

form with long stems that trail across the ground. Leaves are broad-elliptic in shape and plants are covered with both stellate and hirsute hairs, which are particularly dense on the lower stems. By contrast, plants of the *viridis* morphotype (= *Piriqueta viridis* Small (Small, 1933), = *P. caroliniana* var. *glabra* (Long & Lakela, 1971)) are found in relatively mesic sites along the edges of southern slash pine flatwoods. The fine limestone sand soils of these habitats are poorly drained, leading to flooding that can be sustained for several weeks during wetter periods (Abrahamson & Hartnett, 1990). Plants of this morphotype are completely glabrous with narrow-linear leaves and smooth erect stems that are up to 0.5 m tall (Maskas & Cruzan, 2000). Habitats across the hybrid zone are variable, but are primarily xeric sandhill scrub communities. At a few of the hybrid sites the vegetation is more typical of the mesic habitats to the south (Martin & Cruzan, 1999). Both morphotypes and their hybrids retain their phenotypic distinctions when grown in the greenhouse under the same environmental conditions (Martin & Cruzan, 1999; Maskas & Cruzan, 2000; Handy *et al.*, 2004).

Plants of both morphotypes bear large orange-yellow flowers (2–4 cm diameter) and are self-incompatible as a consequence of their distylous breeding system (Ornduff & Perry, 1964; Wang & Cruzan, 1998). The two morphotypes are completely intercompatible and are capable of producing viable F_1 hybrids (Ornduff, 1970; Wang & Cruzan, 1998; Cruzan & Rhode, 2004). Detailed cytological analyses of these taxa are not currently available, but the high viability and fertility of F_1 hybrids indicates that there are probably not any major differences in chromosome arrangements or numbers. While second generation (i.e. F_2 and backcross hybrids) hybrid seeds can be produced (Gavrilets & Cruzan, 1998; Wang & Cruzan, 1998), plants from these crosses have relatively low rates of growth, reproduction, and survival compared to F_1 and parental genotypes (Cruzan & Rhode, 2004).

Objectives

I combined information from patterns of disequilibria and levels of population differentiation to provide insights into the patterns of introgression between two taxa in the *Piriqueta caroliniana* complex. Levels of among-population differentiation and within-population disequilibria among diagnostic genetic markers are used to identify regions of the hybrid zone that have been subject to recent introgression from parental populations and to infer historical patterns of introgression. The information garnered from these analyses corroborates previous results on the direction of hybrid zone expansion and provides an approximate time since initial hybridization for populations in different regions of this broad hybrid zone. The results of these analyses identify distinct regions that are characterized by differences in exposure to recurrent dispersal from parental populations and the inferred relative age of hybrid populations.

Methods

Sampling

Hybrid and parental populations of *Piriqueta* were sampled along an 850 km transect that extends over the length of the Florida peninsula and runs parallel to the Gulf of Mexico coast (Fig. 1). Populations north-west of Lake City Florida and the southern stretch of the Suwannee River were arbitrarily designated as *caroliniana* parental populations, and those south of Lake Okeechobee were arbitrarily designated as *viridis* parental populations. While some of the populations in the parental regions display some admixture (Fig. 1), genotypes found in each of these regions are phenotypically uniform within and among populations (Martin & Cruzan, 1999). Populations were sampled by taking one leaf from all plants in the site up to a maximum of 40 plants (range: 4–40; only five populations had samples < 10). Most populations consisted of 20 or fewer individuals (mean sample size = 15) and smaller populations were distributed across the entire transect. Individual samples within populations were separated by at least 0.5 m to avoid resampling the same genetic individual. Leaves were kept on ice until they were snap frozen in liquid nitrogen and stored at -70°C .

Genetic marker assays

Allozymes Frozen leaves were ground in buffer and the resulting supernatant eluted onto filter paper wicks using previously described methods (Wang & Cruzan, 1998). I conducted a preliminary survey of 14 enzymes on six gel buffer systems on 11% hydrolysed starch gels (Sigma S-4501, Sigma Chemical Co., St Louis, MO, USA). Individual leaf samples were assayed on two buffer systems (morpholine-citrate pH 6.1 and pH 6.8) for six enzymes (6PGD, EST, G6PDH, MDH, PGI, and PGM; Acquaah, 1992) that produced a total of nine resolvable and potentially polymorphic loci using standard procedures (Acquaah, 1992). Loci were interpreted according to the dimeric or monomeric quaternary structure of the enzyme. Each leaf sample was scored for all six loci directly from gels or from digital images.

Diagnostic random amplified polymorphic DNAs (RAPDs) Diagnostic RAPD markers from a previous study (Martin & Cruzan, 1999) were used. Nuclear DNA was isolated using the protocol described by Edwards *et al.* (1991) and purified using the technique described by Maréchal-Drouard and Guillemaut (1995). Plants were assayed for RAPD markers (Williams *et al.*, 1990) using arbitrary 10-base primers provided by Michael Smith Laboratories, University of British Columbia (Vancouver, BC, Canada). At least three parental populations of each morphotype were screened using arbitrary primers (RAPDs) to identify six diagnostic (i.e. nearly fixed in parental populations) markers; two for the *caroliniana* morphotype and four

for the *viridis* morphotype. Markers were checked for repeatability and reliability by modifying the amplification conditions. All leaf samples from the parental and hybrid populations were screened for the same six diagnostic RAPD markers. Each of the markers used in this study were tested for repeatability by changing amplification conditions (Martin & Cruzan, 1999). Data were recorded as the presence or absence of a band for each marker locus.

Data analysis

Hybrid index A hybrid index was calculated for each individual from the RAPD genetic marker data to produce a representation of the genetic composition of each population. I modified the hybrid index proposed by Barton & Gale (1993) by using two normalizing factors to equalize the hybrid scale for the difference in the number of diagnostic markers for each taxon:

$$z = \sum_{i=1}^n \hat{a}_c z_{ci} \sum_{j=1}^m \hat{a}_v z_{vj}$$

Each marker was labeled as 0 or 1, with *caroliniana* markers (z_{ci}) scored as 1 for the absence of a band and *viridis* markers (z_{vj}) given score of 1 for the presence of a band. The hybrid index of each plant was calculated as the weighted average of scores at all six loci, where the unequal scaling factors ($\hat{a}_c = 1/4$ and $\hat{a}_v = 1/8$) produce an index that ranges from 0.0 (for a pure *caroliniana* genotype) to 1.0 (for a pure *viridis* genotype) with the intermediate score of 0.5 corresponding to an F_1 hybrid (all bands present). Note that this index does not necessarily reflect differences in individual marker frequencies so that two individuals with similar hybrid index scores may be characterized by different sets of genetic markers.

Cline analysis The shape of each cline was analysed to compare their relative positions and widths. The center (d_0) and width (w) of the each cline was estimated by fitting the distribution of marker frequencies (p) along the transect to a tanh curve (Butlin *et al.*, 1991). I used the SAS nonlinear least-squares procedure (SAS, 1999) to fit the model

$$p = a + m((1 + \tanh(2(d - d_0)/w))/2) + e$$

(a is the intercept for *viridis*; m is the difference in marker frequency between the morphotypes; d is the distance along the transect; e is the error term). I estimated the four parameters (a , m , w and d_0) using the Marquardt iterative method and allowed the software to calculate partial derivatives. Starting values and increments for each parameter were specified to examine the full range of reasonable parameter values. All other options were left as specified by the software defaults.

Genetic disequilibrium The average level of gametic disequilibrium among diagnostic RAPD markers in each population was

estimated to obtain an indication of patterns of recent gene flow. Diagnostic markers were used because they are expected to be more sensitive to low levels of dispersal (Barton & Gale, 1993). I calculated the disequilibrium (D) for pairs of diagnostic markers using the likelihood procedure described by Hill (1974) for dominant markers. Values of D were standardized to account for allele frequency differences ($D' = D/D_{\max}$) (Lewontin, 1964). For these calculations, the appropriate value of D_{\max} (range: -1 to $+1$) was, for $D < 0$, the larger of the two marker frequencies (m_1 or m_2) and for $D > 0$, the larger of $m_1^*(1 - m_2)$ or $m_2^*(1 - m_1)$ (Hill, 1974). Averages of D' were then calculated among all possible pairs of diagnostic markers (15 pairs total) for each population. I tested whether mean values D' were significantly different from zero by jackknifing t -values for the comparison of each mean to zero, with individual estimates of D' for one pair of marker loci eliminated one at a time (Sokal & Rohlf, 1995). The variance in D' for pairs of loci was higher than the variance among individuals within a population, so jackknifing by dropping individual pairs of loci should provide a more conservative test for significant t -values.

Patterns of population differentiation Diagnostic RAPD markers and allozymes were used to estimate levels of differentiation among populations using the POPGENE software package (Yeh & Boyle, 1997). Since leaf material appropriate for allozyme assays was not available for all populations assayed for RAPD variation, I chose similar subsets of populations for the allozyme and RAPD marker estimates of population differentiation for more accurate comparisons.

To examine the level of differentiation for RAPD markers across the hybrid zone I made separate estimates of G_{ST} for sequentially overlapping groups of four populations starting with the southernmost hybrid population and moving north (using $G_{IS} = 0.0584$ estimated from allozyme data in these same populations with POPGENE). An estimate was made for the group of four southernmost hybrid populations. To form each successive group, the southernmost population was dropped and the next closest population to the north was added. This procedure was repeated until the northern limit of hybridization (as indicated by the distribution of morphological and genetic markers: Martin & Cruzan, 1999) was reached. The relationship between G_{ST} values for each group of four populations and their average latitude was analysed with quadratic and segmented regression models using the NLIN procedure of SAS (SAS, 1999) with average distance among the populations in each group included as a covariate in the analysis. Data points in these regression analyses lacked independence, so I used these results to provide only qualitative assessments of changes in the level of differentiation across the hybrid zone.

Allozyme data were only available for a subset of the populations used in the diagnostic RAPD analyses, so I evenly divided populations in the hybrid zone into three nonoverlapping groups for allozyme analyses of G_{ST} . Each of these three

groups had five populations, and the extent of the southernmost group corresponded to the region that was characterized by high levels of genetic disequilibria (see the Results section). Population differentiation estimates for the allozyme data were also made for seven populations in the *caroliniana* parental region (not all are shown in Fig. 1). Estimates of population differentiation were not conducted with allozyme data in the southern parental populations or with the RAPD data in either parental region because the low level of variability in those populations would have produced estimates near zero.

Results

Distribution of hybrid genotypes

The distribution of hybrids and parental genotypes based on the hybrid index z -scores indicate that pure *viridis* genotypes were only present in the southern portion of the Florida peninsula, and pure *caroliniana* genotypes were restricted to north Florida and southern Georgia (Fig. 1). While several northern populations consisted only of the *caroliniana* morphotype, there were low frequencies of markers associated with the *caroliniana* morphotype in nearly all of the southern *viridis* populations sampled. Populations in central Florida consisted entirely of various hybrids, and with the exception of a few populations in the north, parental genotypes were completely absent from the hybrid zone.

Cline analysis

Analysis of the shape of the RAPD marker clines using the tanh function provided estimates of cline position and width that resulted in a good correspondence between the predicted and observed values across the hybrid zone for the two *caroliniana* markers (Fig. 3). Attempts to fit the tanh model to the four diagnostic *viridis* markers resulted in either failures to converge on an optimal solution or unreasonable estimates of cline width and position (i.e. estimated cline widths were greater than the width of the hybrid zone or cline centers were negative). The lack of fit for these four markers probably resulted from the large variance in frequencies among populations across the northern portion of the hybrid zone (Martin & Cruzan, 1999). Results of the analysis of the frequency of the c28 marker indicates that this cline is located towards the southern end of the hybrid zone ($d_0 = 300.3$ km north of the Florida Keys with a 95% confidence interval of 292.3–308.2) and is relatively narrow ($w = 62.9$ km; 95% confidence interval (CI) = 34.0–91.8; Fig. 3). The intercepts of the c28 curve for *viridis* ($a = 0.03$; 95% CI = -0.08 – 0.14) and *caroliniana* (as indicated by the differential marker frequency in parental populations: $m = 0.96$; 95% CI = 0.84 – 1.08) were not distinguishable from values of zero and one, respectively. The c59 cline, on the other hand, was somewhat further south of the c28 cline ($d_0 = 268.2$ km north, 95%

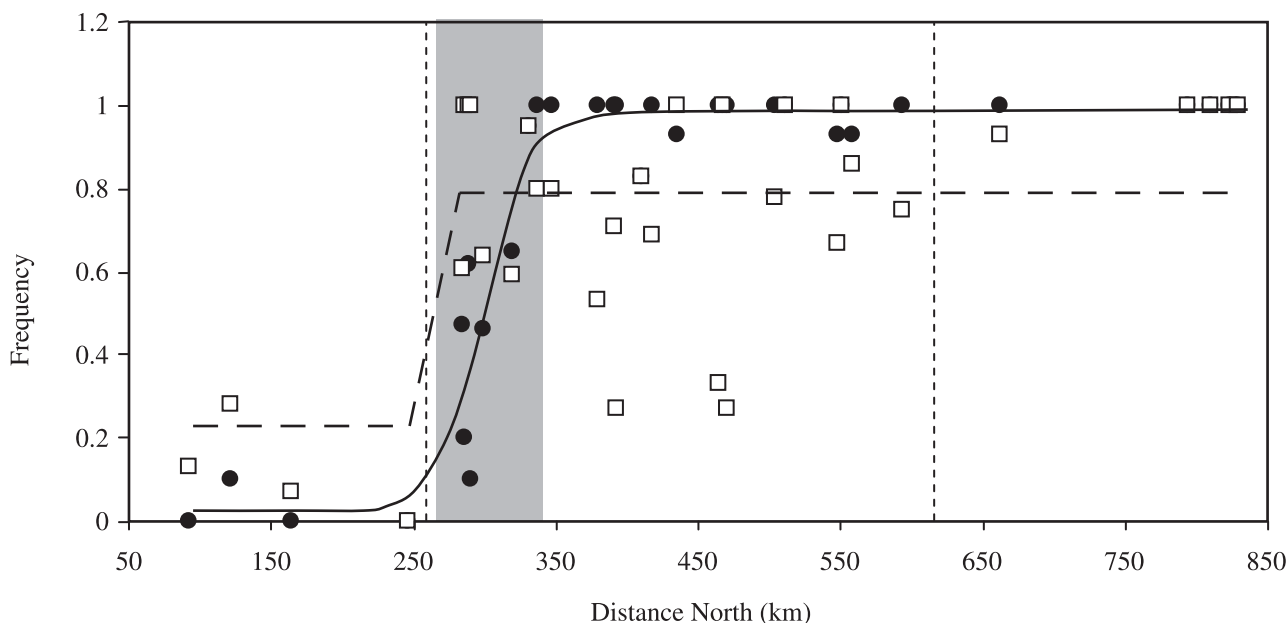


Fig. 3 Position of the c28 (solid line/solid circles) and c59 (dashed line/open squares) clines for the *Piriqueta* hybrid zone in central Florida, USA. Lines are based on the best fit to tanh models (Butlin *et al.*, 1991) using the NLIN procedure of SAS (SAS, 1999). The shaded region indicates the position of the putative tension zone as indicated by the width of these sharp clines. Vertical dashed lines indicate the geographic extent of the hybrid zone. The horizontal axis represents the position of each population along the transect indicated in Fig. 1.

CI = 228.0–308.4) and much narrower ($w = 1.82$ km, 95% CI = 1.42–2.22; Fig. 3). The relatively high frequency variance of this marker across the hybrid zone resulted in nonzero intercepts and large confidence intervals ($a = 0.22$, 95% CI = -0.02 – 0.46 ; and $m = 0.53$, 95% CI = 0.28 – 0.79).

Patterns of disequilibria

Analysis of pairs of diagnostic RAPD markers indicated that strong positive associations among taxon-specific (i.e. intramorphotype) alleles (D') are found only in the southern portion of the hybrid zone (275–400 km north; Fig. 2). When estimates were averaged across diagnostic markers for both morphotypes, the highest levels of positive association were found within the region of the c28/c59 clines and in one population just to the north of them (Figs 2 and 3). With the exception of one population in the central portion of the hybrid zone, which had a significantly negative level of disequilibrium, all disequilibria estimates were significantly positive or near zero. **Nearly all disequilibria estimates were significantly positive at the southern end of the hybrid zone, but in the central and northern regions of hybridization all of the means of estimates of D' were near zero (400–600 km north; Fig. 2).** North of 450 km on the transect, the large majority of individual disequilibria estimates were zero. Variances in disequilibria estimates were relatively low (Fig. 2) and no particular pairs of loci displayed consistently larger or smaller values, so it is unlikely that estimates of D' were affected by linkage among loci.

Patterns of population differentiation

The level of population differentiation (G_{ST}) for overlapping groups of four populations estimated from diagnostic RAPD markers varied across the hybrid zone and displayed a pattern qualitatively similar to the shared allozyme alleles (Fig. 4). In the southern portion of the hybrid zone levels of differentiation were intermediate. They dropped sharply around 360 km north and then gradually increased to very high levels at the northern limit of hybridization.

A segmented regression model (i.e. separate linear and quadratic regressions; Fig. 4) was fitted to these data to provide a qualitative assessment of the level of among population differentiation across the hybrid zone. The segmented model produced a significantly better fit to the data than a single quadratic regression ($F = 13.96$, $P < 0.005$, 1/19 df for the reduction in the error sums of squares by fitting a segmented model). The shared allozyme markers displayed a similar pattern as the diagnostic RAPD markers, with population differentiation lowest in the center, and highest in the northern region of the hybrid zone (Fig. 4). The average distance among populations in each group had no apparent effect on the G_{ST} estimates from RAPD markers ($P > 0.50$ for the effect of average distance among populations in each group on G_{ST}). While the estimates of population structure from overlapping groups of populations for RAPD markers are not completely independent of each other, they serve to provide an indication of the qualitative patterns of differentiation across the hybrid zone.

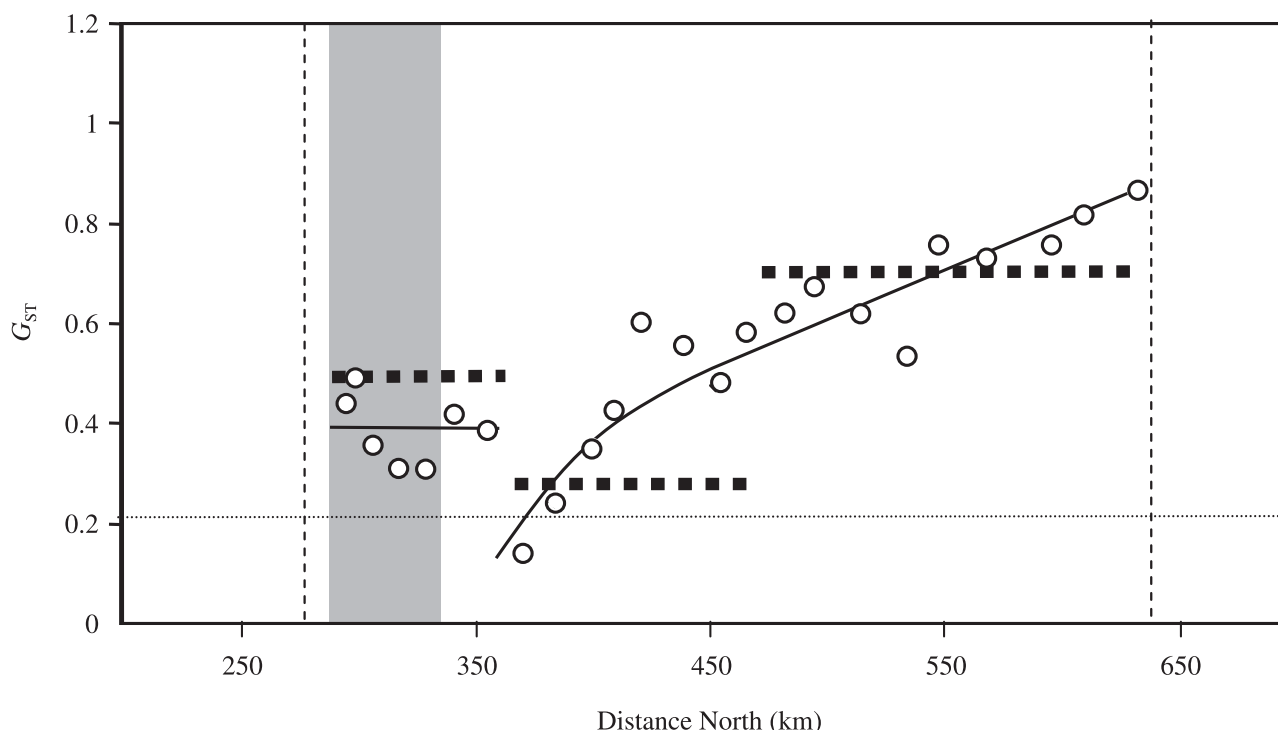


Fig. 4 Levels of population differentiation for diagnostic random amplified polymorphic DNA (RAPD) markers (G_{ST} , solid lines/open circles) and allozymes (thick horizontal dotted lines) across the *Piriqeta* hybrid zone in central Florida. Each dot represents the level of differentiation for a group of four populations, with the location on the horizontal axis corresponding to the southernmost population in each group. Solid lines represent the best fit to the levels of differentiation for RAPD markers using a segmented regression model with the $NLIN$ procedure of SAS (SAS, 1999). The three thick dotted horizontal lines represent G_{ST} estimates from allozyme loci for equally sized groups of populations in each region (south, central and north) of the hybrid zone. The thinner horizontal dotted line indicates the level of differentiation for allozyme markers in the *caroliniana* parental populations. The shaded region indicates the position of sharp clines for *caroliniana* markers (Fig. 3) and the vertical dashed lines indicate the geographic extent of the hybrid zone.

Synopsis

The patterns of among-population genetic differentiation and within-population disequilibria suggest that this hybrid zone can be roughly divided into three separate regions. The southernmost region is characterized by the presence of sharp clines and high levels of genetic disequilibria among diagnostic markers (Figs 2 and 3). Levels of genetic differentiation among populations are intermediate in this region (Fig. 4), probably as a consequence of recurrent gene flow. Populations in the central portion of the hybrid zone have genetic disequilibria estimates near zero (Fig. 2) and levels of genetic differentiation that are close to those found in the northern parental region (Fig. 4). The northernmost region of hybridization is characterized by disequilibria estimates near zero and high levels of among population differentiation (Figs 2 and 4).

Discussion

Levels of genetic disequilibria within populations and differentiation among populations provide a general picture of the contemporary and historical patterns of introgression across this broad hybrid zone. Regions of recent introgression

are characterized by relatively high levels of disequilibria and historical patterns of hybrid zone expansion can be discerned from the degree of population differentiation relative to populations of the parental taxa. The coincidence of the high level of disequilibria and steep clines in south Florida corroborates the hypothesis that selection against early generation hybrid genotypes in this region is responsible for the maintenance of a tension zone. At first this suggestion appears to be in conflict with the observation that this hybrid zone is very broad. However, note that the effects of selection would be expected to be heterogeneous across the genome (Anderson, 1949; Rieseberg & Wendel, 1993; Dowling & Secor, 1997). A number of theoretical analyses have considered conditions that would favor the introgression of neutral or adaptive alleles past a sharp cline that results from negative selection at a second locus (Barton, 1979; Gavrillets & Cruzan, 1998), but empirical investigations that explicitly examine patterns of gene flow past a cline are rare (Marchant *et al.*, 1988; Shaw *et al.*, 1990). The low level of population differentiation in central Florida and high level at the northern extent of the hybrid zone is consistent with the proposal that *viridis* alleles have penetrated past the tension zone and spread northward.

The inferences about patterns of introgression made in this study are dependent on a number of assumptions and conditions. First, I assume that the observed levels of differentiation are largely caused by neutral processes (i.e. gene flow and drift) rather than selection. There are no apparent differences in vegetation composition that correspond to marker frequencies (Martin & Cruzan, 1999) or to levels of differentiation among populations. Second, I assume that dispersal processes are effectively homogeneous across the hybrid zone. This is probably reasonable because the level of fragmentation and the physiognomy of these habitats is relatively homogeneous across this region (pers. obs.). Third, the interpretation of levels of genetic differentiation among populations as an indicator of historical patterns of introgression assumes that hybrid metapopulations have been isolated from long-distance gene flow from parental populations. This is clearly not the case for the southern hybrid populations, which displayed high levels of genetic disequilibrium among diagnostic markers as a result of recent long-distance gene flow from parental populations. For the central portion of the hybrid zone, however, low levels of genetic differentiation and disequilibrium support the hypothesis that these populations have been isolated from introgression from parental populations. The level of genetic differentiation in central Florida compared with other regions of the hybrid zone and parental populations suggests that populations in the center of the hybrid zone have been subject to the homogenizing effects of local gene flow and isolated from long-distance gene flow from parental populations for an extended period of time.

My interpretation of variation in levels of genetic differentiation as an indicator of time since initial hybridization is independent of any particular model of metapopulation structure; gene flow leads to the homogenization of allele frequencies at neutral loci within groups of populations under a wide variety of conditions. Although processes of colonization and extinction in some metapopulations may increase the level of genetic differentiation, making gene flow estimates from allele frequency data unreliable (Whitlock & McCauley, 1999), the inferences of relative times since initial hybridization made here rely solely on comparison of relative levels of genetic differentiation. In doing so I am assuming that the influences of gene flow, colonization, and extinction on population structure are equivalent for hybrid and parental regions. It is possible that higher frequencies of extinction and colonization may occur in hybrid populations if hybrid breakdown were severe, but this would tend to increase rather than decrease the level of genetic heterogeneity in hybrid metapopulations. Given that gene flow is the primary force expected to homogenize allele frequencies among populations (Wright, 1978; Slatkin, 1987; Hedrick, 2004), and assuming that levels of gene flow among local populations are similar across all regions of the hybrid zone, it is reasonable to interpret the relative level of genetic differentiation among hybrid populations as a qualitative assessment of the time since initial hybridization.

Patterns of disequilibrium

High levels of gametic disequilibrium will be generated any time there is movement of pollen or seeds between populations that differ in allele frequencies at multiple loci. Hence, I expect the highest levels of disequilibrium in regions of contact between groups of populations that have the largest differences in allele frequency; specifically, the boundaries between parental and hybrid populations. Based on the relative consistency across individual disequilibrium values within populations, the genetic markers used in this study are probably unlinked and can be used as a reliable indicator of recent gene flow (Barton & Gale, 1993). The pattern of disequilibrium at the southern portion of the hybrid zone is consistent with recent introgression since I expect that gametes and seeds from parental regions will carry only conspecific alleles and will result in positive values of D' . The patterns of disequilibrium observed for *Piriqueta* populations in central Florida provide some clues concerning the recent patterns of gene flow and selection across this broad hybrid zone.

The strong positive values of disequilibrium in populations at the southern end of the hybrid zone are indicative of recent dispersal from parental populations. This region of high disequilibrium corresponds to the position of relatively sharp clines and provides further support for the hypothesis that a tension zone exists in this region (Barton & Gale, 1993). The tension zone model assumes that sharp clines are maintained by a balance between ongoing dispersal from parental regions, which is counterbalanced by selection against hybrid genotypes (Key, 1968; Barton, 1979). In the case of this *Piriqueta* hybrid zone, crosses between plants from the center of the hybrid zone and the *viridis* morphotype produce hybrids (with hybrid maternal parents) that have higher survival and growth than the parental genotypes (Cruzan & Rhode, 2004; unpublished), which is not consistent with the expectations for a tension zone. However, backcross genotypes tend to have much lower rates of growth, reproduction, and survival than parental and F_1 plants under field conditions (Cruzan & Rhode, 2004; unpublished). The lower fitness of second-generation hybrids could effectively reduce the rate of introgression and is likely to be responsible for the maintenance of sharp clines at the southern extent of this broad hybrid zone.

It is notable that levels of disequilibrium remain close to zero in the central and northern portion of the hybrid zone. Some of the slightly positive and negative disequilibrium values in this region may be caused by dispersal among local populations that are fixed for different mixtures of diagnostic alleles from the two parental morphotypes (Martin & Cruzan, 1999). What is more striking is the absence of strong positive disequilibrium in the northern portion of this hybrid zone. If the hybrid zone were continuing to expand north into regions occupied by the *caroliniana* morphotype, then I would expect to see high levels of positive disequilibrium at the northern limit of hybridization owing to genetic exchange between the

northern hybrid and parental *caroliniana* populations. The lack of disequilibria among diagnostic genetic markers in the northern hybrid populations suggests that the northern expansion hypothesized by Martin & Cruzan (1999) has ceased or at least slowed in recent generations.

While high levels of disequilibria among diagnostic markers are expected to occur within tension zones, the region of high disequilibria observed in this *Piriqueta* hybrid zone is broader than the sharp clines at the southern limit of hybridization (i.e. high levels of D' were found in one population just north of the position of the sharp clines). A similar pattern has been observed in a *Bombina* hybrid zone (Szymura & Barton, 1986), where it was interpreted as evidence of infrequent long-distance dispersal (Barton & Gale, 1993). In the present study, it is notable that the extension of high levels of disequilibria occurs in the northern direction only, suggesting that any successful long-distance dispersal is unidirectional. Alternatively, the broad region of high disequilibria at the southern extent of the hybrid zone may represent the effects of a staggered series of sharp clines that cover a broader area than the ones detected. Staggered clines would be expected if the region of hybridization were moving (Moran, 1981), which could be caused by a gradient in the density of individuals or directional gene flow (Barton & Gale, 1993). Hence, either of these causes for the relatively broad region of elevated levels of disequilibria at the southern extent of hybridization is consistent with the hypothesis that introgression into the hybrid zone is asymmetrical, with gene flow occurring primarily from the south.

Patterns of introgression

The pattern of differentiation in diagnostic and shared marker frequencies among populations provides additional clues concerning the historical patterns of introgression north of the tension zone. High levels of population differentiation have been observed in other studies of hybrid zones (Castiglia & Capanna, 1999; Latta & Mitton, 1999) and are a natural consequence of contact between groups of populations with different allele frequencies. In the case of *Piriqueta*, the large differences in genetic marker frequencies among populations that were observed by Martin & Cruzan (1999) have resulted in larger estimates of among-population differentiation in the northern portion of the hybrid zone. This pattern is consistent with the hypothesis that an advancing wave of introgression has traveled across central Florida leaving a gradient of population differentiation in its path.

It is striking that the lowest level of differentiation among populations in diagnostic RAPD and shared allozyme marker frequencies occurred just to the north of the sharp clines and their associated regions of high disequilibria. The lack of strong differentiation among these populations may represent the effects a sustained period of reduced levels of gene flow from the south. Such a pattern would be expected if the sharp

clines at the southern extent of hybridization were acting as an effective dispersal barrier to neutral alleles (Barton, 1979). The monotonic increase in the level of differentiation for diagnostic markers north of this region is consistent with northward expansion of the hybrid zone. Alternatively the hybrid zone may have expanded in both directions, but in that case equally high levels of differentiation would be expected at both the southern and northern extent of the hybrid zone. The position of the sharp clines, patterns of disequilibria, and the gradual increase in the level of differentiation from the center to the northern limit of this *Piriqueta* hybrid zone is consistent with the model of unidirectional introgression proposed by Martin and Cruzan (1999).

We can use the difference in the level of differentiation between the lowest values estimated at the center of the hybrid zone and the northern extent of hybridization to obtain a rough estimate of the time frame for northward introgression (Crow & Aoki, 1984; Whitlock, 1992). The number of generations required for F_{ST} to approach equilibrium can be approximated from the population size (N) and migration rate (m) using the relationship

$$Y' \approx Y(1 - 2m)(1 - 1/2N)$$

(Y is the difference between the observed level of F_{ST} and its expected equilibrium value; Y' is the difference in the subsequent generation) (Crow & Aoki, 1984). The rate of decrease in genetic differentiation is rapid at first but slows as the equilibrium value is approached (Crow & Aoki, 1984). In the case of *Piriqueta* I do not have exact estimates of the migration rate, but I can obtain a range of reasonable values based on gene flow estimates from allozyme data in parental populations where Nm is roughly equal to 1.0. Using a range of population sizes (N probably lies between 25 and 100), estimates of possible values for the number of generations required for G_{ST} to approach equilibrium ($Y' < 0.10$) can be made using numerical evaluations of the above equation ($T = 21\text{--}81$ generations for $N = 50$ and 100, respectively). This analysis suggests that the expansion of the northern portion of this hybrid zone was relatively rapid. For example, although most individuals survive only 3–4 yr, some live at least 10 yr (D. A. Jones, University of Florida, Gainesville, FL, USA, pers. comm.), which will dampen changes in the levels of population differentiation. Using a range of generation times from 5 to 10 yr, these calculations suggest that expansion of this hybrid zone may have occurred as recently as within the last 100–800 yr. However, note that this estimate is based on a number of simplifying assumptions, such as equal levels of diffusion dispersal among all populations. Under conditions of sporadic dispersal among spatially structured populations the time for a return to equilibrium may be substantially longer.

There are several other factors that may have contributed to an underestimate of the time required for introgression based on levels of population differentiation. For example, the level

of differentiation may have been accentuated by population bottlenecks as a result of low fitness in early generation hybrids. (Cruzan & Rhode, 2004; unpublished). The resulting smaller effective population sizes would increase the effects of drift and reduce the level of migration among populations (i.e. less pollen and seeds would be available for dispersal; Husband & Barrett, 1996), which may be responsible for the observation that adjacent hybrid populations in the northern portion of the hybrid zone were often fixed for alternative alleles (Martin & Cruzan, 1999). Additional studies of the demographic consequences of introgression will help clarify the impact of hybrid breakdown on the rate of hybrid zone expansion and provide insights into the conditions leading to the establishment and spread of recombinant hybrid genotypes. Numerical analyses of changes in population differentiation and gametic disequilibria within populations that considers different dispersal modes and the demographic consequences of hybrid breakdown may provide a clearer picture of patterns of introgression in this and other hybrid zones.

Conclusions

The above analyses of patterns of population differentiation and levels of gametic disequilibria suggest that the *Piriqueta* hybrid zone in central Florida probably developed in two stages. The hypothesis that initial contact between these two morphotypes occurred south of the current region of hybridization is supported by the presence of isolated populations in south Florida that possess alleles diagnostic for the *caroliniana* morphotype (Martin & Cruzan, 1999; for a similar situation see Marchant *et al.*, 1988; Shaw *et al.*, 1990). This stage of hybridization would have been marked by the establishment of a narrow hybrid zone with concordant clines, which may have become staggered as the tension zone moved north in response to asymmetrical introgression. The second stage of hybrid zone formation may have been initiated as a result of alleles from the *viridis* morphotype penetrating the tension zone and becoming established in favorable genetic backgrounds. At this point, a northward expansion of the hybrid zone presumably ensued as alleles spread across the central and northern portions of the Florida peninsula. In its current state, the lack of high levels of disequilibria among diagnostic markers at the northern limit of hybridization suggests that hybrid zone expansion may have slowed or ceased, perhaps owing to increased fragmentation of suitable habitat as a consequence of agriculture and urbanization activities.

My ability to infer these historical processes has been facilitated by the relatively low rates of dispersal, which has preserved high levels of population differentiation in regions of recent hybridization, and has allowed the observation of patterns of introgression at an intermediate stage in the development of this hybrid zone. Analyses of levels of genetic differentiation among populations in combination with

patterns of disequilibria within populations have the potential to provide a relatively thorough assessment of patterns of introgression and the historical development of hybrid zones in a wide range of taxonomic groups.

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