Evolution, 54(1), 2000, pp. 137–144

FREQUENCY AND SPATIAL PATTERNING OF CLONAL REPRODUCTION IN LOUISIANA IRIS HYBRID POPULATIONS

JOHN M. BURKE,[1] MARK R. BULGER,[2] RENATE A. WESSELINGH,[3] AND MICHAEL L. ARNOLD[4]
Department of Genetics, University of Georgia, Athens, Georgia 30602-7223
[2]*E-mail: bulger@dogwood.botany.uga.edu*
[3]*E-mail: wesselin@dogwood.botany.uga.edu*
[4]*E-mail: arnold@dogwood.botany.uga.edu*

Abstract.—The plant genera in which natural hybridization is most prevalent tend to be outcrossing perennials with some mechanism for clonal (i.e., asexual) reproduction. Although clonal reproduction in fertile, sexually reproducing hybrid populations could have important evolutionary consequences, little attention has been paid to quantifying this parameter in such populations. In the present study, we examined the frequency and spatial patterning of clonal reproduction in two Louisiana iris hybrid populations. Allozyme analysis of both populations revealed relatively high levels of genotypic diversity. However, a considerable amount of clonality was apparent. Nearly half of all genets (47%) in one population and more than half (61%) in the other had multiple ramets. Furthermore, both populations exhibited relatively high levels of genetic structuring, a pattern that resulted from the aggregation of clonal ramets. The occurrence of clonal reproduction in hybrid populations could not only facilitate introgression through an increase in the number of flowering ramets per genet and/or the survivorship of early generation hybrids, but might also influence the mating system of such populations. Any potential increase in the selfing rate due to cross-pollination among ramets of the same genet may, in turn, increase the likelihood of homoploid hybrid speciation.

Key words.—Clonal reproduction, clonal structure, genotypic diversity, hybrid speciation, Louisiana irises, natural hybridization.

Received December 15, 1998. Accepted July 7, 1999.

Clonal reproduction is a common component of many plant and, to a lesser extent, animal species. In general terms, clonal reproduction is defined as the production of genetically identical organisms from a single ancestral organism by mitosis (King and Stansfield 1990). Mechanisms of clonal reproduction include vegetative spread, production of bulbils and apomictic seed set in plants, and fission and parthenogenesis in animals. Although reviews of the plant and animal literature have revealed that asexual reproduction can be a major component of the life history of certain species, the potential for clonality is not necessarily an accurate predictor of genotypic diversity (Parker 1979; Ellstrand and Roose 1987). Thus, taxa with the capacity for both sexual and asexual reproduction may vary considerably in terms of clonal diversity.

In a recent survey of five major floras, Ellstrand et al. (1996) found that natural hybridization is nonrandomly distributed among plant taxa. Genera in which hybridization is most prevalent tend to be outcrossing perennials with some mechanism for clonal reproduction, most often vegetative spread. This pattern is expected because outcrossers will have more opportunity to hybridize than predominant selfers, perennials are more likely to be found and identified than annuals, and asexual reproduction provides a means for the stabilization of highly heterozygous hybrid genotypes (Grant 1981).

In addition to perpetuating a novel hybrid genotype, the occurrence of clonal reproduction in fertile, sexually reproducing hybrid lineages could have other important evolutionary implications. In cases where hybridization is rare and/or the early generation hybrids have relatively low levels of fertility, clonal reproduction could increase the number of flowering ramets per genet, thereby increasing the opportunity for successful hybrid matings (ramets are defined as clonally produced parts of a plant with their own roots and a potentially independent existence, whereas a genet is comprised of all ramets arising from a single seed; Silvertown and Lovett Doust 1993). Furthermore, clonality may increase the survivorship of a rare hybrid genotype from one year to the next, again improving the chances for successful hybrid matings. In both cases, the ultimate outcome is an increased opportunity for introgression (Stebbins 1959). Perhaps the most important effect of clonal reproduction on the evolution of hybrid populations, however, concerns the influence of clonal structure on the mating system. In particular, it has been suggested that increasing levels of clonal structure will increase the likelihood of self-fertilization in the form of cross-pollination among different ramets of the same genet (i.e., geitonogamy; Handel 1985). This potential increase in the selfing rate is especially important in the context of homoploid hybrid speciation.

The most widely accepted models of homoploid hybrid speciation are the recombinational models of Stebbins (1957) and Grant (1958). Recombinational speciation involves the production of a new homozygous recombinant type for chromosomal and/or genic sterility factors following hybridization between lineages differing by at least two such factors (Stebbins 1957; Grant 1958; Templeton 1981; Rieseberg et al. 1996). Thus, the new recombinant type is interfertile, but at least partially isolated from the parental taxa. Assuming that suitable habitat exists for the establishment of such a recombinant type, this process can lead to the production of a reproductively isolated hybrid species. Because inbreeding leads to an overall increase in homozygosity and can contribute to rapid karyotypic evolution, the importance of inbreeding in recombinational speciation has been emphasized

[1] Present address: Department of Biology, Indiana University, Bloomington, Indiana 47405; E-mail: jburke@bio.indiana.edu.

by both Grant (1958) and Templeton (1981). More recently, McCarthy et al. (1995) used computer simulations to demonstrate that recombinational speciation is indeed more likely as the selfing rate increases.

Alternatively, if two taxa exhibit morphological, physiological, or ecological character differences, hybridization between them can lead to the production of a unique homozygous recombinant type isolated by external, rather than internal, barriers (Grant 1981). Because this process is essentially analagous to recombinational speciation, the mating system and, therefore, the clonal structure of the populations involved, would be expected to play an equally important role. In view of the possible impact of clonal reproduction on the evolution of hybrid populations and the production of hybrid species, it is somewhat surprising that little attention has been paid to quantifying this parameter in hybrid zones. In the present study, we examined the frequency and spatial patterning of clonal reproduction in Louisiana iris hybrid populations.

The Louisiana iris species complex consists of three relatively widespread species (*Iris fulva* Ker-Gawler, *Iris hexagona* Walter, and *Iris brevicaulis* Raf.; Viosca 1935) and a rare endemic diploid hybrid species (*Iris nelsonii* Rand.; Randolph 1966). *Iris fulva* has relatively small, brick red flowers; is predominantly hummingbird pollinated; and is generally associated with the shady understory habitats typical of the banks of bayous of the Mississippi River. *Iris hexagona* has blue flowers; is predominantly bumblebee pollinated; and grows in open, freshwater marshes and swamps across the southeastern coast of the United States. *Iris brevicaulis* has blue flowers; is predominantly bumblebee pollinated; and inhabits mainly drier, oak forest and pasture habitats. *Iris nelsonii* has relatively large, brick red flowers and is found in deep shade in the freshwater swamps of southern Louisiana (Viosca 1935; Randolph 1966; Arnold and Bennett 1993; Cruzan and Arnold 1993). All four species have the capacity for clonal reproduction via vegetative spread. Although these species have relatively wide ranges (with the exception of *I. nelsonii*) and distinct ecological preferences, they occur sympatrically in southern Louisiana, where interspecific matings have led to the production of numerous hybrid populations (Viosca 1935; Riley 1938; Arnold et al. 1990a,b 1991). Furthermore, although the ecology, taxonomy, and evolutionary biology of the Louisiana irises have been studied for well over half a century, the evidence regarding the occurrence of clonal reproduction in natural Louisiana iris populations has been largely anecdotal (but see Bennett 1989). Inferences have generally been based on morphological similarities and differences among individuals.

The goal of the present study was to provide a quantitative description of the frequency and spatial patterning of clonal reproduction in two Louisiana iris hybrid populations. To this end, we (1) estimated levels of genotypic variation within each population; (2) estimated the number and size of clones within each population; (3) analyzed the spatial distribution of ramets and genets within each population; and (4) analyzed the fine-scale genetic structure of the populations.

Materials and Methods

Study Sites

We examined two natural Louisiana iris hybrid populations. The first population, known as ''Foti,'' has previously been shown to consist almost exclusively of hybrids between *I. fulva* and *I. brevicaulis*, with a low frequency of parental-like individuals (Cruzan and Arnold 1993, 1994). The Foti population, which measures 15 m $\times$ 20 m, spans a fenceline separating forest from pasture along the edge of Bayou Teche 6 km north of St. Martinville, St. Martin Parish, Louisiana. In addition to separating forest from pasture, Cruzan and Arnold (1993, 1994) used species-specific RAPD markers to show that this fence marked the transition from an *I. fulva*-like subpopulation (forest) into a more *I. brevicaulis*-like subpopulation (pasture). The second population, known as ''Young's Coulee,'' has previously been shown to consist mainly of *I. fulva*-like hybrid individuals with a low frequency of *I. hexagona* and *I. brevicaulis* RAPD markers (Arnold 1993). The Young's Coulee population, which measures 10 m $\times$ 34 m, is located on a muddy flat along the banks of Young's Coulee approximately 9 km south of the town of Abbeville in Vermilion Parish, Louisiana.

Sample Collection and Electrophoresis

All visible Louisiana iris ramets within each of the two populations were mapped to the nearest cm. Total sample sizes were $n = 269$ and $n = 184$ ramets at Foti and Young's Coulee, respectively. Leaf samples were taken from each mapped ramet, snap frozen in liquid nitrogen, and transported to the laboratory for enzyme extraction. The leaf samples were then crushed under liquid nitrogen with a mortar and pestle. Proteins were extracted and stabilized by the addition of a polyvinylpyrrolidone buffer (Mitton et al. 1979). The crude enzyme extract was absorbed onto filter paper wicks cut from Whatman (Maidstone, England) 3MM paper, placed in 96-well microtest plates, and stored at -70° C.

Samples were electrophoresed on 10% starch gels using four different buffer systems. Only those loci that were polymorphic in at least one of the populations and that exhibited easily interpretable banding patterns were scored; 10 such loci were resolved with eight histochemical stains. Buffer systems and enzyme stains followed the methods of Soltis et al. (1983). Phosphoglucoisomerase (*Pgi-3*) was resolved using a continuous citric acid electrode buffer and a tris-citric acid gel buffer (system 2). Fluorescent esterase (*Fe-1*) and phosphoglucomutase (*Pgm-3*) were resolved using a boric acid–sodium hydroxide electrode buffer and a tris–citric acid gel buffer modified with 0.015 M tris and 0.004 M citric acid (system 6). Menadione reductase (*Mnr-1*) and triose-phosphate isomerase (*Tpi-1, Tpi-3*) were resolved using a discontinuous boric acid–lithium hydroxide electrode buffer and a tris-citric acid gel buffer (a variation of system 8). Fructose-1,6-diphosphate (*F16*), 6-phosphogluconate dehydrogenase (*6Pgd-1, 6Pgd-2*), and shikimate dehydrogenase (*Skdh*) were resolved using a continuous histidine-citrate buffer (system 11). To facilitate consistent and accurate scoring, standards were run on each gel.

Genetic Diversity and Clonal Variation

We estimated the discriminatory power of the genetic data in two ways. First, we used the formula of Aspinwall and Christian (1992) to calculate the probability that ramets of the same multilocus genotype belong to the same genet. Because this probability is greater than 0.99 in both populations (see Results), we assume that all ramets within each population that share a multilocus genotype constitute a genet. Next, following Berg and Hamrick (1994), we calculated the proportion of all possible genotype pairs in each population that differ by two or more loci. This calculation gives the proportion of genotype pairs that would have to be misscored for at least two loci to incorrectly identify two distinct genotypes as members of the same genet.

Deviations from Hardy-Weinberg equilibrium were calculated using Wright's fixation index ($F = 1 - H_o/H_e$; Wright 1922) and tested using χ^2-tests or, in cases with skewed allele frequencies and small sample sizes (*Pgi-3* and *Tpi-1* at Young's Coulee), exact tests (Weir 1996). Significance values were adjusted for multiple comparisons using the sequential Bonferroni procedure of Holm (1979). Because clonal reproduction could cause us to consider some genets more than once, we reduced the dataset to include a single ramet per genet prior to performing these analyses.

The multilocus genotypic diversity of each population was assessed using three diversity measures. The first was the proportion of ramets that were distinguishable on the basis of their multilocus genotype, calculated as the number of distinct genotypes (genets) divided by the total number of ramets sampled; the inverse of this is the mean number of ramets per genet (Ellstrand and Roose 1987). We then estimated D, the complement of the Simpson index corrected for finite sample sizes, as $D = 1 - \sum n_i(n_i - 1)/N(N - 1)$ for $i = 1$ to C, where n_i is the number of ramets belonging to genet i, N is the total number of samples, and C is the number of genets (Pielou 1969; Peet 1974). Values of D range from zero to one, with higher values corresponding to greater genotypic diversity. Finally, genotypic evenness (E), which scales D to the level of polymorphism within each population, was estimated as $E = (D - D_{min})/(D_{max} - D_{min})$, where $D_{min} = (C - 1)(2N - C)/N(N - 1)$ and $D_{max} = N(C - 1)/C(N - 1)$.

Spatial Statistics

The spatial distribution of ramets within each population was analyzed using Ripley's L-statistics (Ripley 1976, 1977). Ripley's $L(t)$ is calculated from the number of ramets in concentric circles of radius t around each ramet. These calculations are generally performed out to a radius of half the shortest side of the plot (7.5 m for Foti and 5 m for Young's Coulee). Furthermore, because of the high density of individuals in our populations, we calculated $L(t)$ at 0.25 m intervals. To assess statistical significance, $L(t)$ values were compared to 95% and 99% confidence envelopes generated from 999 simulations of a population having a Poisson (random) distribution. At a given distance, $L(t)$ is significantly different from zero at $P < 0.05$ (or $P < 0.01$) if the observed value falls above or below the 95% (or 99%) confidence envelope. When $L(t) = 0$, the spatial pattern is random at the scale of t; when $L(t) > 0$ the distribution is clumped; and when $L(t) < 0$ the distribution is uniform. All calculations and simulations were performed using a program developed by P. R. Aldrich (Smithsonian Institution, National Museum of Natural History).

To test for aggregation of clonal ramets, we calculated the average distance among all possible pairs of ramets within each genet as well as the average distance between nonclonemates for each genet. A smaller distance between clonemates than nonclonemates indicates clumping of clonal ramets. These data were analyzed by two-way ANOVA with population and relatedness (i.e., within vs. among genets) as main effects. Both main effects, as well as their interaction, were tested against the residual error.

Fine-Scale Genetic Structure

Following the methods of Loiselle et al. (1995), we estimated the coancestry (f_{ij}) of all possible pairs of individuals within each population from their multilocus genotypes; f_{ij} measures the correlation in the frequencies of homologous alleles at each locus for each pair of mapped ramets (Cockerham 1969). Mean values of f_{ij} were obtained for discrete distance intervals (0.50 m at both sites) by averaging over all pairs of ramets located within that interval. To obtain a multilocus measure of spatial genetic structure, the results were combined over loci by weighting each locus by its expected heterozygosity (H_e). Weightings for each locus were adjusted for unequal sample sizes (i.e., missing genotypes) where appropriate.

To assess statistical significance, f_{ij} values were compared to 95% and 99% confidence envelopes generated under the null hypothesis of no spatial genetic structure. Specifically, intact multilocus genotypes were drawn at random with replacement and assigned to occupied map locations within each population. This procedure was repeated 199 times, with the observed f_{ij} representing the 200th statistic in each distance class. For a given distance class, f_{ij} is significantly different from zero at $P < 0.05$ (or $P < 0.01$) if the observed value falls above or below 95% (or 99%) of these statistics. When $f_{ij} = 0$, there is no significant correlation among individuals at the spatial scale of interest; when $f_{ij} > 0$, individuals in a given distance class are more closely related than expected by chance; and when $f_{ij} < 0$, individuals within a given distance class are less related than expected by chance. All calculations and simulations were performed using a program developed by J. Nason (University of Iowa).

RESULTS

Genetic Diversity

All 10 of the loci that we assayed were polymorphic at Foti, whereas three of the loci (*Mnr-1, Pgm-3,* and *Tpi-3*) were monomorphic at Young's Coulee. Based on the observed levels of variation, the probability that ramets of the same multilocus genotype are members of the same genet is high in both populations (0.9978 and 0.9908 for Foti and Young's Coulee, respectively). Furthermore, over 80% of comparisons between all possible pairs of distinct multilocus genotypes differed by at least two loci in both populations

TABLE 1. Statistics of genotypic diversity in the two hybrid populations. Prop. dist. refers to the proportion of individuals that can be distinguished on the basis of their multi-locus genotype, *D* is the complement of Simpson's index corrected for finite samples, *E* is genotypic evenness, and ramets refers to the mean number of ramets per genet.

Population	Prop. dist.	*D*	*E*	Ramets (SE)
Foti	0.4007	0.9829	0.9681	2.51 (0.33)
Young's Coulee	0.2500	0.9565	0.9509	4.00 (0.50)

(92.32% and 80.61% at Foti and Young's Coulee, respectively). This result indicates that, in the vast majority of cases, we would have had to misscore individuals for at least two loci to incorrectly identify two distinct genotypes as members of the same genet. Fixation indices varied across loci and between populations, but only three of the 17 loci exhibited significant deviations from Hardy-Weinberg equilibrium. Significantly positive values were observed for *6Pgd-2* at Foti ($P < 0.01$) and *Pgi-3* at Young's Coulee ($P < 0.001$), whereas a significantly negative value was observed for *6Pgd-1* at Foti ($P < 0.01$).

Clonal Variation

A total of 107 and 46 unique multilocus genotypes were observed in the Foti and Young's Coulee populations, respectively. The proportions of ramets that had unique multilocus genotypes were, therefore, 0.4007 at Foti and 0.2500 at Young's Coulee (Table 1). Assuming that ramets within each population that share a multilocus genotype belong to the same genet, the number of ramets per genet varied from one to 16 at Foti and from one to 19 at Young's Coulee (Fig. 1). Overall, there were significantly more ramets per genet at Young's Coulee (4.00 ± 0.50, mean ± SE) than at Foti (2.51 ± 0.33; Wilcoxon rank sum test: $\chi^2 = 4.64$, $P = 0.0313$). According to the modified Simpson's index (*D*) and genotypic evenness (*E*), clonal diversity is somewhat higher at Foti than at Young's Coulee; however, clonal diversity is relatively high in both populations (Table 1). Finally, although the forest subpopulation has more ramets per genet (2.88 ± 0.46) than the pasture subpopulation (2.12 ± 0.27), the difference is not significant (Wilcoxon rank sum test: $\chi^2 = 1.31$, $P = 0.2537$).

Spatial Distribution of Ramets and Genets

According to Ripley's *L*-statistics, both populations exhibited a nonrandom distribution of ramets (Figs. 2A, B). Specifically, the *L*(*t*)-values fell above the 99% confidence envelope at all spatial scales at both Foti and Young's Coulee. This pattern is indicative of a significantly clumped distribution at both sites.

Overall, the average distance between members of the same genet was significantly less than the average distance among nonclonemates at both Foti and Young's Coulee (1.49 ± 0.43 m vs. 6.69 ± 0.29 m and 4.43 ± 0.57 m vs. 11.45 ± 0.45 m, respectively, $P \leq 0.001$; Table 2). There was also significant variation among populations ($P \leq 0.0001$). This effect was due, at least in part, to the higher overall density of ramets at Foti (0.89 ramets/m^2) compared to Young's Coulee (0.54 ramets/m^2). Finally, the population × relatedness in-

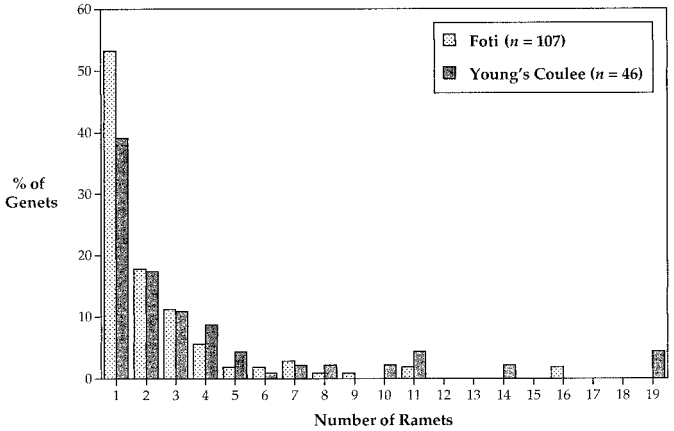

FIG. 1. Frequency distribution of Louisiana iris genet size at Foti ($n = 107$) and Young's Coulee ($n = 46$). Genet sizes were classified according to their number of ramets.

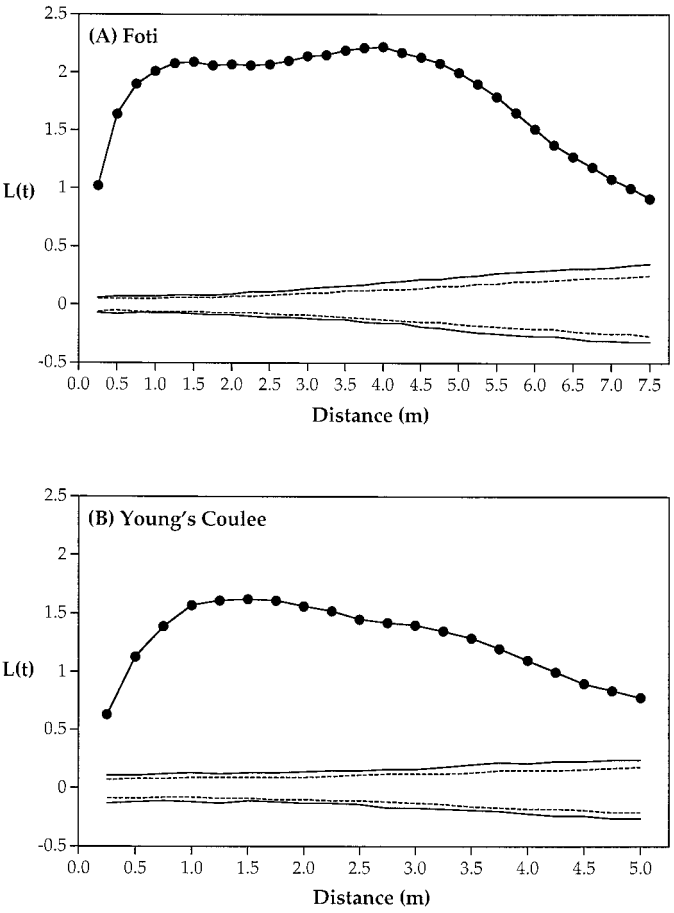

FIG. 2. Ripley's *L*(*t*) plots for all Louisiana iris ramets in the: (A) Foti population ($n = 269$), and (B) Young's Coulee population ($n = 184$). Closed circles give the observed values of *L*(*t*). The solid lines give the upper and lower 99% confidence envelopes around the null hypothesis of $L(t) = 0$. The dashed lines give the upper and lower 95% confidence envelopes around the null hypothesis of $L(t) = 0$. Note that the scale of the x-axis differs among plots.

TABLE 2. ANOVA of the effect of population (Foti vs. Young's Coulee), relatedness (within vs. among genets), and their interaction on interplant distance. All effects were tested against the residual error.

Source	df	SS	MS	*F*	*P*
Population	1	1709.95	1709.95	186.50	≤0.0001
Relatedness	1	679.55	679.55	74.11	≤0.0001
Population × relatedness	1	37.84	37.84	4.13	0.0434
Error	226	2072.15	9.17		

teraction was significant ($P = 0.0434$). This interaction was caused by a greater disparity between the within- and among-genet distances at Young's Coulee relative to Foti.

Fine-Scale Genetic Structure

Analyses of fine-scale genetic structure in the two populations revealed a significant positive correlation among near neighbors (Figs. 3A, B). In the Foti population, this correlation extended to a distance of approximately 2 m, whereas the pattern extended out to approximately 4 m at Young's Coulee. Furthermore, there was an overall tendency toward negative coancestry values beyond approximately 10 m in both populations. Although the spatial scale of this pattern was similar in the two populations, it is important to note that the degree of relatedness (i.e., the magnitude of f_{ij}) was much higher at Young's Coulee than at Foti, especially at smaller distances (< 2 m). When considered separately, the Foti pasture and forest subpopulations both exhibited similar patterns, with positive correlations at smaller spatial scales and a tendency toward negative correlations at larger distances (Figs. 3C, D). It is interesting to note, however, that the degree of relatedness differed markedly between the pasture and forest. The pasture subpopulation was characterized by relatively small (but significantly positive) values of f_{ij} at small scales, whereas the forest subpopulation exhibited a much stronger pattern of relatedness, which was similar in magnitude to the Young's Coulee population.

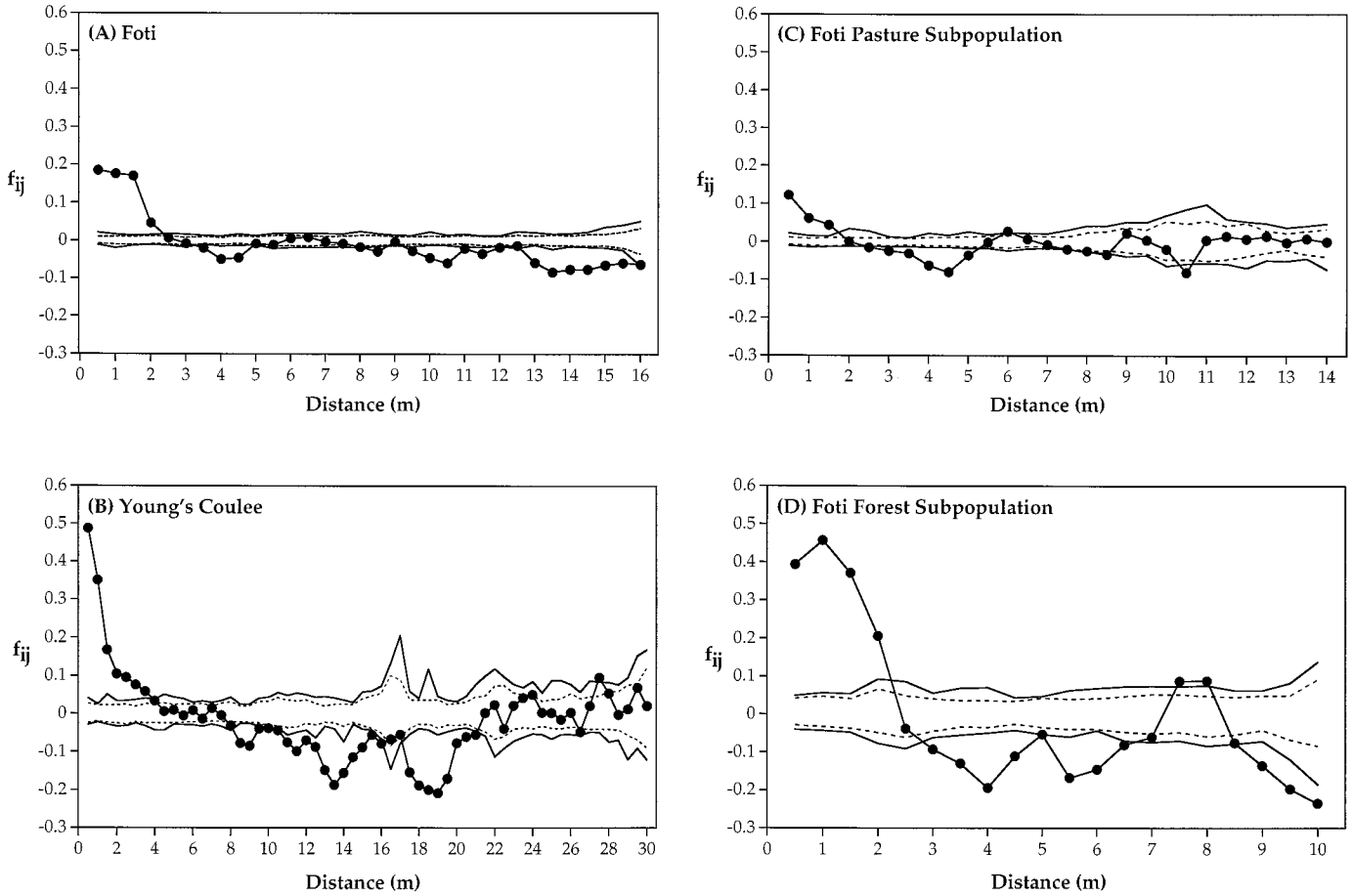

FIG. 3. Coancestry (f_{ij}) plots for all Louisiana iris ramets in the: (A) Foti population ($n = 269$), (B) Young's Coulee populations ($n = 184$), (C) Foti pasture subpopulation ($n = 183$), and (D) Foti forest subpopulation ($n = 86$). Closed circles give the observed values of f_{ij}. The solid lines give the upper and lower 99% confidence envelopes around the null hypothesis of $f_{ij} = 0$. The dashed lines give the upper and lower 95% confidence envelopes around the null hypothesis of $f_{ij} = 0$. Note that the scale of the x-axis differs among plots.

Discussion

In a review of clonal variation in plant species, Ellstrand and Roose (1987) reported that, on average, the proportion of individuals that were distinguishable was 0.17, the mean *D*-value was 0.62, and the mean *E*-value was 0.68. When these values are compared to those in Table 1, it is clear that our populations contain substantially more clonal variation than populations of the average clonal species. However, the values reported by Ellstrand and Roose (1987) come from primarily obligate clonal species, where sexual reproduction is rare or absent. In contrast, the Louisiana irises produce fertile hybrids and sexual reproduction is prevalent in natural populations (e.g., Arnold et al. 1993; Cruzan and Arnold 1994; Hodges et al. 1996). Therefore, the relatively high levels of clonal variation within our populations are likely due to ongoing sexual reproduction within both populations. This being said, it is important to note that there was a considerable amount of asexual reproduction in both populations. In fact, nearly half of all genets at Foti and more than half at Young's Coulee had multiple ramets (47% and 61%, respectively; Fig. 1).

The nonrandom spatial distribution of ramets in both populations seems likely to result, at least in part, from clonal reproduction. The role of clonal reproduction in determining the distribution of ramets becomes clearer in view of the spatial relationships of ramets within and among genets. In particular, the smaller distance among ramets within genets when compared to non-clonemates is indicative of clumping, or aggregation of clonal ramets (Table 2). Of course, it is possible that habitat heterogeneity contributes to the nonrandom distribution of ramets. For example, if the populations consist of a patchwork of suitable and unsuitable habitat, ramets will tend to aggregate in the suitable patches regardless of whether they are derived from a common clonal ancestor. It seems that this effect would be more important at larger spatial scales (i.e., several meters), over which vegetative spread is much less likely to occur. Finally, it is worth noting that some genotypes consist of widely separated ramets within a population. This is especially true at Young's Coulee, where six genotypes occur in patches separated by more than 10 m. Although it is possible that these patches are not actually derived from a common clonal ancestor, the high probability (> 0.99 in both populations) that ramets sharing a multilocus genotype are, in fact, members of the same genet suggests that these individuals are clonally derived. This finding is significant in that vegetative spread does not appear to be the only means of clonal dispersal in these populations. Rather, it seems likely that rhizomes are capable of being moved throughout the populations, perhaps due to breakage and subsequent dispersal during floods or other times of disturbance. Ultimately, this sort of dispersal would make individual genets less susceptible to destruction by localized stochastic events (e.g., fallen trees, pest infestation, etc.).

The apparent effect of asexual reproduction on population structure is further illustrated by the analysis of fine-scale genetic structure (Figs. 3A, B). The observed pattern of positive coancestry at reduced spatial scales accompanied by negative coancestry at larger scales is consistent with relatively high levels of clonal structuring. Thus, assuming that asexual reproduction is occurring and that clonal ramets are clustered, one would expect many near neighbor pairs to share a multilocus genotype. In contrast, shared genotypes among more distantly separated pairs would be very unlikely. This being said, the role of limited dispersal and/or localized inbreeding in producing the observed fine-scale genetic structure cannot be entirely ruled out. However, the magnitude of the f_{ij} values at Foti and, in particular, Young's Coulee are much larger than might be expected in the absence of clonal reproduction. For example, assuming random mating, the coancestry estimates are a measure of the inbreeding coefficient between related individuals with an expected value of 0.25 for full-sibs and 0.125 for half-sibs (Cockerham 1969; Loiselle et al. 1995). At Foti, individuals that occur within 2 m of each other exhibit coancestry values that are similar to those expected in full-sib arrays; the coancestry values are even higher at Young's Coulee. In addition, the occurrence of significant, positive coancestry values over a larger spatial scale at Young's Coulee than at Foti seems to be a function of the fact that ramets of a single genet are, on average, more dispersed at Young's Coulee, again implicating clonal reproduction. Finally, it is interesting to compare the fine-scale genetic structure of the forest and pasture subpopulations at Foti (Figs. 3C, D). In comparison to the pasture subpopulation, the forest subpopulation exhibits much more extreme values of coancestry. These values are similar to those observed at Young's Coulee and may, in part, result from the slightly (but not significantly) higher number of ramets per genet in the forest. It is difficult to say what factors are responsible for this pattern, but interspecific developmental differences and/or habitat disturbance may play a role. As described in the Materials and Methods, both Young's Coulee and the forest subpopulation consist mainly of *I. fulva*-like individuals. In addition, both Young's Coulee and the forest subpopulation occur in relatively undisturbed habitats. In contrast, the pasture subpopulation, which consists mainly of *I. brevicaulis*-like individuals, has experienced considerable habitat disturbance due to grazing.

Taken together, the results of the present study indicate that clonal reproduction may have a substantial impact on the evolution of Louisiana iris hybrid populations. In view of the widespread occurrence of hybridization and introgression between these species, it appears that hybridization events have long-lasting effects on the populations involved. Although the establishment of F_1 hybrids is an extremely rare event in natural iris populations (Arnold et al. 1990a,b, 1991, 1992; Nason et al. 1992; Arnold 1993; Cruzan and Arnold 1993), it seems likely that the clonal nature of these plants will provide repeated opportunities for the production of advanced-generation hybrids (e.g., backcrosses, F_2, F_3, etc.). In fact, greenhouse and field transplant experiments indicate that F_1 hybrids between *I. fulva* and *I. hexagona* survive and reproduce clonally as well as, or significantly better than, both of their parents (Emms and Arnold 1997; Burke et al. 1998). However, perhaps the most important effect of clonal reproduction on the evolution of hybrid populations relates to the influence of clonal structure on the mating system.

As noted in the introduction, increasing levels of clonal structure will increase the likelihood of self-fertilization

among ramets of the same genet (Handel 1985). This change in the mating system, in turn, affects the likelihood of hybrid speciation; increased selfing leads to an increase in the probability of recombinational speciation (Grant 1958; Templeton 1981; McCarthy et al. 1995). It is therefore interesting to note that, if we were to assume random mating among ramets within a population and to ignore the possible effects of spatial structure and phenological differences, we would expect approximately 2% of all matings at Foti and 5% at Young's Coulee to occur between ramets of the same genet. Although these levels seem low, it must be kept in mind that they are extremely conservative estimates. The actual impact of clonal reproduction is likely to be much higher, at least to the extent that pollination is localized and ramets of the same genet have similar flowering times. In fact, a variety of studies have revealed a tendency for pollen to move preferentially among near neighbors (e.g., Levin and Kerster 1968, 1974; Schlising and Turpin 1971). In addition, recent work in the Louisiana irises indicates that nearly 80% of pollinator transitions from one flower to the next occur among nearest neighbors (R. A. Wesselingh and M. L. Arnold, unpubl. data). Although pollen carryover can diminish the effects of such pollinator behaviors (e.g., Thomson and Plowright 1980), matings among near neighbors in clonally structured populations will produce an overall increase in the selfing rate. Interestingly, Cruzan et al. (1994) reported significantly higher levels of selfing (25% vs. 10%) in *I. fulva* than in *I. hexagona.* Although little is known about the patterns of clonal reproduction in natural *I. hexagona* or *I. hexagona*-like hybrid populations, their findings could, in part, result from relatively higher levels of clonal structure in *I. fulva* or *I. fulva*-like hybrid populations.

In view of the above discussion, it is noteworthy that Arnold (1993) suggested that a population such as Young's Coulee, which (1) contains numerous individuals that are genotypically identical and phenotypically similar to *I. nelsonii* individuals (Randolph 1966; Arnold 1993); (2) is adjacent to the area in which *I. nelsonii* occurs (Randolph et al. 1967); and (3) exhibits relatively high levels of clonal structure, may have given rise to *I. nelsonii.* It must be kept in mind, however, that there is no evidence of hybrid sterility in the Louisiana irises, thereby making it unlikely that *I. nelsonii* arose via recombinational speciation. Rather, it seems more likely that *I. nelsonii* arose as a new recombinational type isolated by external, rather than internal barriers. In other words, hybridization may have led to the production of a unique homozygous recombinant type for physiological or ecological character differences between the parental species, thereby allowing *I. nelsonii* to invade a novel habitat (i.e., deep shade in freshwater swamps).

In summary, our findings suggest that clonal reproduction is a potentially important, but often overlooked, aspect of hybrid zone evolution. To gain a better understanding of the role of clonal reproduction in the evolution of hybrid populations, we first need additional data regarding the frequency and pattern of clonal reproduction in such populations. Furthermore, experimental and/or comparative studies of the effects of differing levels of clonal reproduction on the mating system will provide critical data for an evaluation of the possible role of clonal reproduction in homoploid hybrid speciation.

Acknowledgments

The authors thank A. Bouck, J. Johnston, K. Holsinger, E. Kentner, A. Schwarzbach, J. Williams, and two anonymous reviewers for comments on an earlier version of the manuscript; J. Vogel for assisting with the field work; the Foti family for access to their property; and M. Burke and J. Hamrick for technical assistance. This research was supported by the American Iris Society Foundation and National Science Foundation grant DEB-9703853 (to MLA). JMB and MRB were supported by the NSF/USDA/DOE Plant Molecular Evolution Training grant BIR-9220329.

Literature Cited

Arnold, M. L. 1993. *Iris nelsonii*: origin and genetic composition of a homoploid hybrid species. Am. J. Bot. 80:577–583.

Arnold, M. L., and B. D. Bennett. 1993. Natural hybridization in Louisiana irises: genetic variation and ecological determinants. Pp. 115–139 *in* R. G. Harrison, ed. Hybrid zones and the evolutionary process. Oxford Univ. Press, New York.

Arnold, M. L., B. D. Bennett, and E. A. Zimmer. 1990a. Natural hybridization between *Iris fulva* and *I. hexagona*: patterns of ribosomal DNA variation. Evolution 44:1512–1521.

Arnold, M. L., J. L. Hamrick, and B. D. Bennett. 1990b. Allozyme variation in Louisiana irises: a test for introgression and hybrid speciation. Heredity 65:297–306.

Arnold, M. L., C. M. Buckner, and J. J. Robinson. 1991. Pollen mediated introgression and hybrid speciation in Louisiana irises. Proc. Nat. Acad. Sci. 188:1398–1402.

Arnold, M. L., J. J. Robinson, C. M. Buckner, and B. D. Bennett. 1992. Pollen dispersal and interspecific gene flow in Louisiana irises. Heredity 68:399–404.

Arnold, M. L., J. L. Hamrick, and B. D. Bennett. 1993. Interspecific pollen competition and reproductive isolation in *Iris.* J. Hered. 84:13–16.

Aspinwall, N., and T. Christian. 1992. Clonal structure, genotypic diversity, and seed production in populations of *Filipendula rubra* (Rosaceae) from the north central United States. Am. J. Bot. 79:294–299.

Bennett, B. D. 1989. Habitat differentiation of *Iris fulva* Ker-Gawler, *Iris hexagona* Walt., and their hybrids. Ph.D. diss., Louisiana State University, Baton Rouge, LA.

Berg, E. E., and J. L. Hamrick. 1994. Spatial and genetic structuring of two sandhills oaks: *Quercus laevis* and *Quercus margaretta* (Fagaceae). Am. J. Bot. 81:7–14.

Burke, J. M., S. E. Carney, and M. L. Arnold. 1998. Hybrid fitness in the Louisiana irises: analysis of parental and F_1 performance. Evolution 52:37–43.

Cockerham, C. C. 1969. Variance of gene frequencies. Evolution 23:72–84.

Cruzan, M. B., and M. L. Arnold. 1993. Ecological and genetic associations in an *Iris* hybrid zone. Evolution 47:1432–1445.

———. 1994. Assortative mating and natural selection in an *Iris* hybrid zone. Evolution 48:1946–1958.

Cruzan, M. B., J. L. Hamrick, M. L. Arnold, and B. D. Bennett. 1994. Mating system variation in hybridizing irises: effects of phenology and floral densities on family outcrossing rates. Heredity 72:95–105.

Ellstrand, N. C., and M. L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. Am. J. Bot. 74:123–131.

Ellstrand, N. C., R. Whitkus, and L. H. Rieseberg. 1996. Distribution of spontaneous plant hybrids. Proc. Nat. Acad. Sci. 93: 5090–5093.

Emms, S. K., and M. L. Arnold. 1997. The effect of habitat on parental and hybrid fitness: reciprocal transplant experiments with Louisiana irises. Evolution 51:1112–1119.

Grant, V. 1958. The regulation of recombination in plants. Cold Spring Harbor Symp. Quant. Biol. 23:337–363.
———. 1981. Plant speciation. Columbia Univ. Press, New York.
Handel, S. N. 1985. The intrusion of clonal growth patterns on plant breeding systems. Am. Nat. 125:367–384.
Hodges, S. A., J. M. Burke, and M. L. Arnold. 1996. Natural formation of *Iris* hybrids: experimental evidence on the establishment of hybrid zones. Evolution 50:2504–2509.
Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6:65–70.
King, R. C., and W. D. Stansfield. 1990. A dictionary of genetics. Oxford Univ. Press, New York.
Levin, D. A., and H. W. Kerster. 1968. Local gene dispersal in *Phlox.* Evolution 22:130–139.
———. 1974. Gene flow in seed plants. Evol. Biol. 7:139–220.
Loiselle, B. A., V. L. Sork, J. Nason, and C. Graham. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). Am. J. Bot. 82:1420–1425.
McCarthy, E. M., M. A. Asmussen, and W. W. Anderson. 1995. A theoretical assessment of recombinational speciation. Heredity 74:502–509.
Mitton, J. B., Y. B. Linhart, K. B. Sturgeon, and J. L. Hamrick. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. 70:86–89.
Nason, J. D., N. C. Ellstrand, and M. L. Arnold. 1992. Patterns of hybridization and introgression in oaks, manzanitas and irises. Am. J. Bot. 79:101–111.
Parker, E. D., Jr. 1979. Ecological implications of clonal diversity in parthenogenetic morphospecies. Am. Zool. 19:753–762.
Peet, R. 1974. The measurement of species diversity. Annu. Rev. Ecol. Syst. 5:285–307.
Pielou, E. C. 1969. An introduction to mathematical ecology. Wiley-Interscience, New York.
Randolph, L. F. 1966. *Iris nelsonii*: a new species of Louisiana iris of hybrid origin. Baileya 14:143–169.
Randolph, L. F., I. S. Nelson, and R. L. Plaisted. 1967. Negative evidence of introgression affecting the stability of Louisiana *Iris* species. Cornell Univ. Agric. Exp. Sta. Mem. 398:1–56.
Rieseberg, L. H., B. Sinervo, C. R. Linder, M. C. Ungerer, and D. M. Arias. 1996. Role of gene interactions in hybrid speciation: evidence from ancient and experimental hybrids. Science 272: 741–745.
Riley, H. P. 1938. A character analysis of colonies of *Iris fulva, Iris hexagona* var. *giganticaerulea* and natural hybrids. Am. J. Bot. 25:727–738.
Ripley, B. D. 1976. The second-order analysis of stationary point processes. J. Appl. Prob. 13:255–266.
———. 1977. Modelling spatial patterns. J. R. Stat. Soc. B 39: 172–192.
Schlising, R. A., and R. A. Turpin. 1971. Hummingbird dispersal of *Delphinium cardinale* pollen treated with radioactive iodine. Am. J. Bot. 58:401–406.
Silvertown, J. W., and J. Lovett Doust. 1993. Introduction to plant population biology. Blackwell, Oxford.
Soltis, D. E., C. H. Haufler, D. C. Darrow, and G. J. Gastony. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern J. 73:9–27.
Stebbins, G. L., Jr. 1957. The hybrid origin of microspecies in the *Elymus glaucus* complex. Cytologia Suppl. 36:336–340.
———. 1959. The role of hybridization in evolution. Proc. Am. Phil. Soc. 103:231–251.
Templeton, A. R. 1981. Mechanisms of speciation: a population genetic approach. Annu. Rev. Ecol. Syst. 12:23–48.
Thomson, J. D., and R. C. Plowright. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera.* Oecologia 46:68–74.
Viosca, P., Jr. 1935. The irises of southeastern Louisiana: a taxonomic and ecological interpretation. Bull. Am. Iris Soc. 57:3–56.
Weir, B. S. 1996. Genetic data analysis II. Sinauer Associates, Inc., Sunderland, MA.
Wright, S. 1922. Coefficients of inbreeding and relationship. Am. Nat. 56:330–338.

Corresponding Editor: K. Holsinger