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NUCLEAR AND CYTOPLASMIC CONTRIBUTIONS TO INTRASPECIFIC DIVERGENCE IN AN ANNUAL LEGUME

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Abstract.—The genetic architecture of trait differentiation was evaluated between two ecologically distinct populations of *Chamaecrista fasciculata.* Individuals from Maryland and Illinois populations were crossed to create 10 types of seed: Maryland and Illinois parents, reciprocal F_1 and F_2 hybrids, and backcrosses to Maryland and to Illinois on reciprocal F_1 hybrids. Reciprocal crosses created hybrid generation seeds with both Maryland and Illinois cytoplasmic backgrounds. Experimental individuals were grown in a common garden near the site of the Maryland population. In the garden, plants from the Illinois population flowered, set fruit, and died earlier than those from Maryland, likely reflecting adaptations to differences in growing season length between the two populations. Although reproductive components at the flower and whole plant level differed between the two populations, reproductive output as measured by fruit and seed production was similar. Cytoplasmic genes had a subtle but pervasive effect on population differentiation; hybrids with Maryland cytoplasm were significantly differentiated from those with Illinois cytoplasm when all characters were evaluated jointly. The nuclear genetic architecture of population differentiation was evaluated with joint scaling tests. Depending on the trait, both additive and nonadditive genetic effects contributed to population differentiation. Intraspecific genetic differentiation in this wild plant species appears to reflect a complex genetic architecture that includes the contribution of additive, dominance, and epistatic components in addition to subtle cytoplasmic effects.

Key words.—Cytoplasmic effects, dominance, epistasis, genetic architecture, nonadditive genetic variation, population differentiation.

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A basic tenet of the neo-Darwinian synthesis is that evolution studied at the within- and between-population levels provides insight into the processes responsible for macroevolution. Within this framework, most evolutionary studies have focused on the role that selection plays in generating diversity. The type and amount of genetic variation that mediates an organism's response to selection has received less attention. Although there is no doubt as to the fundamental role of natural selection in the evolutionary process (Endler 1986), there is far less unanimity among evolutionary biologists on the genetic structure of trait differentiation (e.g., Eldredge and Gould 1972; Charlesworth et al. 1982; Gottlieb 1984, 1985; Coyne and Lande 1985; Orr and Coyne 1992). In particular, the relative roles of nonadditive genetic effects, including within-locus interactions (dominance) and between-locus interactions (epistasis), and additive genetic effects are relatively unknown (Whitlock et al. 1995; Fenster et al. 1997). Last, the importance of non-Mendelian genes, such as cytoplasmic factors, to adaptive evolution is also understudied.

The genetic architecture underlying traits, specifically the contribution of additive, dominance, and epistatic gene action, is of interest because it may provide insight into the process of evolution. For example, if species act as panmictic units, as proposed by Fisher (1930), then an allele will be screened by selection across all genetic backgrounds and its additive effects will determine its evolutionary fate. In contrast, if species are highly substructured and drift alters allele frequencies within subpopulations, then alleles will be under selection in different genetic backgrounds (Wright 1931). This selection of genes in a genetic context, proposed by Wright (1931, 1932), increases the relative contribution of epistatic interactions to trait genetic architecture. Quantifying the genetic architecture of trait expression will help establish the importance of population genetic structure to the evolutionary process (Wade and Goodnight 1998). Nonadditive genetic effects are also relevant to our understanding of the formation of new species because epistatic differentiation often contributes to species boundaries (Dobzhansky 1937; Orr 1995; Gavrilets and Hastings 1996; Rieseberg 1997). However, the extent to which population-level processes, in other words, epistatic population genetic differentiation, contribute to speciation genetics is relatively unknown. Finally, recent studies on genetic redundancy have demonstrated that the same phenotype may have different genetic structures (Cohan 1984; Fenster and Barrett 1994; Burch and Chao 1999; Fenster and Galloway 2000), reflecting the contribution of nonadditive gene effects. Thus, populations may respond to selective pressures by different genetic mechanisms (Wade and Goodnight 1998). The prevalence of nonadditive genetic effects will give insight into the potential frequency of genetic redundancy.

Limits to our understanding of the type of genetic architecture underlying trait differentiation also include the relative contribution of cytoplasmic effects. Cytoplasmic maternal inheritance has been noted since the rediscovery of Mendelian genetics (Correns 1909, cited in Grant 1975), yet there have been very few studies that have documented cytoplasmic effects on characters involved in ecological differentiation (but see Galloway and Fenster 1999). Although a number of studies have demonstrated that cytoplasmic effects can be important in reproductive isolation between species (Grant 1975; Breeuwer and Werren 1995; Hutter and Rand 1995) and male sterility in plants (Couvet et al. 1990),

we are largely ignorant of the contribution of cytoplasmically inherited genes to population divergence. Given the potential for cytoplasmic genes to enhance fitness and the response to selection (Kirkpatrick and Lande 1989) there is a need for more studies to quantify the role of the cytoplasm in adaptive population differentiation.

Previous work on *Chamaecrista fasciculata*, an annual legume, found widely separated populations to be locally adapted (Galloway and Fenster 2000). Through a comparison of the parental populations with F_1, F_2, and F_3 interpopulation hybrids, we found that populations were differentiated for epistatic gene interactions that contribute to fitness in nature (Fenster and Galloway 2000). Additional backcross generations that had combinations of cytoplasmic and nuclear genes from different populations demonstrated that cytoplasmic genes may also contribute to local adaptation through their interaction with nuclear genes (Galloway and Fenster 1999). In total, these results suggest that gene interactions, both nuclear and cytonuclear, contribute to adaptive differences in fitness between *C. fasciculata* populations. Epistatically based genetic differentiation for fitness between populations may result from epistatic differentiation in fitness-related traits. Alternatively, epistatically based fitness differentiation may reflect interactions among the traits that contribute to fitness independent of the genetic basis of those characters. To fully understand the implications of gene interactions in this species for the process of evolution, it is necessary to evaluate the genetic architecture of traits that in combination contribute to individual fitness. Here we evaluate both the nuclear and cytoplasmic genetic contributions to differentiation of phenological and morphological characters to gain greater insight into the role of genetic architecture in determining adaptive evolution.

Materials and Methods

Two ecologically distinct populations of *C. fasciculata* were chosen to evaluate the genetic architecture of population differentiation. *Chamaecrista fasciculata* Michx. is a self-compatible, predominately outcrossing, annual legume of eastern North America (Irwin and Barneby 1982; Fenster 1991b, 1995). One population was located at the margin of an agricultural field in Beltsville, Maryland; the other was located at Gooselake Prairie, a tall-grass prairie near Morris, Illinois. The growing season is longer in Maryland, with plants germinating several weeks earlier and dying from frost several weeks later than in Illinois (pers. obs.). Previous work revealed a home-site fitness advantage when both populations were grown in Maryland but not when individuals were grown in Illinois (Galloway and Fenster 1999, 2000).

Crosses were conducted under controlled conditions to create five generations of seeds. A single individual from each of 15 maternal families from each population was grown in the greenhouse. Single donor crosses were made between randomly chosen pairs of individuals within each site to create parental seed and between the populations to produce F_1 hybrids. Pairs were reassigned each day pollinations were conducted. Reciprocal crosses between the populations created hybrid seed with the same nuclear genetic composition, but different cytoplasmic genes. Reciprocal hybrids were treated separately in further crosses. A single individual of the F_1 and parental generations for each maternal family was then grown in the greenhouse. The F_1 individuals were crossed to one another to produce F_2 seed. The parental populations were used as pollen donors on F_1 plants to create backcrosses to the Maryland population (BCM) and to the Illinois population (BCI). Last, within- and between-population crosses were conducted on the parental individuals to recreate parental and F_1 seed with the same number of generations in cultivation as the F_2 and backcross lines. This crossing design resulted in a total of 10 types of seed: Maryland and Illinois parental seed, and reciprocal F_1, F_2, BCM, and BCI hybrids. Maternal lines were crossed in an attempt to maintain outcrossing in these small populations. However, as a consequence of conducting crosses between the maternal families each generation, the families are not genetically independent of one another.

Experimental individuals were grown in a common garden. Seeds from each family and seed type were hand-scarified and germinated in the greenhouse. At the two-leaf stage, seedlings were transplanted to a garden plot outside the University of Maryland greenhouse, approximately 10 km from the site of the Maryland population. With the exception of the F_2 generation, five plants from each of 10 maternal families were transplanted for each seed type; one into each of five blocks. The number of F_2 seedlings was increased to 10 per family (two per block) to accommodate anticipated increases in variance in the recombinant hybrid generation. In total, 600 seedlings were transplanted ([8 seed types $\times$ 50 seedlings] + [2 seed types $\times$ 100 seedlings]). Seedlings were stratified by replicate within a block (10 replicates/block, one per family). This planting design resulted in columns (blocks) and rows (replicates) that were used to account for microenvironmental variation. Before transplanting, seedlings were gently rinsed of potting soil, blotted dry, and weighed. They were then planted 25 cm apart; plants dead or missing in the first 2 weeks were replaced. Twenty-one plants died prior to flowering and were not included in the harvest measurements. The garden was weeded and mulched 2 weeks after transplant, and watered during a prolonged dry period when a number of plants wilted.

Phenological and morphological characters were measured on each individual. Plants were checked every third day for the onset of flowering, fruit production, and death. Three phenological intervals were calculated, number of days from: (1) transplant to flowering; (2) onset of flowering to fruit production; and (3) onset of fruit production to death. Plants were harvested when dead. Three vegetative characters were measured: (1) growth (number of leaves produced in an 11-day period, 3 weeks after transplant); (2) harvest aboveground vegetative biomass and (3) degree of branching (branch weight/[mainstem weight + branch weight]). Reproductive characters were measured both at the fruit and the whole plant level. Ovules were counted in three early-developing fruits. Seed number and mean seed weight were quantified for the first three ripe fruits. The number of open flowers per plant was counted weekly and summed across the season to estimate flower production. Fruit production was determined by counting enlarged pedicels on harvested plants. The proportion of initiated fruits to mature was quan-

tified by dividing fruit production by the number of mature fruit plus those aborted (aborted fruit remain attached to the plant).

Statistical Analysis

Initial analyses were conducted on all variables to remove environmental effects. Analysis of (co)variance (AN[C]OVA; Proc GLM, SAS Institute 1990) with row and block as factors was conducted for each character to remove microenvironmental effects due to location in the garden. Replacement of individuals in the transplant process was also included as a factor in the growth analysis, and damage prior to harvest (e.g., loss of major branches) was included as a factor in the analyses of flower production and branching pattern. There were substantial phenotypic correlations ($r \geq 0.80$) in the F_2 and parental generations between vegetative biomass, flower production, and fruit production. To reduce correlations between characters, vegetative biomass was included as a covariate in the calculation of residuals for flower production and dropped from independent analysis. Fruit production was not altered because it estimates fitness. The magnitude of the correlations between the remaining characters averaged $r = 0.18 \pm 0.02$ (SE). Residuals from these AN(C)OVAs were composed predominately of genetic effects and unexplained error and were used in all further analyses.

Differentiation between the parental populations for phenological and morphological characters was evaluated using ANOVA (Proc MIXED, SAS Institute 1996) on residuals of individual traits with population and family (nested within population) as factors. Family was treated as a random effect. Cytoplasmic genetic effects were determined by comparing the reciprocal hybrids of the F_1, F_2, BCM, and BCI generations using multivariate analysis of variance (MANOVA) with residuals of all characters as dependent variables and cytoplasm type (MD or IL), family (nested within cytoplasm type), and hybrid generation as independent variables. Standardized canonical coefficients from the MANOVA were used to evaluate the relative contribution of each response variable to the differences between the cytoplasm types (Scheiner 1993). The MANOVA analysis was conducted using Proc GLM (SAS Institute 1990) and family was treated as a random effect. Growth, flower production, and fruit production were square-root transformed, whereas branching pattern and proportion fruit matured were logit transformed to improve normality. Finally, all plant-level characters (i.e., excluding ovule number per fruit, seed number per fruit, and seed weight) were used in a path analysis to determine the relationship between traits and fitness (Mitchell 1993).

Joint scaling tests were used to evaluate the genetic architecture of population differentiation for phenological and morphological characters. The joint scaling test, developed by Cavalli (1952) and Hayman (1960; see also Mather and Jinks 1982; Lynch and Walsh 1998), uses least-square regression techniques to fit the best line to the generation means. We hierarchically fit genetic models for each variable. Initially, we tested the contribution of additive genetic effects to population differentiation. If genetic differences between the populations are predominately due to additive effects, the F_1 and F_2 generation means will be equivalent to the midparent value (average of the two parents) and the backcross generations to the average of the midparent and their respective parent (e.g., Fig. 1F). We then used a goodness-of-fit test to compare the observed generation means with those predicted under the additive model (Hayman 1958). If there was no significant deviation of fit between the observed and expected means ($P > 0.05$), the model of additive genetic population divergence was accepted.

If the additive model provided a poor fit ($P < 0.05$), we then fit a genetic model that included both additive and dominance effects to the data. If differentiation between the populations is largely due to dominance, the hybrid generations, particularly the F_1, will resemble one of the parental populations more than the other (e.g., Fig. 1G; additive $\times$ additive epistasis in addition to dominance influences F_1 expression; Lynch and Walsh 1998). Note that these comparisons of line means will only reveal a net directional dominance. Opposing dominance effects that cancel each other out will not be detected, therefore findings of differences in dominance between populations are likely robust. (Similarly line means will only reveal a net directional epistasis.) Due to segregation, the F_2 generation will have half the heterozygosity of the F_1 and therefore is expected to be intermediate between the F_1 and the midparent value. However, if gene interactions influence a trait, the F_2 and backcross generation means will differ from their expected values because segregation in the production of gametes will disrupt gene interactions. If the additive-dominance model of differentiation provided a poor fit to the data, a model including the contribution of additive, dominance, and digenic components of epistasis (additive $\times$ additive, additive $\times$ dominance, dominance $\times$ dominance) to population differentiation was tested. However, the adequacy of this model could not be evaluated because the number of generation means is the same as the number of parameters estimated. Therefore, we do not know if higher-order gene interactions contribute to population differentiation (e.g., Mather and Jinks 1982). When epistatic interactions contribute to population divergence, additive and dominance estimates are unreliable (Hayman 1958, 1960). The significance of individual parameter estimates was determined with *t*-tests; both individual and sequential-Bonferroni-adjusted (over all tests) *P*-values are presented.

Joint scaling tests were conducted on family means of the residuals of each trait to maintain statistical independence of the data. As a consequence, the sample size for each line was 10, in contrast to 50 if individual plants had been used. The results are therefore somewhat conservative. Untransformed data were analyzed; tests of transformed data (unpubl.) indicated that the choice of scale does not influence the results. The two cytoplasm types for each generation were pooled in the joint scaling tests. Pooling the reciprocal hybrids also results in a somewhat conservative test for dominance and epistasis because the error variance of the hybrid generations may be inflated.

RESULTS

Phenotypic Differentiation between Populations

Phenological, reproductive, and vegetative traits differed between the Maryland and Illinois populations, although lev-

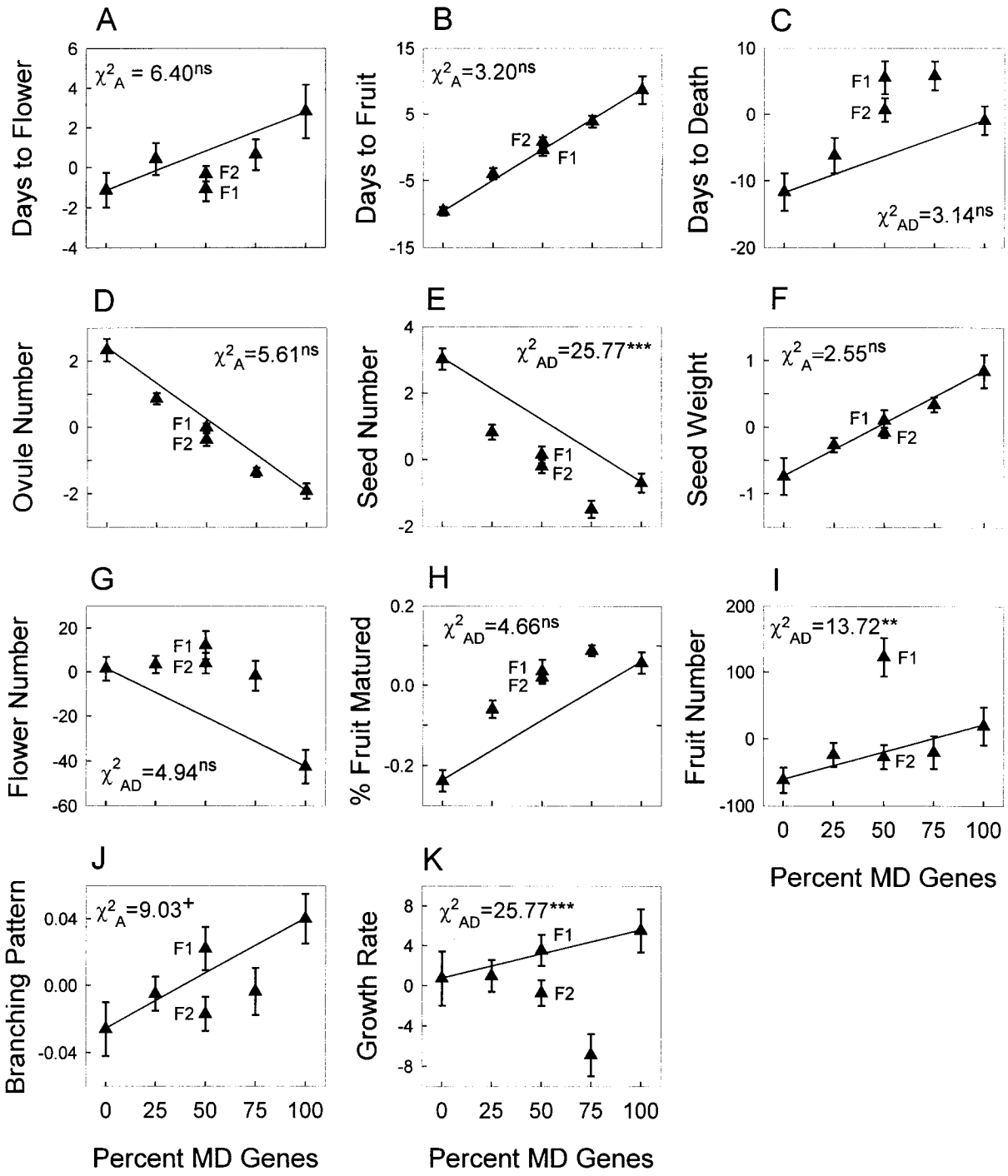

FIG. 1. Means (± 2 SE) of phenological and morphological traits for Maryland and Illinois populations and four generations of hybrids of *Chamaecrista fasciculata* grown in a common garden in Maryland. Goodness-of-fit tests (χ^2) for the best fit model of the architecture of genetic differentiation are presented: A, additive (df = 4); AD, additive-dominance (df = 3). A significant χ^2 statistic indicates that the model is a poor fit to the data; lack of fit of the AD model indicates the presence of epistatic differentiation between populations. A line joins the Maryland and Illinois parental populations. If genetic differences between the populations are due to genes of additive effect, the hybrid population means should fall on the line. Data are means of residuals after removing environmental effects. See Table 1 for significance levels.

els of differentiation were smaller for the fitness estimate, fruit production. Illinois plants were phenologically ahead of those from Maryland; they had shorter intervals between transplant and flowering, flowering and fruit production, and fruit production and death (Table 1). The cumulative effect was that Illinois plants died earlier (128 days from transplant compared to 160 days for Maryland), with most dying before the killing frost. Fruit and seed characters differed between

TABLE 1. Comparison of phenological and morphological traits for the parental generation of *Chamaecrista fasciculata* from Illinois (IL) and Maryland (MD) grown in a common garden in Maryland. *F*-values (df = 1, 18) and *Z*-values from mixed-model ANOVA comparing populations and families within each population are presented with means of raw data.

Variable	Population *F*	Family(Pop) *Z*	Mean (SE) MD	Mean (SE) IL
Phenology (days)				
Planting to flower	5.79*	1.69*	56.85 (1.32)	52.68 (0.89)
Flower to fruit	68.93***	1.69*	51.84 (2.14)	33.61 (0.81)
Fruit to death	10.52**	0.27	53.54 (2.25)	43.30 (2.87)
Reproductive				
Per fruit				
Ovule number	98.93***	2.10*	10.58 (0.26)	14.90 (0.34)
Seed number	77.23***	0.15	8.05 (0.34)	11.92 (0.40)
Seed weight/(mg)	21.49***	0	8.32 (0.25)	6.58 (0.29)
Whole plant				
Flower number	24.66***	1.39+	—[1]	—
% fruit matured	53.60***	0	75.50 (2.44)	45.57 (2.93)
Fruit number	3.58+	0.83	262.69 (32.73)	186.10 (14.18)
Vegetative				
Branch pattern	16.07***	0	0.80 (0.02)	0.74 (0.01)
Growth rate	1.66	0	41.30 (2.17)	35.67 (2.83)

+$0.05 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[1]Analysis on flower number adjusted for plant size (means in Fig. 1), so whole plant means are not presented.

the sites, with Illinois plants producing more ovules and more, lighter seeds per fruit than Maryland plants (Table 1). Illinois individuals also produced more flowers per unit biomass, but matured a substantially smaller proportion of the fruits they initiated and consequently produced marginally fewer fruit than Maryland plants. Growth rate did not differ between the two populations, however, Maryland individuals were more highly branched than those from Illinois (Table 1). Families within each population differed for days to flowering and to fruit production and for ovule number, likely indicating a combination of both within-population genetic variation and maternal effects for these characters (Table 1). All phenological, vegetative, and whole plant reproductive traits contribute to fitness as estimated by fruit production (Fig. 2).

Cytoplasmic Differentiation between Populations

A comparison of the reciprocal hybrids found that cytoplasm type significantly influenced phenological and morphological characters. When all characters were included in a MANOVA, there was a difference between individuals with Illinois cytoplasm and those with Maryland cytoplasm, and this difference was consistent across the four hybrid generations (cytoplasm × generation was not significant, Table 2A). Characters also varied among families, however, family variation was not consistent across all hybrid generations (Table 2A). When univariate tests were conducted on each variable separately (analyses not shown), cytoplasm type only influenced seed number per fruit ($F_{1,32} = 4.26$, $P < 0.05$). Hybrid individuals with Maryland cytoplasm produced

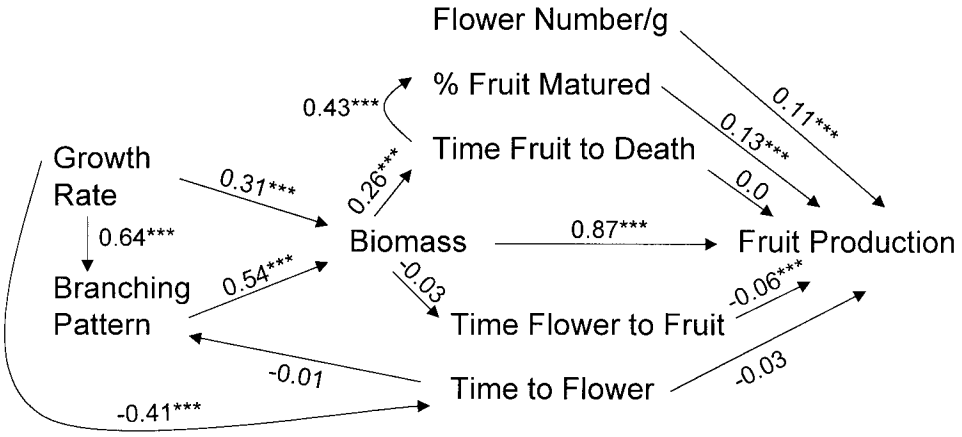

FIG. 2. A schematic diagram representing the relationship between measured traits and fruit production, an estimate of fitness. Standardized regression coefficients (see Table 1 for significance levels) from multiple regressions are provided to evaluate the strength of the relationship among traits. Fruit-level reproductive traits were omitted from the diagram because they are expected to largely influence fitness as estimated by seed production.

TABLE 2. Multivariate analysis of variance to compare reciprocal hybrids between Illinois and Maryland *Chamaecrista fasciculata* populations. Nuclear effects of four hybrid generations (F_1, F_2, BCM, BCI) were compared in Illinois and Maryland cytoplasmic backgrounds. The standardized canonical coefficients describe the relative contribution of individual traits to the overall cytoplasm effect. (A) MANOVA factor effects. (B) Standardized canonical coefficients for contribution of traits to the cytoplasm effect. Sign refers to the direction of the effect (positive, MD cytoplasm increases trait; negative, MD cytoplasm decreases trait).

Source	df[1]	Wilks' lambda	*F*	*P*
A.				
Cytoplasm	11, 25	0.379	3.72	0.0031
Family(cytoplasm)	385, 318	3.32×10^{-5}	1.38	0.0015
Generation	33, 80	0.059	3.93	0.0001
Cytoplasm × generation	33, 80	0.482	0.683	0.89
Family(cyto) × generation	407, 3647	0.260	1.22	0.0029
B.				
Phenology		Reproductive		
Planting to flower	0.157	Ovule number/fruit		−0.248
Flower to fruit	−0.643	Seed number/fruit		0.766
Fruit to death	0.418	Seed weight		0.069
Vegetative		Flower number		0.391
Growth rate	−1.388	% fruit matured		−1.187
Branching pattern	0.211	Fruit number		0.815

[1] Degrees of freedom for the numerator, denominator.

more seeds than those with Illinois cytoplasm (in contrast to the parental generation, Table 1). If seed number is removed from the model, hybrids with Illinois cytoplasm are still significantly differentiated from those with Maryland cytoplasm ($F_{10,26} = 2.70$, $P < 0.02$). Growth rate and percent fruit matured, followed by fruit number, seeds per fruit, and time from flower to fruit, made the largest contribution to the expression of cytoplasmic differences between the populations (Table 2B). Seed number per fruit and fruit number are greater in individuals with Maryland cytoplasm, whereas growth rate, percent fruit matured, and time from flower to fruit are greater in plants with Illinois cytoplasm. Note that these effects of the cytoplasmic genes frequently oppose those found in the parental generation (cf. Table 1)

Nuclear Genetic Differentiation between Populations

The genetic architecture of population differentiation for phenological traits included additive and dominance gene action. Population differences for the intervals from transplant to flowering and from flowering to first fruit were associated with additive gene action (Table 3, Fig. 1A, B). The additive model is a particularly good fit for the time from flowering to first fruit (Fig. 1B). Dominance also contributed to differences between populations for the time interval between initiation of fruit and death (Table 3, Fig. 1C), with Maryland genes for later death being dominant over Illinois genes.

Ovule number per fruit and seed weight differences between the populations were best explained by an additive model, whereas seed number per fruit differences included additive, dominance, and epistatic (dominance × dominance) genetic effects (Table 3, Figure 1D–F). Flower number (adjusted for plant size) and the proportion of fruit matured were best explained by a genetic model that included additive and dominance differences between populations (Table 3, Figure 1G, H). Illinois genes for a large number of flowers per unit plant size were dominant over Maryland genes, whereas Maryland genes that mature a larger fraction of fruit were dominant over Illinois genes. Although differences in the number of fruits between populations was only marginally significant, a joint scaling test revealed additive, dominance, and epistatic genetic differentiation between populations for this trait. This genetic divergence is demonstrated by the substantial heterosis expressed in the F_1 generation and fewer fruits than expected in the F_2 and backcross generations (Fig. 1I).

The best-fit models of population differentiation for vegetative characters included additive for branching pattern and additive-dominance-epistasis for growth rate (Table 3, Fig. 1J, K). Similar to fruit production, growth rate exhibited little phenotypic differentiation between populations, but strong genetic differentiation. The epistatic effects included both additive × dominance and dominance × dominance components (Table 3). In summary, the architecture of nuclear genetic differentiation varied among characters and included additive, dominance, and epistatic components.

DISCUSSION

Phenotypic Differentiation between Populations

Phenological differences between the Maryland and Illinois populations may reflect adaptations to local growing conditions. In the common-garden plot, the time interval between each phenological stage was shorter for Illinois plants, resulting in a compressed life cycle relative to Maryland individuals. Genetic differentiation of phenological characters is likely a response to local selective environments because the growing season in northern Illinois is shorter than in Maryland (pers. obs.). Previous work in another Illinois population of *C. fasciculata* found phenotypic selection for earlier flowering and fruiting over 2 years (Kelly 1992). A reciprocal transplant experiment between the two populations found a home-site fitness advantage for Maryland individuals when planted in their native environment over 2 years (Galloway and Fenster 1999, 2000), supporting an adaptive role for divergence in phenology.

TABLE 3. The architecture of genetic differentiation between Illinois and Maryland populations of *Chamaecrista fasciculata*. Joint scaling tests were used to fit genetic models of population differentiation including additive (A), additive and dominance (AD), or additive-dominance-epistasis (ADE). See Figure 1 for goodness-of-fit tests used to determine the best fit model. Parameter estimates of genetic effects are given for best-fit models (*a*, additive; *d*, dominance) and the significance of each evaluated with *t*-values (italics). For individual *t*-tests, $^{+}0.05 < P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; *** and $^{(**)}$ significant at $\alpha < 0.05$ after a sequential Bonferroni adjustment.

Variable	Best-fit model	a[1]	d	$a \times a$	$a \times d$	$d \times d$
Phenology (days)						
Planting to flower	A	1.17				
		1.89^{+}				
Flower to fruit	A	9.20				
		*15.19****				
Fruit to death	AD	6.65	12.62			
		*4.22****	*4.26****			
Reproductive						
Per fruit						
Ovule number	A	−2.13				
		*−14.66****				
Seed number	ADE	−2.30	−1.53	−0.51	−0.44	4.44
		*−6.64****	*−1.39*	*−0.48*	*−1.08*	*2.58**
Seed weight	A	6.89×10^{-4}				
		*5.69****				
Whole plant						
Flower number	AD	−17.03	32.82			
		*−4.33****	*4.37****			
% fruit matured	AD	0.15	0.15			
		*9.78****	*4.66****			
Fruit number	ADE	3.62	164.69	20.32	−36.86	270.61
		0.12	*1.71*	*0.22*	*−1.05*	*1.74*
Vegetative						
Branch pattern	A	0.025				
		*2.28***				
Growth rate	ADE	−7.86	−8.49	−8.90	−10.28	34.05
		−3.27$^{(**)}$	*−1.15*	*−1.26*	*3.47*$^{(**)}$	*2.89***

[1] *t*-test df; $a = 56$, $d = 37$, $a \times a = 56$, $a \times d = 18$, $d \times d = 37$.

Although reproductive characters differed between the two populations in the common garden, levels of fruit and seed production were more similar. Individuals from the Illinois population had more, smaller ovules and seeds per fruit than those from Maryland. While Illinois plants were smaller, they had more flowers per unit plant size. However, a smaller fraction of those flowers became mature fruit. In combination, plant size, flower production, and fruit maturation rates resulted in more similarity in the level of fruit production between populations than of its component characters. This interaction among characters in their contribution to fruit production is clear from the schematic diagram (Fig. 2). The contribution of character interactions to fitness suggests that suites of associated characters are likely to change in response to selection.

These two populations expressed differentiation in fitness as measured by number of fruit produced per seed planted under Maryland field conditions. However, *C. fasciculata* populations transplanted shorter distances, up to 100 km, often have limited phenotypic variation in fitness despite genetic differentiation for this character (Fenster and Galloway 2000; Galloway and Fenster 2000). The present study suggests that in these populations characters underlying fitness vary and contribute to the genetic differentiation, although the interaction of these traits results in little overall fitness differentiation.

Cytoplasmic Differentiation between Populations

Cytoplasmic genes contributed to phenotypic differentiation between Maryland and Illinois *C. fasciculata* populations over a suite of characters. Only seed number per fruit was significantly affected by cytoplasm type when reciprocal crosses for the four hybrid generations were compared. Based on this result, cytoplasmic genes appear to contribute little to phenotypic differences between the populations. However, the hybrids with Maryland cytoplasm were highly differentiated from those with Illinois cytoplasm when all characters were considered jointly. It appears that there are subtle cytoplasmic effects due to correlations between characters that form a pattern when examined across a number of traits (Scheiner 1993).

Cytoplasmic and nuclear genes often had opposing effects on phenotypic expression. For example, Illinois cytoplasm was strongly associated with greater growth rates and a larger percentage of fruit matured, whereas individuals with Maryland cytoplasm had more seeds per fruit and greater fruit production. In contrast, in the parental generation Maryland individuals matured a larger percentage of their fruits and Illinois plants produced more seeds per fruit. Growth rate and fruit production did not differ between the parental populations. These differences in phenotypic expression between the parental populations (which differ in cytoplasmic and

nuclear genes) and the hybrid generations (which differ in cytoplasmic genes) suggest that cytoplasmic genes influence phenotype expression in the opposite direction (although to a lesser extent) than nuclear genes for these traits. As a consequence, cytoplasmic effects may retard or accelerate selection response in these populations, depending on the direction of selection. A field experiment with these same populations (but different hybrid generations) also found that cytoplasmic and nuclear genes had opposing effects on phenotypic expression in two of the three comparisons in which there were significant cytoplasmic effects and no cytoplasmic-nuclear interaction (Galloway and Fenster 1999).

It is unclear whether these cytoplasmic effects are adaptive, perhaps reflecting selection for an optimum, or maladaptive and constrain the evolution of these populations. A study using second generation backcrosses between these Illinois and Maryland *C. fasciculata* populations found that individuals with matching cytoplasmic and nuclear genes (i.e., from the same location) had greater vegetative biomass and fitness than those with mismatched genomes (e.g., Maryland cytoplasmic genes and Illinois nuclear genes; Galloway and Fenster 1999), suggesting that the cytoplasmic effects are adaptive and likely coevolved with nuclear genetic effects. In total, these studies indicate a role for cytoplasmic genes in intraspecific phenotypic differentiation in *C. fasciculata.* Because all seeds used in this study were from plants grown for several generations in a common environment, maternal environmental effects are not expected to be associated with specific cytoplasm types. Cytoplasmic effects are therefore likely genetic, attributable to either the chloroplast or mitochondrial genomes and their interactions with nuclear genes.

Cytoplasmic genes have also been found to influence the phenotype, especially in combination with nuclear genes in other work (e.g., Beavis and Frey 1987; Clark and Lyckegaard 1988; Fos et al. 1990; Burke et al. 1998). In the few cases studied, cytoplasmic effects in plants appear to be associated with mtDNA (Pollak 1991; Saumitou-Laprade et al. 1994). There are few examples (other than male sterility) where the effects of intraspecific cytoplasm variation on fitness and life-history traits have been documented in wild plants. However, it seems likely that cytoplasmically inherited genes would be associated with trait differentiation in angiosperms, given the very restricted dispersal of seeds (McCauley 1994, 1998) and maternal uniparental inheritance of cytoplasmic factors.

Nuclear Genetic Differentiation between Populations

The architecture of nuclear genetic differentiation between a Maryland and an Illinois *C. fasciculata* population included additive and nonadditive genetic effects. Strictly additive genetic changes between populations were found for time from transplant to flowering and from flowering to fruit production, as well as ovule number, seed weight, and branching pattern. Additive and dominance effects contributed to population differentiation for the time between fruit production and death, flower number, and percent fruit matured. Dominance differentiation was also documented for fitness and its components for between these *C. fasciculata* populations (Fenster and Galloway 2000). Dominance differentiation suggests that limited gene flow in *C. fasciculata* (Fenster and Sork 1988; Fenster 1991a,b,c; Fenster and Dudash 1994) results in populations becoming fixed for distinct deleterious alleles. Finally, epistatic interactions contributed to population differentiation for seed number per fruit, fruit number, and growth rate. Although the phenotypic expression of growth rate did not differ between the Illinois and Maryland parental generations, the decreased mean growth rate of the F_2 and one of the backcross relative to the parents indicates that the populations are differentiated for gene interactions.

The contribution of epistatic interactions to intraspecific differentiation of wild plants has received little study (but see Shore and Barrett 1990; Parker 1992) relative to additive and dominance genetic effects. In contrast, the genetic architecture of intraspecific differentiation has been well studied in agricultural and horticultural plants (reviewed in Mather and Jinks 1982; Lynch and Walsh 1998). Typically these studies have been conducted with the goal of detecting additive and additive $\times$ additive variation to exploit in selection experiments. Indeed, the presumption of additive genetic effects forms the basis of a number of models of microevolution (e.g., Lande and Arnold 1983; Coyne et al. 1997). However, studies of intraspecific genetic architecture typically find a substantial contribution of nonadditive genetic effects (see agricultural references above and Vetukhiv 1953; Burton 1990; Brown 1991; Hard et al. 1992, 1993; Parker 1992; van Treuren et al. 1993; Andersson 1996; Armbruster et al. 1997; Hatfield 1997; Lair et al. 1997). Nonadditive genetic variation may result in unpredictable or asymmetrical responses to selection (e.g., Merila and Sheldon 1999), in particular for characters like growth rate in which population means are indistinguishable but the genetic basis underlying these means differs (see also Fenster and Galloway 2000).

The genetic architecture of population divergence varied among characters for *C. fasciculata.* There was little evidence that the mode of genetic differentiation was associated with specific types of characters. Variation in genetic architecture among characters (e.g., Armbruster et al. 1997; Hatfield 1997) or within a character across populations (Armbruster et al. 1997; Lair et al. 1997) is common. Our results are similar to a study that examined differentiation in fitness components between local and distant populations of the pitcher-plant mosquito (Armbruster et al. 1997) in demonstrating that the genetic divergence of fitness is not necessarily congruent to the genetic divergence of its components. Phenological, vegetative, and whole plant reproductive characters all contribute to fitness as measured by fruit production. These characters largely demonstrate additive and dominance differences between populations, whereas epistatic interactions differ between populations for fruit production. It is possible that interactions between the traits that in combination contribute to fitness give rise to epistatic differences for fitness. Differences between the populations for reproductive characters that interact multiplicatively to influence fitness (discussed above at the phenotypic level) support this idea.

Within- and between-locus nonadditive genetic effects contributed to differentiation for fitness and fitness components under field conditions for a number of *C. fasciculata* populations, including those used in the present study (Fens-

ter and Galloway 2000). In the common garden, epistasis contributed to genetic differentiation for fruit production. Fruit production of F_2 and F_3 individuals in the field in Maryland also revealed epistatic differentiation between the Maryland and Illinois populations (Fenster and Galloway 2000). This consistency of expression of genetic architecture across sites is surprising in light of the environmental differences between the sites. The common garden supported on average 20-fold greater vegetative biomass than the field plot despite experiencing many similar elements of the Maryland selective environment (e.g., season length, temperature, rainfall). Consistent genetic architecture across environments is in contrast to other findings (e.g., Blows and Sokolowski 1995; Armbruster et al. 1997).

Evolutionary Consequences

Cytoplasmic genes and a variety of modes of nuclear gene action contribute to genetic differentiation for presumably adaptive traits between *C. fasciculata* populations. Trait-dependent variation in the genetic architecture of population differentiation implies that different, and likely independent, evolutionary changes underlie different characters. In *C. fasciculata,* epistatically based differentiation for fitness (Fenster and Galloway 2000) appears to reflect both underlying epistatic genetic differentiation for the traits correlated with fitness as well as interactions among traits that contribute to fitness. Our results demonstrate that interactions at the phenotypic and genetic levels contribute to character evolution. Thus, both genetic differentiation and trait evolution may be strongly context dependent in *C. fasciculata.*

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