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POPULATION DIFFERENTIATION IN AN ANNUAL LEGUME: GENETIC ARCHITECTURE

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Abstract.—The presence or absence of epistasis, or gene interaction, is explicitly assumed in many evolutionary models. Although many empirical studies have documented a role of epistasis in population divergence under laboratory conditions, there have been very few attempts at quantifying epistasis in the native environment where natural selection is expected to act. In addition, we have little understanding of the frequency with which epistasis contributes to the evolution of natural populations. In this study we used a quantitative genetic design to quantify the contribution of epistasis to population divergence for fitness components of a native annual legume, *Chamaecrista fasciculata.* The design incorporated the contrast of performance of F_2 and F_3 segregating progeny of 18 interpopulation crosses with the F_1 and their parents. Crosses were conducted between populations from 100 m to 2000 km apart. All generations were grown for two seasons in the natural environment of one of the parents. The F_1 often outperformed the parents. This F_1 heterosis reveals population structure and suggests that drift is a major contributor to population differentiation. The F_2 generation demonstrated that combining genes from different populations can sometimes have unexpected positive effects. However, the F_3 performance indicated that combining genes from different populations decreased vigor beyond that due to the expected loss of heterozygosity. Combined with previous data, our results suggest that both selection and drift contribute to population differentiation that is based on epistatic genetic divergence. Because only the F_3 consistently expressed hybrid breakdown, we conclude that the epistasis documented in our study reflects interactions among linked loci.

Key words.—Coadaptation, drift, epistasis, Fisher-Wright controversy, heterosis, inbreeding depression, outbreeding depression, selection, shifting balance.

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The extent to which epistasis does or does not contribute to genetic divergence is pivotal to a number of evolutionary genetic models, some of which date back to the inception of Neodarwinism (e.g., Fisher 1930; Wright 1931). More recently, epistatic differentiation has been invoked to play an important role in the ability of populations to respond to selection (reviewed in Wade and Goodnight 1998) and the maintenance of genetic variation (Gimelfarb 1989; Goldstein and Holsinger 1992). Epistasis is also central to many theories of speciation (Dobzhansky 1937; Stebbins 1950; Mayr 1954; Grant 1981; Carson and Templeton 1984; Howard 1994; Gavrilets and Hastings 1996; Arnold 1997; Rieseberg 1997; Burke et al. 1998) and demonstration of an important role of epistasis in intraspecific differentiation may remove the need for ad hoc explanations for the evolution of epistatic interactions at the interspecifc level (Wade and Goodnight 1998). Furthermore, epistasis may play an important role in conservation genetics if it contributes to outbreeding depression (Fenster and Dudash 1994; Frankham 1995; Dudash and Fenster 2000). Therefore, we would like to know whether intraspecific epistatic differentiation is prevalent and whether models of evolution must incorporate this complexity.

If epistasis contributes to population genetic differentiation, what processes are responsible for population divergence? Although selection alone may cause populations to diverge epistatically (Dobzhansky 1937; Orr 1995; Gavrilets and Hastings 1996), perhaps the most frequently cited evolutionary model that includes epistasis, Wright's (1931, 1932) vision of the adaptive landscape, incorporates both drift and selection as leading to epistatic differentiation. There is abundant evidence for the role of natural selection in population divergence (Endler 1986; Futuyma 1997), and empirical work over the last 40 years has demonstrated that species exhibit significant population genetic structure, and thus the opportunity for drift to influence evolution (Hastings and Harrison 1994). This latter conclusion is based on studies documenting small population sizes (e.g., Levin 1981; Fenster 1991a,b), heterosis in progeny of interpopulation crosses (e.g., Wallace 1955; Fenster 1991b; reviewed in Waser 1993), and population differentiation for neutral molecular markers (Slatkin 1987; Hamrick and Godt 1989). Although the conditions for drift to influence evolution have been demonstrated, there are only several well-documented cases where drift has been shown to contribute to the evolution of adaptations (reviewed in Coyne et al. 1997). Thus, we have a limited understanding of the way in which drift interacts with selection and whether selection acts on gene interactions. This is surprising because it is the selection of genes in a genetic context, or the way these genes interact epistatically within specific genetic backgrounds (e.g., genes selected for their joint effects on fitness, Falconer and Mackay 1996), that Wright (1931, 1932) modeled as being so important. The evolution of populations, according to Wright, represents the evolution of favorably coadapted genes (Goodnight 1995; Wade and Goodnight 1998). Studies that simultaneously quantify population structure and the genetic basis and adaptive nature of population differentiation are needed if we are to understand whether we need to incorporate drift and epistasis into models of evolution.

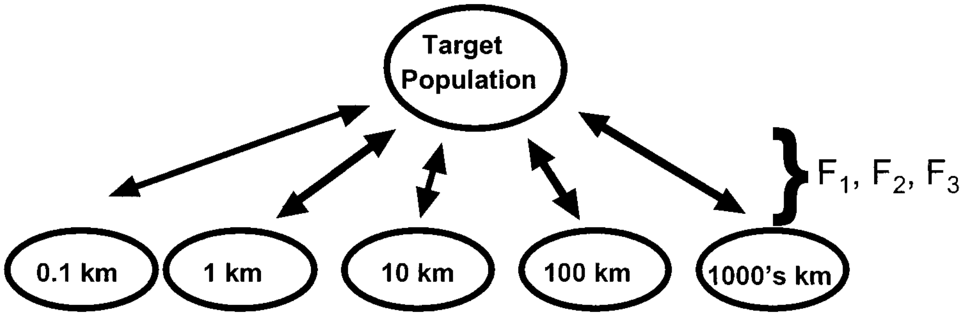

FIG. 1. Crossing design to examine the spatial scale of local adaptation, inbreeding, and epistatic differentiation between populations. The design was replicated for three target populations.

Several recent reviews of these alternative conceptions of the evolutionary process conclude that there are too few field-based studies that address selection on gene interactions, interactions between selection and drift, and the genetic architecture of adaptive evolution to evaluate either vision (Whitlock et al. 1995; Coyne et al. 1997; Fenster et al. 1997; Wade and Goodnight 1998). Although laboratory or common-garden studies can provide a detailed understanding of the genetic mechanisms underlying adaptation, studies in nature are necessary to fully understand how genetic architecture may dictate evolution because the interaction between natural selection and genetic variation ultimately determines the evolutionary process.

In a companion paper (Galloway and Fenster 2000), we demonstrated limited evidence for a home-site advantage or local adaptation at the scale of populations separated by $\leq$ 100 km. However, it is still possible that there is genetic differentiation in the way the same populations are adapted to their environments, especially if fitness differentiation is largely due to nonadditive gene action. Here we examine the contribution of nonadditive genetic variation to population differentiation for fitness and its components in an outcrossing annual, *Chamaecrista fasciculata.* We ask (1) do patterns of dominance differ among populations, suggesting a role for drift in population divergence, and (2) does epistasis contribute to population divergence for fitness components? Because gene flow is limited in *C. fasciculata* (Fenster 1991a,b; Fenster and Dudash 1994), we expect the processes of population differentiation and the type of gene action may change with increasing distance between populations. Thus, we ask our questions within a spatial context to determine how the genetic architecture and the influence of selection and drift on population differentiation scale with distance.

MATERIALS AND METHODS

Study Organism and Study Site

Chamaecrista fasciculata Michx., partridge pea (= *Cassia fasciculata*; Irwin and Barneby 1982), is a self-compatible, mostly outcrossing (Fenster 1991a, 1995), annual legume native to eastern North America. There are no pre- or post-mating sterility barriers between populations separated by up to 2000 km. Fuller description of characteristics such as habitat and life history can be found in Galloway and Fenster (2000).

Five populations in each of three regions were selected to quantify local adaptation (Galloway and Fenster 2000), population inbreeding, and the contribution of epistasis to population genetic differentiation in *C. fasciculata.* The three regions span the species range in eastern North America. In each region a target or home-site population was chosen. The remaining four populations in each region were located at increasing distances from the target population (0.1, 1, 10, 100 km). Seeds were collected separately by maternal family in each population.

Crossing Design

Each of the target populations was crossed to populations in the same state and to the other target populations for a total of six interpopulation crossing distances (Fig. 1). From 20–40 maternal families of field-collected seed were grown to flowering in the greenhouse for each population. Upon flowering, single-donor crosses were made both within and between populations. Within-population crosses were conducted between randomly selected individuals from the 20–40 different maternal families for each parental population. Within-population crosses represented crosses between individuals that were within several Wright neighborhoods of one another based on previous gene flow estimates (Fenster 1991a,b). Each population was also reciprocally crossed with its local target population. The Maryland and Kansas target populations were crossed to Illinois and to each other to generate 1000-km and 2000-km crossing distances, respectively. Because of the intermediate location of Illinois, two 1000 km crosses were conducted, one to Maryland and the other to Kansas. These crosses resulted in parental seed (crossing distance 0) and F_1 hybrids between populations 0.1, 1, 10, 100, 1000, and 2000 km apart.

Crosses were conducted for two more generations to produce F_2 and F_3 hybrids between populations and parental seed that had experienced equivalent cultural conditions to hybrids. F_1 plants within a seed type were randomly crossed to one another to produce F_2 individuals. F_2 seeds were then grown and randomly crossed to produce third generation hybrid seeds (F_3). Reciprocal hybrids were crossed separately for all generations, producing two groups of F_1, F_2, and F_3 hybrids that share the same nuclear genes, but differ in their cytoplasmic genes. In the field experiments each reciprocal cross was evenly represented in the hybrid generations. Parental and F_1 seed was produced on second generation parental individuals so that the parental, F_1, and F_2 seeds all experienced two generations of controlled crossing. The F_3 seeds represent a third generation of crosses in the greenhouse. As a result of recreating F_1 and parental seed, all generations of seed were produced in the same season (summer 1994). This design reduces maternal effects carried over from the field environment in the parental generation.

The crossing protocol was also designed to reduce inbreeding and genetic drift. Pollinations were conducted using single donors to minimize loss of genetic variation due to pollen competition. To further reduce inbreeding during our crossing regime, crosses were conducted between maternal families. Between 10 and 15 maternal families were grown for each seed type for all generations.

Field Experiment to Quantify Performance

For two years experimental seeds were planted into natural vegetation in field plots located within the target population in each region. See Galloway and Fenster (2000) for planting design and procedures. Experimental seeds for each seed type were weighed by block (1995, 12 blocks) or overall (1996, 36 blocks) so that seed size effects could be statistically factored out in assessment of performance. A total of 43 seed types were planted in each block (6 crossing distances $\times$ 2 reciprocals/distance $\times$ 3 hybrid generations $=$ 36 hybrid seed types $+$ 7 parental populations). These seed types were represented by at least 10 maternal families for each block except for nontarget parental seed in 1996, when only seven families for nontarget parentals were grown in each block. Each block in 1995 had 30 seeds for each parental and F_1 seed type and 40 seeds for each F_2 and F_3 crossing distance (10,440 seeds/site). In 1996, 10 seeds/target parental; 7 seeds/nontarget parental; 10 F_1, 10 F_2, and 10 F_3 seeds/crossing distance were planted in each block (8136 seeds/site). Sample sizes were greater in the recombinant hybrid generations (F_2, F_3) compared to the F_1 and parents to compensate for an expected increase in phenotypic variance.

Four fitness components were measured for each seed type in each block: percent germination, percent survivorship, and mean biomass and fruit production of surviving individuals. Cumulative fitness was quantified as the number of fruit produced per seed planted, which incorporates both survivorship and reproduction. Individuals that either did not germinate or died prior to reproduction were assigned a fruit production (and thus fitness) of zero. Full details of methods to measure fitness and its components are provided in Galloway and Fenster (2000). In total, fitness and its components was quantified in approximately 55,000 individuals across the two years and three sites of the study. Although plots were fenced with poultry wire, in Illinois in 1995 there was almost complete herbivory by prairie voles at the time the plants began to produce fruit. As a consequence, results from that site for biomass, fruit production, and cumulative fitness are presented for 1996 only.

Statistical Analysis

Analysis of covariance (ANCOVA) was used to evaluate whether drift and epistatic interactions contribute to population divergence for percent germination, mean vegetative biomass, mean fruit production, and cumulative fitness. For each crossing distance the fitness of the two parental populations was averaged to create a midparent (MP) value. To meet the assumptions of ANCOVA (e.g., minimize heteroscedasticity and missing values), all analyses were conducted on block means. Year (1995, 1996), planting location (Maryland, Illinois, Kansas), crossing distance (0.1, 1, 10, 1000, 2000 km), generation (MP, F_1, F_2, F_3), and block (nested within year and planting location) were included as main effects in the analysis and seed weight as a covariate for all traits but fruit production. Because vegetative biomass and fruit production are highly correlated ($r > 0.79$, on average), biomass was included as a covariate in the analysis of fruit production. Survivorship from seedling stage to fruit production was defined as a dichotomous variable and analyzed using log-linear categorical analysis with maximum likelihood to estimate parameters (PROC CATMOD, SAS Institute, 1990). Additional description of the survivorship analysis can be found in Galloway and Fenster (2000). With the exception of block, all effects were fixed. For all characters there were significant two- and three-way interactions with planting location (not shown); therefore, analyses were conducted separately for each planting location. Germination rate and cumulative fitness were also analyzed for each year because of interactions between planting location and year. If ANCOVA revealed a significant interaction between generation and distance, additional analyses were conducted for each crossing distance.

Divergence among parental populations due to drift was assessed by the presence of heterosis in the F_1 generation. We tested for heterosis using a priori linear contrasts between the F_1 and the MP generation. To quantify the contribution of epistasis to population differentiation, a priori contrasts were constructed to compare the recombinant hybrid generations (F_2 and F_3) to the average of the F_1 and MP generation. The standard error of the epistasis contribution was constructed by linear combination of the appropriately weighted variance estimates of the generation means that contribute to the estimate, that is, MP, F_1, F_2 or MP, F_1, F_3 (Lynch 1991; Lynch and Walsh 1997). This test of epistasis assumes that the F_2 and F_3 have equivalent heterozygosity and heterozygosity intermediate to the parents and F_1. We tested this assumption by evaluating heterozygosities for four polymorphic allozyme loci in 20–30 individuals of each seed type. Averaged across the four loci, F_1 progeny had greater heterozygosity than the parents, and F_2 and F_3 progeny had equal heterozygosities with each other and intermediate to the parents and the F_1 (unpubl. data), conforming to the assumptions of our tests. Linear contrasts among the generations were also conducted by combining crossing distances to quantify main trends in the data that reflected the summed generation effects. We also conducted contrasts separately for each distance in 1996, when a significant distance-by-generation interaction was found. Sequential Bonferroni correction (Rice 1989) was used to minimize Type II errors ($n = 3$ for each ANOVA, $n = 18$ when contrasts were conducted for each crossing distance). All contrasts reported as significant remained significant after sequential Bonferroni correction, unless otherwise noted.

TABLE 1. Analysis of covariance on percent germination to evaluate the role of drift and epistasis in genetic differentiation between *Chamaecrista fasciculata* populations separated by six distances. Distance refers to the particular crossing combination between target population and populations of increasing distance from the target. The experiment was replicated using populations from Kansas, Maryland, and Illinois, and germination was evaluated under natural conditions in each location over two years. Drift was evaluated by comparing the midparent (MP) to the F_1 generation. Epistatic differentiation between populations was determined by comparing the recombinant hybrid generations (F_2, F_3) to the average of the MP and F_1 values. Seed weight is a covariate.

Source	df	Kansas *F*	Maryland *F*	Illinois *F*
Year	1	13.32***	1.58	0.06
Distance	5	0.96	2.17+	6.65***
Year × distance	5	1.31	3.83**	1.21
Generation	3	12.87***	59.79***	5.62***
Year × generation	3	0.95	4.90**	0.11
Distance × generation	15	1.63+	2.01*	1.54+
Year × distance × generation	15	0.85	1.13	0.83
Block (year)	46	8.16***	11.35***	15.03***
Seed weight	1	15.89***	3.07+	4.81*
Error df		1843	1838	1570
Linear contrasts:				
Heterosis:				
F_1 vs. MP	1	0.13	74.08***	0.88
Epistatic effects:				
F_2 vs. (MP + F_1)/2	1	0.23	52.74***	0.12
F_3 vs. (MP + F_1)/2	1	30.87***	9.47**	12.36***

+ $0.1 > P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Genetic and Evolutionary Inferences Based on Hybrid Performance

The performance of the F_1 relative to either parent may represent various underlying genetic phenomena. The fitness of the F_1 may be equal to the MP value if the populations have genetically diverged and the basis of the differentiation is additive gene action. This would result in the dilution of genes associated with adaptation and will cause the loss of fitness of the F_1 relative to the home parent, in the case of local adaptation, leading to performance intermediate to the two parental performances. Enhanced performance of the F_1 relative to either parent or to the MP value represents heterosis that may be due to dominance, overdominace, and/or the expression of positive additive-by-additive interactions that are serendipitously created through the interpopulation crosses (Hill 1982; Lynch 1991; Lynch and Walsh 1997). If heterosis is due to dominance, then drift is may be responsible for the genetic differentiation associated with the enhanced performance of the F_1, which results from the masking of deleterious recessive alleles. The F_1 may also have lower fitness than the MP value if genes conferring local adaptation are recessive or if there is heterozygote disadvantage due to negative intra- and/or interlocus interactions resulting from combining genes from different populations.

As with the F_1 performance, the performance of the F_2 and F_3 may represent a number of underlying genetic phenomena that may be operating simultaneously. The F_2 and F_3 performance will equal the MP value and the F_1 if the basis of genetic differentiation between the two parental populations is additive. In this case fitness less than the home parent is expected due to the dilution of genes associated with local adaptation. The F_2 and F_3 may have fitness greater than either parent or the MP value due to heterosis, especially if heterosis is observed in the F_1 generation. The underlying genetic basis of the enhanced performance may, as in the F_1, be due to dominance or overdominance associated with intralocus interactions or due to favorable gene interactions.

Contrasts of the performance of the F_2 and F_3 relative to the MPs and F_1 allows for dominance and epistatic effects to be distinguished from one another. In the absence of epistasis, the performance of the F_2 and F_3 will be intermediate to the F_1 and MP value because the segregating generations have half the heterozygosity and thus half the fitness gain relative to the F_1 (Cockerham 1980; Hill 1982; Lynch 1991; Lynch and Walsh 1997). However, if groups of genes interact and these interactions differ between populations (i.e., epistasis), then the segregating F_2 and F_3 generations may have fitness more than or less than (MP + F_1)/2. F_2 and F_3 performance more than or less than (MP + F_1)/2 indicates genes combined from different populations have either a positive or negative effect on fitness, respectively. This test for epistasis is independent of the underlying genetic basis of F_1 performance (Lynch and Walsh 1997). The differences in fitness between the segregating F_2 and F_3 generations and the F_1 is then a result of two phenomena: (1) the reduction of heterozygosity due to Mendelian segregation; and (2) recombination. If the evolution of population differentiation represents the evolution of linked interacting genes, then any process allowing more recombination, including the creation of additional segregating generations, will reveal the presence of more epistasis.

RESULTS

Percent Germination

Significant generation effects were observed at all three sites (Table 1). Separate analyses for generation effects were conducted in Maryland for each year because significant year-by-generation interactions were detected (Table 1). Based on the F_1 versus MP contrast, F_1 heterosis for germination rate

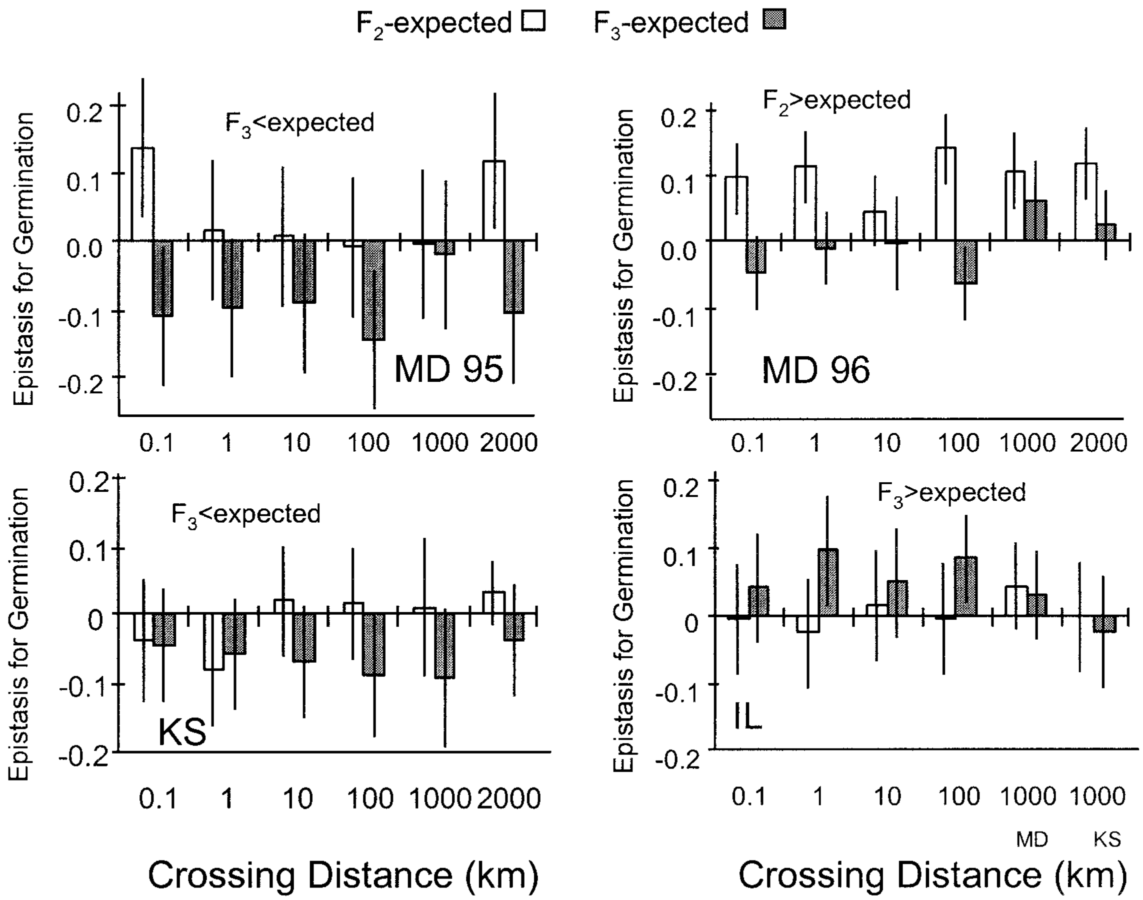

FIG. 2. Deviation of F_2 and F_3 hybrids from the average of the F_1 and midparent values to determine the contribution of epistasis to population differentiation for germination rates. Significant deviations from zero (noted in the body of the figure) indicate epistatic differentiation between the populations of *Chamaecrista fasciculata.* Experiments were conducted in nature and replicated in Maryland (MD), Illinois (IL), and Kansas (KS). Crosses are among populations separated by six distances. Bars represent two standard errors. Missing bars indicate a value of zero.

was observed for both years in Maryland ($F_{1,468} = 9.87$, $P < 0.01$; $F_{1,452} = 102.39$, $P < 0.001$, 1995 and 1996, respectively). However, the expression of heterosis for germination rate was greater in 1996 for Maryland (1995: F_1 67% > MP 58%; 1996: F_1 68% > MP 52%). Epistatic interactions for germination differ between populations in all three locations. In Maryland in 1995 ($F_{1,468} = 17.97$, $P < 0.01$) and in Kansas over both years, fewer F_3 seeds germinated than expected based on the average of the MP and F_1 generation (Fig. 2). In contrast, germination exceeded expectations for the F_3 in Illinois and the F_2 in Maryland in 1996 ($F_{1,1452} = 46.22$, $P < 0.001$, Fig. 2). Nonsignificant or only marginally significant crossing distance-by-generation effects indicate that crossing distance did not influence the degree of dominance or epistatic differentiation between populations in any planting location (see also Fig. 2).

Survivorship

A significant generation-by-year effect in Kansas (Table 2) was manifested as generations not differing for survivorship in 1995 ($\chi^2 = 6.55$, df = 3, $P = 0.08$), but differing in 1996 ($\chi^2 = 90.43$, df = 3, $P < 0.001$, analyses not shown). The F_1 hybrids had greater survivorship than the parental generation mean, indicating heterosis in between-population crosses in Kansas in 1996 ($\chi^2 = 71.95$, df = 1, $P = 0.0001$, analysis not shown) and in Maryland over both years (Table 2, Fig. 3). The survivorship of the recombinant hybrids was greater than the expected MP-F_1 average for both F_2 and F_3 generations in Maryland (Table 2) and in Kansas in 1996 (F_2 $\chi^2 = 48.80$, df = 1, $P = 0.0001$; F_3 $\chi^2 = 39.59$, df = 1, $P < 0.0001$; Fig. 4). Generation differences were largely due to low levels of survivorship of the parental generation (Fig. 3). As with germination, there was no evidence that the degree of nonadditive differentiation between populations varied with crossing distance (Table 2, distance-by-generation not significant and therefore not in model).

Vegetative Biomass

Nonadditive genetic effects contributed to population differentiation for vegetative biomass in Kansas and Maryland. These effects were consistent across years, as demonstrated by the lack of significant interactions involving both year and generation (Table 3). For both planting locations the F_1 biomass was greater than the average of the two parental populations, indicating heterosis (Table 3, Fig. 5). The pattern of epistatic differentiation varied between Maryland and Kansas.

TABLE 2. Log-linear analysis of survivorship to evaluate the role of drift and epistasis to genetic differentiation of *Chamaecrista fasciculata* populations sampled over six distances and planted in three locations over two years. The models vary among the three planting locations; factors omitted from models are indicated by a dash. Factors not significant for any site and year combination are not included in the table. See Table 1 for details.

Source	df	Kansas χ^2	Maryland χ^2	Illinois[1] χ^2
Generation	3	37.14***	49.55***	3.63
Distance	5	14.01*	43.01***	3.03
Year	1	6.11*	5.94*	15.64***
Distance × year	5	11.16*	—	—
Distance × generation × year	15	—	—	27.05*
Generation × year	3	33.76***	—	—
Block	14	59.36***	168.27***	140.96***
Linear contrasts:				
Heterosis:				
F_1 vs. MP	1	35.36***	45.93***	
Epistatic effects:				
F_2 vs. $(MP + F_1)/2$	1	7.28**	11.86***	
F_3 vs. $(MP + F_1)/2$	1	3.50	7.42**	
Likelihood ratio: χ^2		132.00	397.61	359.50
df		352	360	345

[1] Contrasts not conducted because generation effect was not significant.

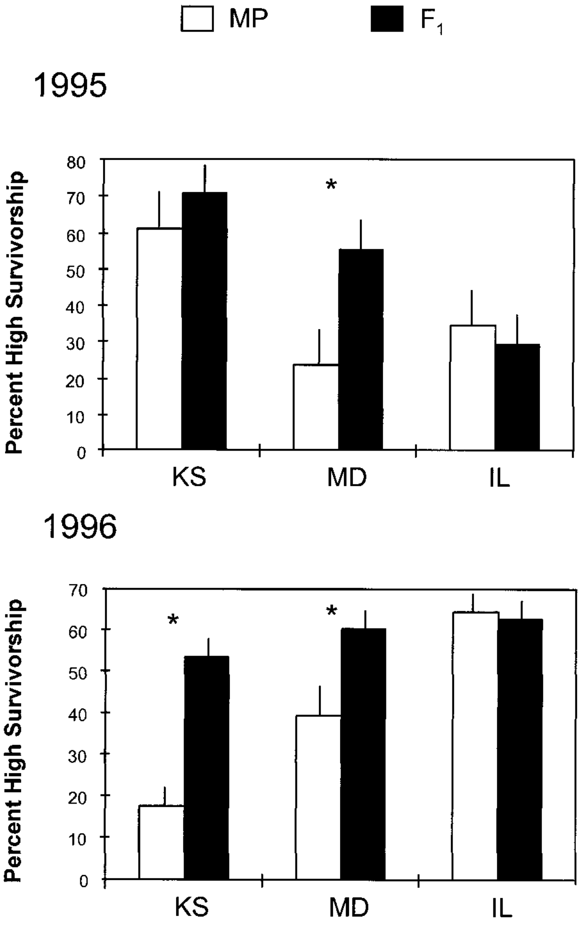

FIG. 3. Contrasts of F_1 of interpopulation crosses and midparent (MP) values to determine the contribution of drift to population differentiation for high survivorhip in *Chamaerista fasciculata.* Because the definition of ''high survivorship'' differs between planting locations, it is only meaningful to compare the magnitude of survivorship among generations within each location. F_1 that are significantly different from MP are starred. Bars represent two standard errors. See Figure 2 for details.

In Maryland, the F_2 generation had greater biomass than the expected MP-F_1 average, whereas in Kansas the F_3 generation had lower biomass than expected (Table 3, Fig. 6). Significant distance-by-generation interactions in Maryland and Illinois demonstrate that the magnitude of the generation effect varies with crossing distance (Table 3). However, this variation does not scale in a simple way with distance (Fig. 6).

Fruit Production

Fruit production adjusted for plant size demonstrates no hybrid vigor, but appreciable hybrid breakdown was found in both Maryland and Illinois across years (Table 4). Both recombinant hybrid generations in Maryland and the F_3 in Illinois had lower than expected fruit production (Fig. 7).

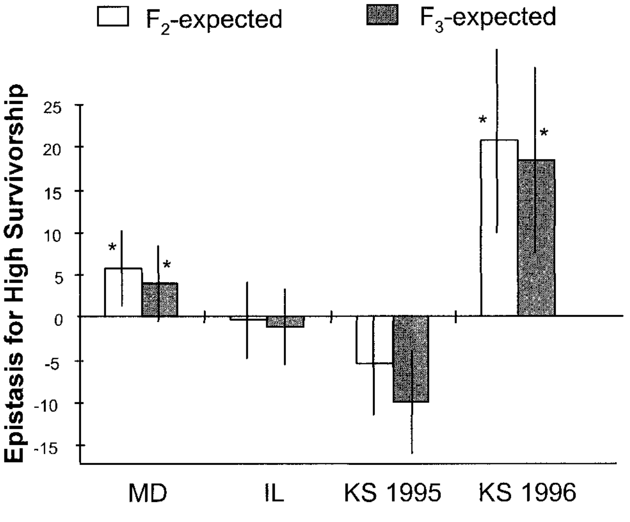

FIG. 4. Deviation of F_2 and F_3 hybrids from the average of the F_1 and midparent values to determine the contribution of epistasis to population differentiation for high survivorship in *Chamaecrista fasciculata.* F_2 and F_3 that are significantly different from $(F_1 + MP)/2$ are starred. Bars represent 2 standard errors. See Figure two for details.

TABLE 3. Analysis of covariance on vegetative biomass to evaluate the role of drift and epistasis to genetic differentiation of *Chamaecrista fasciculata* populations separated by six distances. See Table 1 for details.

Source	df	Kansas *F*	Maryland *F*	Illinois[1] *F*
Year	1	22.17***	4.77*	—
Distance	5	38.55***	37.75***	11.27***
Year × distance	5	1.67	5.83***	—
Generation	3	20.33***	25.33***	1.47
Year × generation	3	2.27+	1.07	—
Distance × generation	15	1.41	1.81*	1.90*
Year × distance × generation	15	0.55	0.72	—
Block (year)	46	9.30***	12.29***	10.72***
Seed weight	1	11.25***	22.62***	2.09
Error df		1840	1181	1101
Linear contrasts:				
Heterosis:				
F_1 vs. MP	1	17.87***	55.51***	
Epistatic effects:				
F_2 vs. $(MP + F_1)/2$	1	0.19	10.01**	
F_3 vs. $(MP + F_1)/2$	1	24.86***	1.51	

[1] No year factor in Illinois analysis; contrasts not conducted because generation not significant.

Because plant size is included as a covariate, differences in fruit production reflect either variation in flower production rates per unit plant size, successful fertilization, or successful fruit development (the amount of early- and late-stage fruit abortion differed between Maryland and Illinois target populations in another study; L. F. Galloway and C. B. Fenster, unpubl. data). In Kansas, the influence of generation on fruit production depends on crossing distance (Table 4). Only the F_3 generation of the 100-km cross differs from its expected performance, producing more fruit than expected (analysis not shown).

Cumulative Fitness

In 1995, fitness of F_1 hybrids was greater than the MP value in both Maryland and Kansas (Table 5A, Fig. 8). Linear contrasts of the 1996 data indicated that the F_1 outperformed the MP only in Maryland when the effects were summed across crossing distance (Table 5B). Because the performance of the generations varied among crossing distances for all planting locations in 1996 (a significant distance-by-generation effect, Table 5B) we evaluated heterosis separately by distance to determine if there were any patterns associated with scale. In Maryland in 1996, after sequential Bonferroni correction, only the 0.1-, 1000-, and 2000-km crosses continued to exhibit significant F_1 heterosis (analyses not shown), whereas the F_1 of the 10- and 100-km crosses were just shy of exhibiting significant heterosis after sequential Bonferroni correction ($P < 0.07$, $P < 0.10$, respectively). There was no evidence of heterosis in Illinois in 1996. Further scrutiny of the distance-by-generation interaction in Kansas in 1996 revealed that only the longest interpopulation cross produced significant heterotic progeny after sequential Bonferroni correction (analysis not shown). Thus, with the exception of Kansas 1996, we could not detect any consistent scale effects on the expression of heterosis on cumulative fitness.

Reduced cumulative fitness of the F_3 generation compared to the expected average of the MP and F_1 values demonstrated widespread epistatic differentiation between populations. Linear contrasts on the generation factor in the ANCOVA on fitness found the F_3 did not perform as well as expected (in the absence of epistasis) in Kansas and Maryland over both years (Table 5, Fig. 9). Because of the significant interaction of generation with distance for all sites in 1996 (Table 5B), we also conducted individual contrasts for each of the contrasts involving the F_2 and F_3 for the 1996 data. The F_3 performance is generally lower than the expected value in 1996 for Kansas and Maryland. However, this difference remained significant for only four of the contrasts after sequential Bonferroni correction (all in Kansas, crosses between populations 0.1, 100, 1000 and 2000 km apart; analyses not shown) and there was no pattern with distance.

In contrast, the F_2 generation performance in Kansas and Maryland did not differ from expected in 1995. In Maryland in 1996 the F_2 over all distances significantly outperformed the expected MP-F_1 average. Investigation of the distance-by-generation interaction in Maryland in 1996 revealed that most of the unexpected high performance of the F_2 is due to the F_2 performance of the two shortest distances, but only the shortest remained significantly greater than the $(F_1 + MP)/2$ after sequential Bonferroni correction. In Illinois there was no overall effect of generation (Table 5). In summary, for both years for almost all crosses the F_3 performance is less than expected and less than that of the F_2 ($P < 0.001$, sign test). However, there is no pattern of increasing epistatic differentiation between populations with increasing distance.

DISCUSSION

Inbreeding and Genetic Drift

F_1 hybrids had greater germination rates, survivorship, and biomass relative to the parental average. Heterosis of yield is due mostly to differential growth; fruit production adjusted for plant size did not express heterosis in any location. This expression of heterosis was also manifested as higher cumulative fitness of the F_1 relative to the average of the two parents, but was strongly environmentally dependent. We interpret the expression of F_1 heterosis as demonstrating that

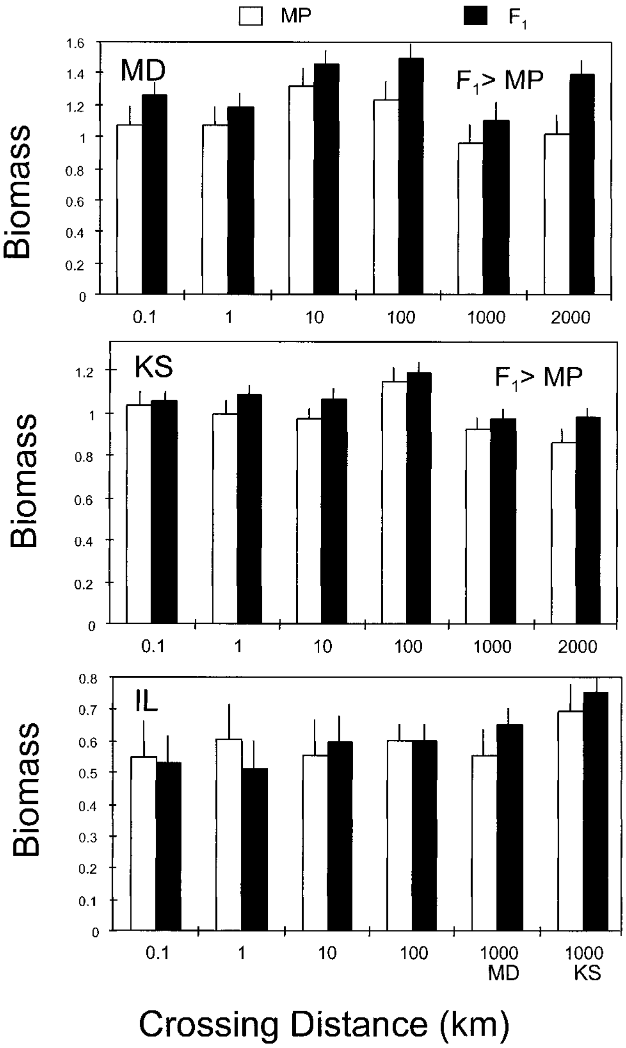

FIG. 5. Contrasts of F_1 of crosses among populations of *Chamaecrista fasciculata* with the midparent (MP) to determine the contribution of drift to population differentiation for biomass. Least square means (adjusted for seed weight) of sqrt transformed biomass are presented. See Figure 2 for details. The difference in fitness between MP and F_1 were uniformly significant across the two years for Maryland and Kansas and are noted in the body of the figure. Bars represent two standard errors.

populations are partially inbred, probably due to limited gene flow and consequent drift effects (Falconer and Mackay 1996). Given the empirical support for a simple dominance basis for heterosis rather than overdominance (Charlesworth and Charlesworth 1987; Crow 1992; Dudash et al. 1997; Dudash and Carr 1998), it is likely that populations contain different deleterious alleles and crosses between genetically differentiated populations result in increased heterozygosity (masking deleterious recessive alleles), resulting in vigorous F_1 progeny. It is possible that overdominance contributes to some of the observed F_1 heterosis, but only marker assisted approaches (e.g., Stuber et al. 1992), which are presently being employed, are likely to distinguish the joint contributions of overdominant and dominance based F_1 heterosis in *C. fasciculata.* Epistasis may also contribute to F_1 heterosis (e.g., Lynch and Walsh 1997, Table 9.5). However, it seems

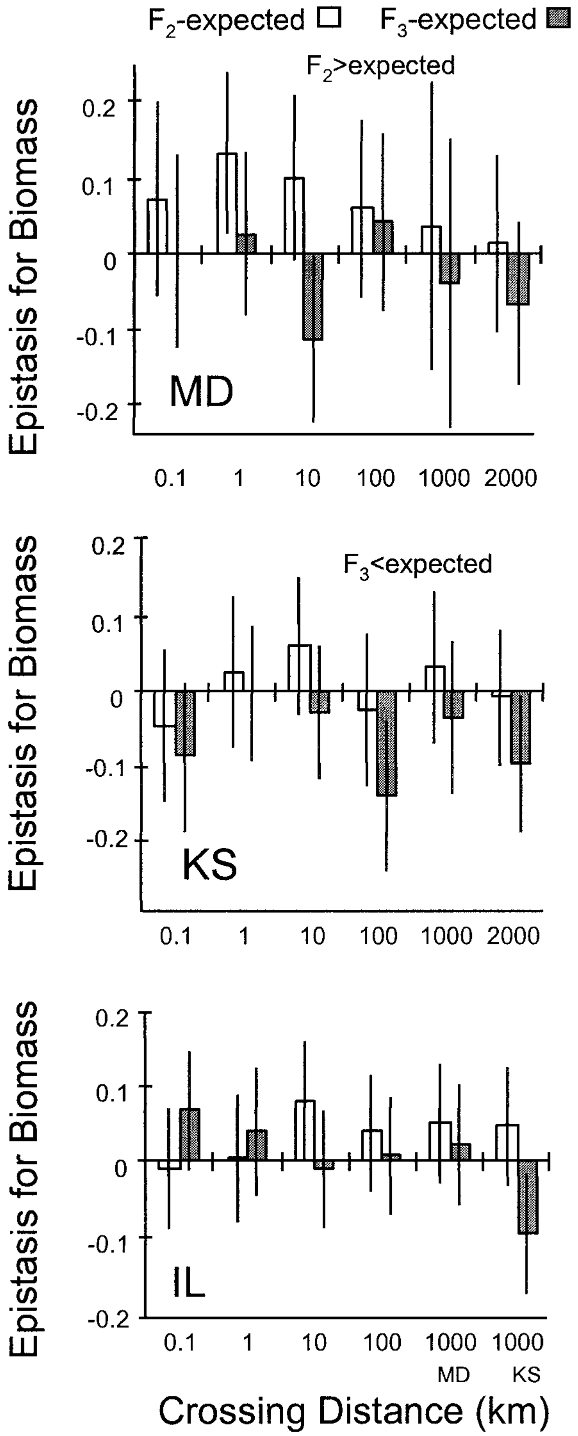

FIG. 6. Deviation of F_2 and F_3 hybrids from the average of the F_1 and midparent values to determine the contribution of epistasis to population differentiation of vegetative biomass in *Chamaecrista fasciculata.* Significant deviations from zero (noted in the body of the figure) indicate epistatic differentiation between the populations. Means are adjusted for seed weight. Missing bars indicate a value of zero. Bars represent two standard errors. See Figure 2 for details.

TABLE 4. Analysis of covariance on fruit production to evaluate the role of drift and epistasis to genetic differentiation of *Chamaecrista fasciculata* populations separated by six distances. Vegetative biomass is included as a covariate. See Table 1 for details.

Source	df	Kansas *F*	Maryland *F*	Illinois *F*
Year	1	126.34***	190.85***	—[1]
Distance	5	115.39***	12.45***	10.99***
Year × distance	5	15.86***	5.91***	—
Generation	3	2.82*	4.51**	3.01*
Year × generation	3	1.56	2.33+	—
Distance × generation	15	2.35**	0.79	0.41
Year × distance × generation	15	1.29	0.42	—
Block (year)	46	6.82***	14.41***	13.30***
Biomass	1	6250.38***	4936.70***	885.67***
Error df		1840	1811	1101
Linear contrasts:				
Heterosis:				
F_1 vs. MP	1	2.52	1.44	0.02
Epistatic effects:				
F_2 vs. (MP + F_1)/2	1	1.00	12.03***	0.02
F_3 vs. (MP + F_1)/2	1	2.55	6.86**	6.40*

[1] No year factor in Illinois analysis.

unlikely that epistasis contributes to enhanced performance of the F_1 when the general conclusion based on the F_3 performance is that combining genes from different populations leads to the disruption of a well-integrated genotype (see below). Thus, we suggest that if gene interactions do contribute to F_1 performance, then they do so negatively. Where the F_1 outperformed the MP, it frequently outperformed both parents (Fenster and Galloway 2000a), indicating that the loss of fitness due to the fixation of deleterious alleles may be substantial.

Similar findings of enhanced performance of F_1 progeny of interpopulation crosses relative to their parents has been observed in previous work in *C. fasciculata* (Fenster 1991b) and in a number of other studies, for example, *Drosophila* (Wright et al. 1942; Wallace 1955), *Phlox* (Levin 1977, 1984), *Sabatia* (Dudash 1990), *Scabiosa* (van Turen et al. 1994), and other examples cited in Waser (1993), suggesting that fixation of deleterious alleles within populations due to drift may be a general phenomenon. Large environmental effects on the expression of inbreeding depression found here and in other studies (Dudash 1990; Pray et al. 1994; Lynch and Walsh 1997) imply that deleterious mutations may experience extreme variation in purifying selection, which may also account for their high frequency.

Contribution of Epistasis to Population Genetic Differentiation

We observed disruption of gene interactions for germination (Maryland and Kansas in 1995), vegetative biomass (Kansas), and fruit production (Maryland and Illinois). Five of the six examples of hybrid breakdown are in the F_3 generation. The one example of F_2 breakdown, fruit production in Maryland, also demonstrated F_3 breakdown. The cumulative effect was the consistent manifestation of the disruption of gene interactions for fitness in the F_3 generation in Maryland and Kansas. These results indicate that gene interactions affect all components of the life history. Even crosses among populations separated by only hundreds of meters result in a disruption of fitness and its components across all phases of the life history. Elsewhere (Galloway and Fenster 1999; Fenster and Galloway 2000b) we have also documented nuclear-by-cytoplasmic interactions such that genotypes perform best in a subset of environments when cytoplasm and nuclear genes are derived from the same population. Overall, our results are consistent with the notion that each of the populations of *C. fasciculata* represents a different adaptive combination of alleles, relative to the target populations, that is, a gene likely to increase fitness in the genetic background of the source population may decrease fitness when placed in a different genetic background.

Our results corroborate earlier work documenting inbreeding and genetic coadaptation in *Drosophila* (Wallace 1953; Brnic 1954; Wallace and Vetukhiv 1955; Anderson 1968) and conform to more recent studies using marker assisted techniques (Clegg et al. 1978; Cavener and Clegg 1981; Burton 1987, 1990; Hard et al. 1992, 1993; Palapoli and Wu 1994; Doebley et al. 1995; Lark et al. 1995; Rieseberg et al. 1995; Armbruster et al. 1997; Hatfield 1997; Li et al. 1997; Routman and Cheverud 1997) and others (reviewed in Whitlock et al. 1995; Fenster et al. 1997). Our observation of epistasis contributing to divergence at a very local scale has also been observed in a number of other studies (Templeton et al. 1976; Price and Waser 1979; Burton 1987, 1990; Waser and Price 1989, 1994; Parker 1992; Deng and Lynch 1996). Our work adds to these contributions by demonstrating that epistatic interactions are expressed under natural field conditions.

The findings of the earlier *Drosophila* experiments that quantified a large effect of linkage on the expression of hybrid breakdown are especially relevant (Brnic 1954; Wallace 1955; Wallace and Vetukhiv 1955; Ohta 1980). In our study, negative epistasis is mostly observed in the F_3 and less consistently in the F_2 generation. It is unlikely that the additional greenhouse generation experienced by the F_3 contributed to inbreeding effects because both F_2 and F_3 generations had similar heterozygosity levels, as revealed by the allozyme survey. Rather, an additional round of recombination appears to be necessary to disrupt putatively linked epistatically interacting genes. This may explain why our results are in contrast to the outcome of a large-scale agricultural field study with maize, where no differentiation in epistatic interactions between cultivars were detected through the F_2 generation (Moll et al. 1965). Recombination disrupts the evolution of coadapted gene complexes (Turner 1967). Our results of coadaptation among linked genes conforms to theoretical predictions that the evolution of interacting gene systems is facilitated where recombination is limited (Wade and Goodnight 1998).

Four of the seven cases of enhanced recombinant hybrid performance for fitness components were for survivorship and four were found in Maryland. Only the F_2 in Maryland in 1996 had a significantly greater performance than expected in the absence of epistasis. These results suggest that better than expected performance of recombinant hybrids may be attributed to the chance bringing together of groups of alleles that in combination enhance fitness. Similar findings of enhanced recombinant hybrid performance have been seen in other studies. For example, better than expected performance for viability and development time was found in the F_2 generation of crosses between *D. melanogaster* populations (Blows and Sokolowski 1995). These type of results demonstrate a possible microevolutionary basis for speciation that includes both the generation of genome-wide negative epistasis among loci expressed in species hybrids (Dobzhansky 1937; Gavrilets and Hastings 1996) and the formation of chance novel recombinants with high fitness following intra- or interspecific crosses resulting in adaptive evolution (Stebbins 1950; Grant 1981; Rieseberg 1997; Burke et al. 1998).

Scale of Population Differentiation

If genetic differentiation between populations follows an isolation-by-distance model and if the amount of heterosis and epistasis is a product of the genetic divergence between lines or populations (Lynch 1991; Orr 1995; Falconer and Mackay 1996), then levels of dominance and epistasis are expected to increase with distance. There was little evidence that nonadditive genetic variation increased with distance between populations. Changes in the contribution of dominance and epistasis to population differentiation with distance was determined by the distance-by-generation effect in the analyses of variance. This interaction was significant for germination (Maryland in 1996), vegetative biomass (Maryland and Illinois), fruit production (Kansas), and cumulative fitness (Maryland in 1996, Illinois in 1996, and Kansas in

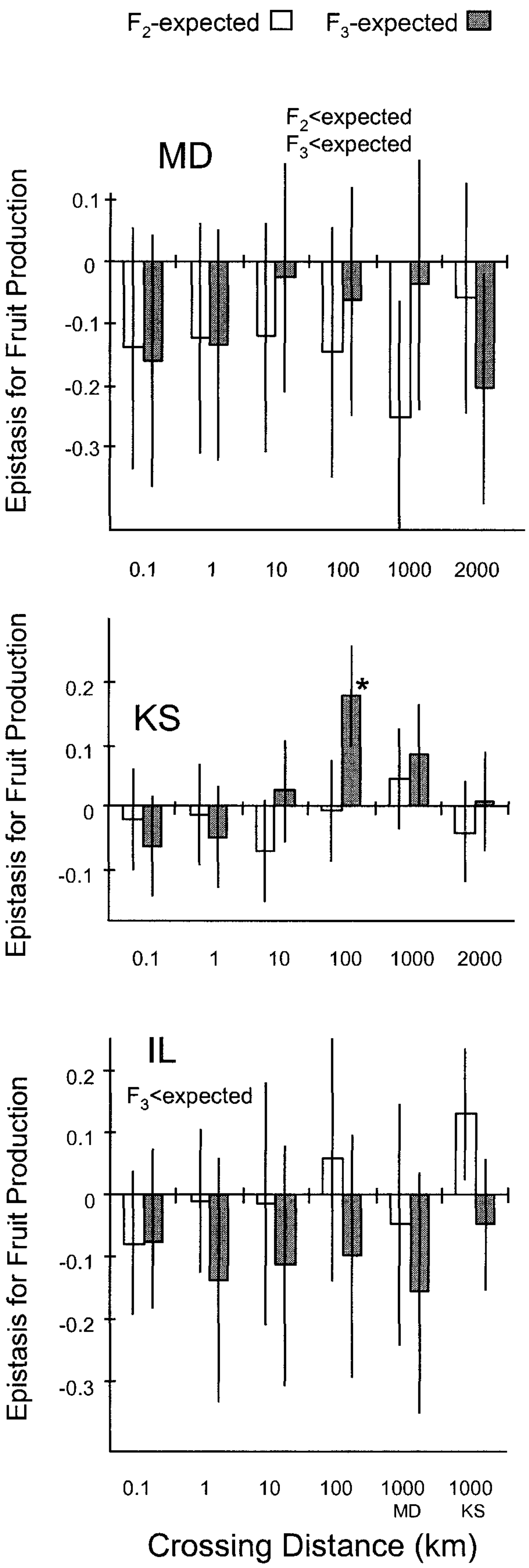

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FIG. 7. Deviation of fruit production of F_2 and F_3 hybrids from the average of the F_1 and midparent values to determine the contribution of epistasis to population differentiation of *Chamaecrista fasciculata.* Significant deviations from zero (noted in the body of the figure) indicate epistatic differentiation between the populations. Although over all distances there was no deviation of the F_2 and F_3 from zero in Kansas, investigation of the generation-by-distance interaction revealed that the performance of the F_3 of the 100-km cross was significantly greater than the $(F_1 + MP)/2$ value (indicated by an asterisk; $P < 0.05$ following sequential Bonferroni correction). Means are adjusted for vegetative biomass. Bars represent two standard errors. See Figure 2 for details.

TABLE 5. Analysis of covariance to evaluate the roles of drift and epistasis in differentiation for cumulative fitness of *Chamaecrista fasciculata* populations separated by six distances. Seed weight is included as a covariate. See Table 1 for details.

A. 1995

Source	df	Kansas *F*	Maryland *F*
Distance	5	6.17***	2.69*
Generation	3	20.87***	15.56***
Distance × generation	15	1.35	0.91
Block	11	11.51***	8.98***
Seed weight	1	4.87*	6.61*
Error	441[1]		
Linear contrasts:			
Heterosis:			
F_1 vs. MP	1	30.71***	13.36***
Epistatic effects:			
F_2 vs. (MP + F_1)/2	1	1.33	0.03
F_3 vs. (MP + F_1)/2	1	12.38***	14.31***

B. 1996

Source	df	Kansas *F*	Maryland *F*	Illinois[2] *F*
Distance	5	13.04***	22.63***	1.87+
Generation	3	29.92***	55.63***	0.32
Distance × generation	15	3.40***	2.12**	1.95*
Block	35	6.78***	12.57***	18.11***
Seed weight	1	17.48***	19.09***	9.32**
Error	1452			
Linear contrasts:				
Heterosis:				
F_1 vs. MP	1	2.64	101.81***	0.21
Epistatic effects:				
F_2 vs. (MP + F_1)/2	1	3.53	6.95**	0.03
F_3 vs. (MP + F_1)/2	1	67.15***	16.12***	0.53

[1] df error for Kansas equals 465.

[2] Crossing distances differ in Illinois, with the two longest crosses of Illinois to Kansas and to Maryland both representing distances of 1000 km.

1996). Although in these analyses differentiation between the generations differs with distance, this tends to be associated with particularly low (e.g., Fig. 6, KS F_3 100 km) or high (Fig. 7, KS F_3 100 km) hybrid performance of one crossing distance compared to the others rather than a pattern of differentiation that corresponds with changes in distance. The one exception is vegetative biomass in Maryland and cumulative fitness in Maryland in 1996, where the F_2 exceeds expectations more frequently in short crosses ($\leq$ 100 km) than longer crosses. This last result may indicate that genes that have been tested under roughly the same local conditions are more likely to result in favorable interactions. Lack of a consistent scale effect for nonadditive genetic differentiation between populations may not be surprising in light of limited scale effects in comparisons of the parental populations (Galloway and Fenster 2000). Although there is some evidence from other studies for scale-dependent changes in nonadditive genetic differentiation (Moll et al. 1965; Burton 1990; Blows 1993; Waser and Price 1994), others have found it lacking (Lair et al. 1997). These contrasting findings likely reflect the genetic processes that underlie population establishment as well as population differentiation.

The relationship between heterosis, epistasis, and interparent distance may not be linear for several reasons. Natural populations do not evolve in uniform environments, thus the expression of nonadditive genetic variation may be affected by a balance between the dilution of adaptive parental genes and the expression of heterosis and hybrid breakdown due to dominance and epistasis (e.g., Moll et al. 1965). Our observation of F_1 heterosis expressed at all crossing distances is consistent with the limited evidence for local adaptation in *C. fasciculata* and indicates that the masking of deleterious recessive alleles provides greater benefit than the decrease in fitness expected with loss of local adaptation. The degree of heterosis expressed due to dominance effects can also be complicated by the expression of epistasis in the F_1 (see above). Thus, if negative additive-by-additive interactions do contribute to F_1 performance, then heterosis gained by population divergence may be also be counteracted by increased disruption of coadapted genes. Furthermore, if gene flow is very limited, then genetic relatedness among individuals falls off exponentially with distance (Malecot 1969) and there is an exponential decrease of allelic lethal genes with distance separating populations (Yokoyama 1979). Because gene flow is very limited in *C. fasciculata*, differences in relationship among populations may be undetectable in terms of differences in performances of the F_1. Finally, interpopulation distance may not be related to genetic differentiation if colonization follows an island model, which may be consistent with our results, at least within the scale of tens of kilometers. Corroborating these last two points, an allozyme survey of populations at a similar spatial scale indicates that as much population differentiation occurs at the level of between populations at local levels as at higher levels (Fenster 1988; Fenster and Dudash 1994; L. F. Galloway, C. Hardy, and C. B. Fenster, unpubl. data). It is possible that all of the above

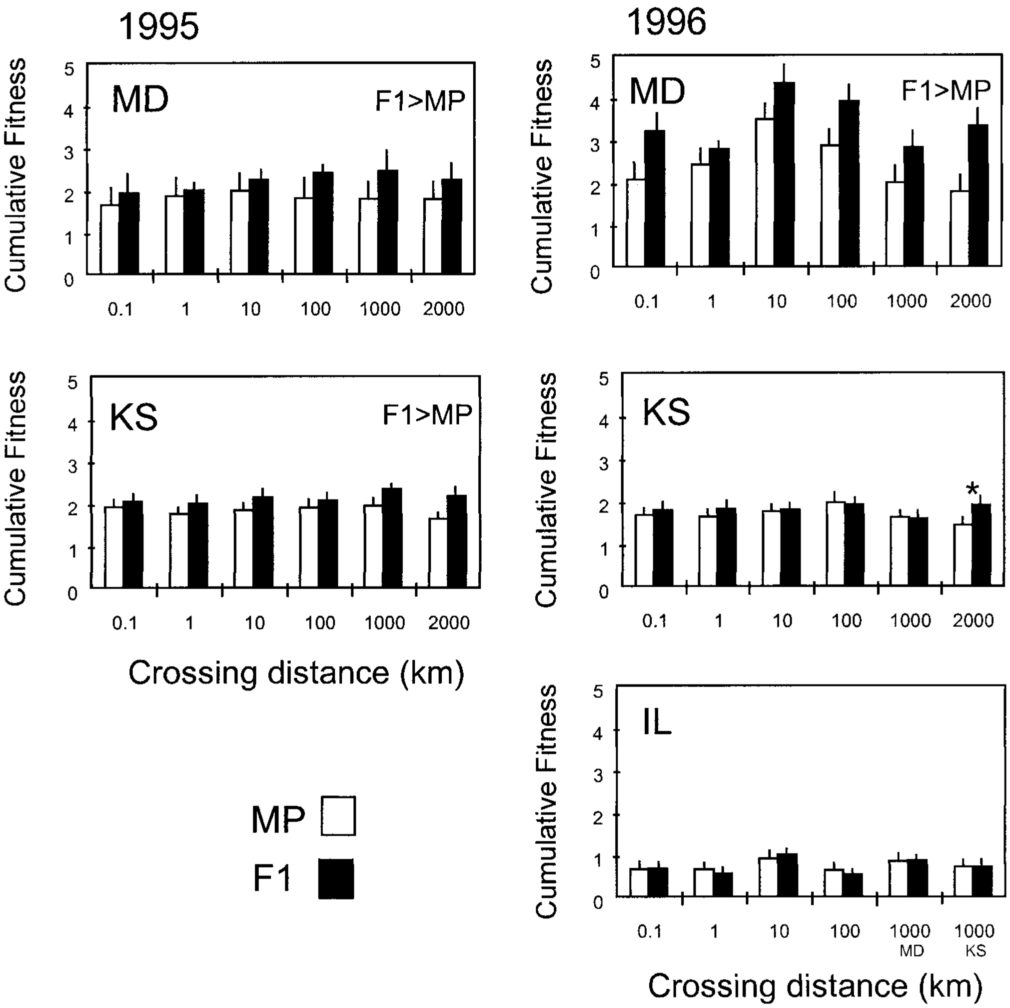

FIG. 8. Contrasts of F_1 of crosses among populations of *Chamaecrista fasciculata* with the midparent (MP) to determine the contribution of drift to population differentiation for fitness. Square-root-transformed means adjusted for initial seed size are presented. The difference in fitness between MP and F_1 was significant for each of the two years of the study in Maryland and for Kansas in 1995 (noted in the body of the figure). Although there was no overall F_1 heterosis in Kansas in 1996, investigation of the generation-by-distance interaction revealed that the longest distance cross yielded F_1 heterosis (indicated by an asterisk; $P < 0.05$ following sequential Bonferroni correction). Bars represent two standard errors.

phenomena simultaneously contribute to the relationship between the expression of heterosis, epistasis, and distance separating the populations.

Population Divergence and the Interaction among Selection, Drift, and Epistasis

Epistatic loss of fitness in the F_3 of interpopulation crosses where local adaptation was observed (Galloway and Fenster 2000), for example, between Kansas and Maryland, suggest that epistasis may contribute to a population's response to local environmental selective pressures or that epistasis is an outcome of population structure and additive substitution of alleles due to selection (Dobzhansky 1937; Gavrilets and Hastings 1996). However, the expression of epistasis in the F_3 even where the parentals demonstrated no evidence of local adaptation (Galloway and Fenster 2000) also demonstrates that epistasis can contribute to fitness differentiation independent of whether that differentiation is associated with local selection pressures. A number of theoretical models demonstrate that different favorable gene interactions can evolve with similar selective pressures if selection acts on different genetic backgrounds arising from drift. In one of the earliest models combining drift and selection (Wright 1935; recently extended by Barton 1986, 1989) limited gene flow leads to different combinations of genes in different populations. With similar optimizing selection pressures on populations, different genes are selected to produce similar phenotypes. Drift combined with uniform directional selection pressures could also lead to epistatic differentiation (Cohan 1984). Both of these processes may reflect lack of consistent local adaptation among populations of *C. fasciculata* that we observed at the scale of $\leq$ 100 km (Galloway and Fenster 2000). Another stochastic process, the random incorporation of novel mutations across populations that have epistatic effects on subsequent mutations (Mani and Clarke 1990), may also give rise to the observed loss of fitness in

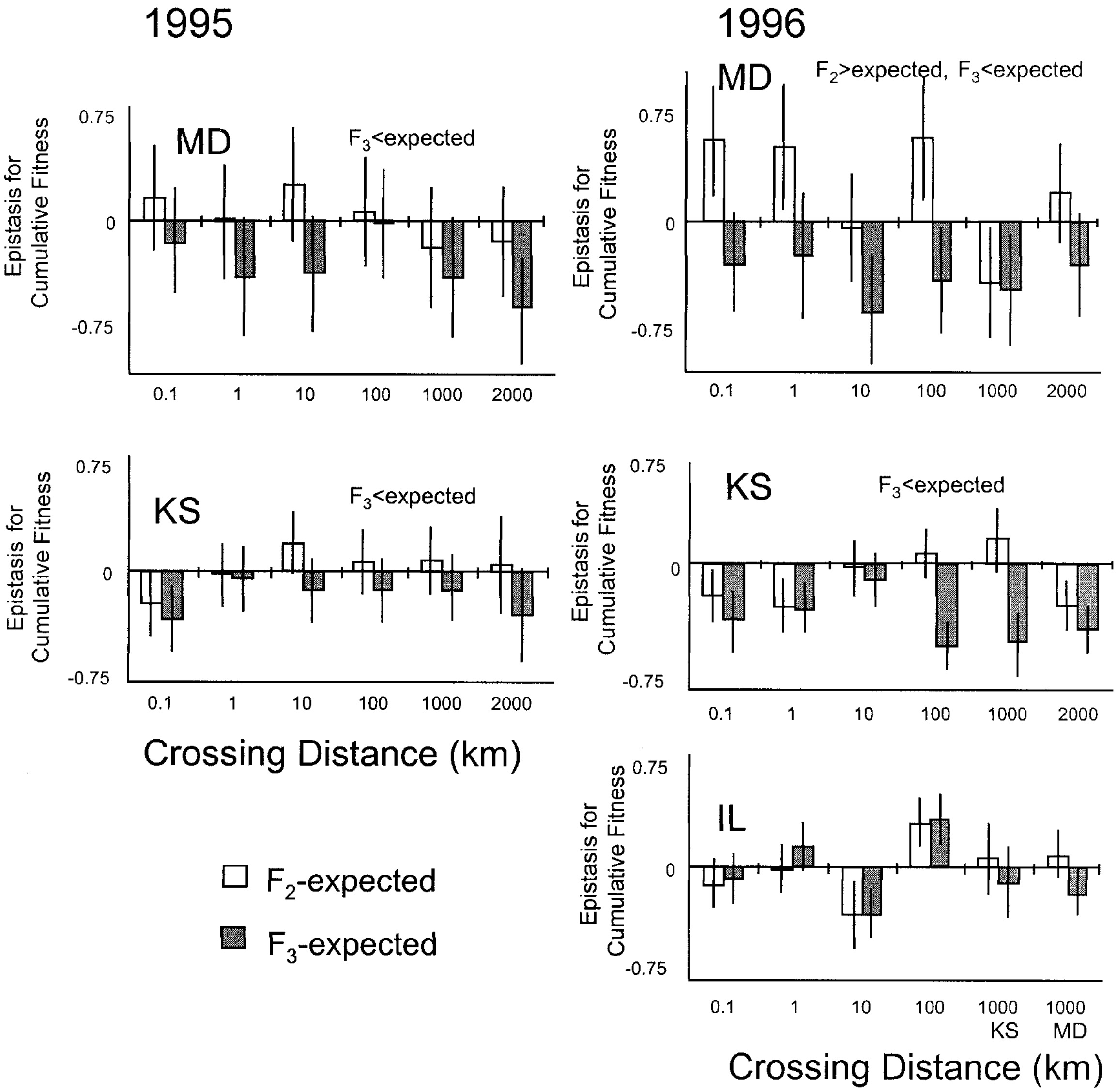

FIG. 9. Deviation of F_2 and F_3 hybrids from the average of the F_1 and midparent generations to quantify the contribution of epistasis to population differentiation for cumulative fitness. Significant deviations from zero (noted in the body of the figure) indicate epistatic differentiation between the populations of *Chamaecrista fasciculata.* Square-root-transformed means adjusted for initial seed size are presented. Bars represent two standard errors. See Figure 2 for details.

the F_3 generation. In addition, it is possible that the disruption of positive gene interactions in the F_3 of the interpopulation crosses may reflect a process of fixation of deleterious mutations due to drift followed by fixation of epistatic compensatory mutations, as recently demonstrated in experimental populations of RNA virus ϕ6 (Burch and Chao 1999). The unexpected fitness loss of the F_3 may also be due to epistatic interactions among deleterious mutations brought together by the interpopulation crosses, but this seems less likely given the limited empirical evidence that interactions among mutations are uniformly negative (Dudash et al. 1997; Elena and Lenski 1997).

Independent of the precise forces responsible for epistatic divergence, the evolution of gene interactions in populations of *C. fasciculata* suggests that each population represents a unique and independent genetic solution imposed by selection (i.e., genetic redundancy; Cohan 1984; Futuyma 1997). Although there is much evidence that evolution is redundant, it is less clear that epistatic interactions underlie this redundancy. Artificial selection experiments demonstrate that drift or the incorporation of novel mutations into different lines may constrain populations to evolve different genetic responses (Cohan and Hoffmann 1989; Cohan et al. 1989; Korona et al. 1994; Travisano et al. 1995). These different responses sometimes represent the evolution of interacting coadapted gene complexes (Mourad 1965; Kitagawa 1967; Enfield 1977; Malmberg 1977; Lenski 1988; Blows 1993; Blows and Sokolowski 1995; Burch and Chao 1999), but also may represent the fixation of alleles at different loci with additive effect. The documentation of disruption of epistatic interactions in the F_3 presented here mirrors the breakdown of presumably adaptive characters observed in other studies. For example, between-population crosses result in larger retina size in the blind cave fish (Wilkens 1971), breakdown of pesticide resistance in houseflies (King 1955), the recovery of the wild-type breeding system in *Eichhornia paniculata* (Fenster and Barrett 1994), and others (Cohan 1984). This breakdown may reflect either the fixation of recessive alleles at different loci (e.g., *E. paniculata*, Fenster and Barrett 1994) or interlocus interactions. Our results differ from many of the above examples because we have explicitly demonstrated that epistasis contributes to the variety of genetic solutions to selection pressures imposed by the environment.

One of the motivating reasons behind our study was to document the relevancy of epistasis to the evolution of natural populations. Whether or not epistasis contributes to population divergence was one of the distinguishing features of Wright's (1931) and Fisher's (1930) alternative visions of the evolutionary process. Although three important features of Wright's shifting balance theory—genetic drift (Fenster 1991a,b), adaptive differentiation (Fenster 1997; Galloway and Fenster 2000), and epistasis for fitness—are present in *C. fasciculata,* our results do not prove one of the defining trademarks of shifting balance, namely that population differences have evolved by crossing a fitness valley (Coyne et al. 1997). In other words, the multilocus interactions that we detected in our experiments do not necessarily demonstrate that populations of *C. fasciculata* are sitting on peaks completely surrounded by a landscape of genetic combinations of lower fitness. With intermediate environments or gene frequencies it is just as possible that simple selection on the individual effects of genes as opposed to selection on gene interactions may have resulted in populations becoming differentiated for epistatic interactions (Dobzhansky 1937; Maynard Smith 1989; Orr 1995; Gavrilets and Hastings 1996). In this case, with ridges connecting peaks, it may not be necessary to invoke populations crossing through a fitness valley. Theoretical investigations demonstrate that where ridges and valleys are both present, populations are much more likely to evolve along the ridges (Wagner et al. 1994; Barton 1996). Furthermore, the environmental dependence of gene expression demonstrated by variation in parental performance (Galloway and Fenster 2000) and fluctuations in the amount of heterosis and epistasis expressed across the life history in different sites and years suggests that the fitness surface is fluid for populations of *C. fasciculata,* which may facilitate peak shifts (Whitlock 1995, 1997). Thus, a fitness valley one year may be a peak or a ridge in another. Therefore, our results may be concordant with both Fisher's (1930) and Wright's (1931) vision of the evolutionary process in that populations may always evolve to increase fitness, but that different populations have different genetic starting points because of drift and epistasis.

The prevalence of epistatic differentiation suggests that the evolutionary process is complex within *C. fasciculata.* The presence of epistasis also implicates population structure or small population size as being an important determinant of evolutionary process, because panmixia would not allow the formation of the observed gene interactions. Thus, the evolution of populations of *C. fasciculata* appears to reflect ''a series of small experiments'' (Wade 1992) where ''context is of the essence'' (Lewontin 1974). The future challenge will be to determine the generality of our results and whether the genetic architecture of population differentiation described here contributes to diversification at the species level.

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