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INTRASPECIFIC HYBRIDIZATION AND THE RECOVERY OF FITNESS IN THE NATIVE LEGUME *CHAMAECRISTA FASCICULATA*

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Abstract.—Genetic incompatibilities and low offspring fitness are characteristic outcomes of hybridization between species. Yet, the creative potential of recombination following hybridization continues to be debated. Here we quantify the outcome of hybridization and recombination between adaptively divergent populations of the North American legume *Chamaecrista fasciculata* in a large-scale field experiment. Previously, hybrids between these populations demonstrated hybrid breakdown, suggesting the expression of adaptive epistatic interactions underlying population genetic differentiation. However, the outcome of hybridization ultimately rests on the performance of even later generation recombinants. In experiments that compared the performance of recombinant F_6 and F_2 generations with nonrecombinant F_1 and parental genotypes, we observed that increasing recombination had contrasting effects on different life-history components. Lifetime fitness, defined as the product of survivorship and reproduction, showed a strong recovery of fitness in the F_6. The overall gain in fitness with increased recombination suggests that hybridization and recombination may provide the necessary genetic variation for adaptive evolution within species. We discuss the mechanisms that may account for the gain in fitness with recombination, and explore the implications for hybrid speciation and phenotypic evolution.

Key words.—*Chamaecrista fasciculata*, epistasis, fitness, heterosis, hybridization, inbreeding depression, recombination.

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Novel genetic variation introduced to populations through gene flow can improve a population's response to natural selection (Fisher 1930). Accordingly, botanists recognized early on the importance of gene flow and hybridization in evolution and speciation (Rieseberg 1995). The selective consequence of gene flow between populations or species is a function of the genetic architecture contributing to adaptation and fitness in the different populations or species (Wright 1940, 1951; Barton 2001). Specifically, epistasis can reduce the benefit of hybridization and gene flow when beneficial genic combinations are disrupted (Fenster et al. 1997 and references therein). Ultimately, the acquisition of sufficient genetic differentiation, particularly when epistatic, can contribute to reproductive isolation and speciation, as in the expression of Dobzhansky-Muller incompatibilities (Dobzhansky 1937; Muller 1940; Mayr 1970; Wright 1977; Orr 1995). Consequently, gene flow between taxa carrying different sets of interacting loci, and subsequent recombination in the hybrid generations, will result in hybrid breakdown and a loss of fitness. Thus, the potential for introgression or even transgressive segregation depends in part on the contribution of epistasis to the genetic architecture differentiating populations or species (Anderson 1968; Arnold 1997; Rieseberg 1997; Barton 2001).

However, epistasis has also been invoked as a creative force, where novel interactions among loci can generate new phenotypes that exhibit improved fitness, as in Wright's shifting balance hypothesis (Wright 1931; Whitlock et al. 1995; Burch and Chao 2004). Similarly, gene flow and recombination can allow for the disruption of negative epistasis, which may be fixed through drift. Lastly, epistasis has been implicated in the de novo evolution of species following hybridization due to epistatic incompatibilities between the new hybrid species and either progenitor (Barton 2001). However, despite the theoretical potential for epistasis as a creative force, very few studies have empirically demonstrated a creative role for epistasis in adaptive evolution or speciation. Experiments that explicitly test for the effect of epistasis on adaptation and fitness, particularly in native environments, will contribute significantly to our understanding of the genetic architecture underlying adaptation and speciation.

A robust test of the outcome of the role of epistasis in hybridization is through the use of reciprocal transplant experiments, in which recombinant hybrids are field tested along with their parents in each parental environment (Lynch and Walsh 1998; Rundle and Whitlock 2001). Here we report on field-based experiments that examine the role of epistasis in the genetic architecture of adaptation and response to hybridization in the legume *Chamaecrista fasciculata*. We use reciprocal transplants in a generation means analysis that allows us to explicitly examine the effect of increasing recombination between differentiated genomes on fitness and adaptation and to infer the relative contribution of epistasis to this process. Prior work with the same populations comparing F_2 and F_3 fitness demonstrated that epistasis made a frequent and significant contribution to local adaptation (Fenster and Galloway 2000a). That study inferred epistasis' contribution to adaptive differentiation via hybrid breakdown implying beneficial synergism within populations and suggested that the interacting loci were linked. We expand upon that work by testing later generation recombinants (F_6) to further elucidate linkage relationships and by controlling for maternal effects among recombinant generations. Thus, we employ a large-scale field-based experiment to quantify the contribution of epistasis to adaptation in native environments

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and the implications of epistasis for the outcome of hybridization and recombination.

Methods and Materials

Study Organism and Study Site

Chamaecrista fasciculata ($2n = 2x = 16$) is an annual legume native to eastern North America, whose range extends from Massachusetts south to Florida and west to Minnesota and Texas. It is found in a variety of natural habitats that are prone to disturbance: sand dunes, prairies, and savannahs. Although highly outcrossing, gene flow in *C. fasciculata* is extremely limited due to limited pollen dispersal (Fenster 1991a,b; Fenster et al. 2003), consequently populations are inbred, yet with local adaptation of populations occurring at the scale of >100 km (Fenster and Galloway 2000a). We employed the same field sites as used before, in Kansas and Maryland, which represent the extremes of the distribution of the species along an east-west gradient. The Kansas site is in a tallgrass prairie located in Konza Prairie in the Flint Hills of Kansas. The Maryland site is at a sandy margin of an agricultural field at the U.S. Department of Agriculture, Beltsville, Maryland. From the two study sites, genotypes were collected in fall 1992 from more than 20 maternal lines and are the source of the Maryland and Kansas parents used in the crosses described below.

Crossing design

In our experiment, we evaluated a set of fitness components for the two parental populations (Maryland and Kansas), an F_1 hybrid and two recombinant hybrid generations (F_2 and F_6). Thus, five genotypes were employed in the experiment. The parental generations represent a sampling of parental genotypes (approximately 20 maternal families) collected as seed from the respective sites in the fall of 1992. To generate the recombinant hybrids, we initiated a regime of random mating beginning with intermating the parental strains from Kansas and Maryland to produce an F_1 generation. We used a regime of random mating, but maintained the identity of the source population of the female parent, which we termed maternal cytotype. Thus, we had two cytotypes, Kansas and Maryland. This allowed us to balance the proportional representation of Kansas and Maryland genotypes in our crosses through equal composition of Maryland and Kansas maternal cytotypes (with origin of the maternal parent or cytotype noted with a subscript K or M). Maintenance of both Kansas and Maryland cytotypes also allowed us to test for cytonuclear disequilibria as a form of epistasis that may contribute to fitness. For both cytotypes we maintained 20 maternal lines throughout all crosses. Thus, we randomly intermated F_{1K} with F_{1M} to produce an F_{2K} and F_{2M} generation and for each subsequent generation we continued random intermating within each hybrid generation until we generated a recombinant F_5 generation (Fig. 1).

Reciprocal crosses between the populations were initiated in the summer of 1993, and by 1999 the F_5 generation was created. In the summer of 2000, the Kansas and Maryland parentals and the F_1, each representing the most recent regeneration of these generations, were grown simultaneously

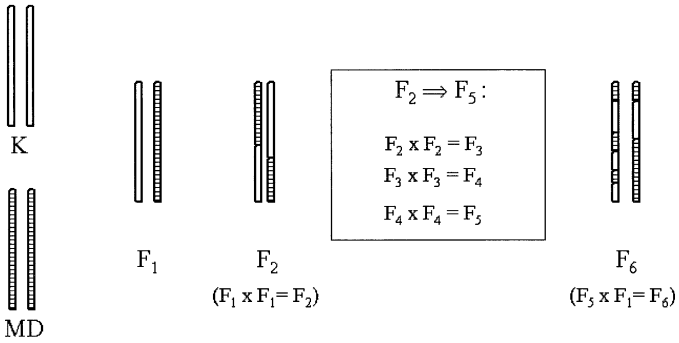

FIG. 1. A schematic of the crossing design, presented via a chromosome diagram. The combinations of Maryland and Kansas genomic material are represented by hatched and open bars, respectively. The degree of mixing of hatched and open bars on each chromosome represents the expected amount of recombination for each generation. Random intermating within a generation was used to generate subsequent generations in all cases except the F_6, which was generated by backcrossing the F_5 onto F_1 female plants. See the Materials and Methods section on crossing design for more detail.

with the F_5. To control for maternal effects, the F_2 was generated as normal by intermating F_1 plants whereas the F_6 was generated by crossing F_5 plants (as pollen parent) back to F_1 plants (maternal parent). In this way, both the F_2 and F_6 generations shared the same maternal parent (F_1 plants) and differences between the two generations could not be ascribed to maternal effects. Because each recombinant generation was produced through random intermating, heterozygosity was high throughout each generation, minimizing selection on genetic load. In addition, we eliminated fertility selection by allowing each maternal line to contribute equally to the next generation. Throughout the study we routinely distinguish between recombinant (F_2 and F_6 generations) and nonrecombinant generations (two parental types and the F_1), where recombinants have chromosomes that are mixtures of the two parental types.

Field Experiments to Quantify Fitness

Hand-scarified seeds from each of the five generations (the parents, F_1, F_2, and F_6) were planted into native field sites in both Maryland and Kansas, in the exact locations from which each of the parental populations were originally collected. The experiment was replicated in 2001 and 2002. Twenty blocks were established within each of the two sites. Each block was approximately 1 m^2, and all 20 blocks were planted in an area of approximately 500 m^2. Within each of the 20 blocks, 140 seeds were planted, consisting of the following types: 20 Kansas parentals, 20 Maryland parentals, 20 F_1 (10 of each cytotype), and 40 each of the F_2 and F_6 (20 of each cytotype). Sample sizes in the F_2 and F_6 generations were twice as large to compensate for the expected increase in phenotypic variance in the recombinant generations. Within each block, the distribution of individuals was random with respect to generation. The total number of seeds planted within each site was 2800, and the total planted over the course of the experiment in two sites for two seasons was 11,200.

All seeds were planted in late March for both the 2001

and 2002 field seasons in Maryland and Kansas, respectively. Seed germination occurred simultaneously with the native population. Four characters related to fitness were measured during and after the growing season: germination, survivorship, fruit production, and dry plant biomass. For each of these characters, we estimated means for each generation for each block. Germination and survivorship were scored for each individual six weeks after planting. We refer to this six-week census as germination rate. Plants grew through the summer until they were fruiting in September when they were harvested. Survivorship from the six-week census to harvest was generally high, averaging >91% per year and site. Following harvesting and drying, we measured the number of fruit produced and dry weight biomass for each collected plant. Individuals that died were neither alive at the six-week census nor present at time of harvest and were assigned a zero for fruit production. However, individuals that were harvested but that did not produce fruit or were standing dead in place were included in the estimate of biomass. Because fruit production was highly correlated with biomass (r^2 = 0.92) and biomass could be more accurately measured than fruit production (because the fruit explosively dehisce), only biomass is reported here. Only individuals with a nonzero biomass were used in analyses to quantify genetic effects on biomass. We also calculated a multiplicative measure of lifetime fitness defined as the product of germination and biomass. This fitness measure represents lifetime fitness for an individual, encompassing juvenile (germination) and adult (adult biomass) estimates of fitness.

Quantifying Epistasis

We quantified the contribution of epistasis to adaptation via a generation means contrast (Lynch and Walsh 1998). This involves comparing the fitness of the two recombinant generations, F_2 and F_6, with what we term a composite generation. The composite generation is defined as the average of the two parents with the F_1 (i.e., composite generation = [((K parent + MD parent)/2) + F_1]/2). This composite generation represents the contribution of additive and dominant gene action to performance (Lynch and Walsh 1998). The contribution of epistasis to trait differentiation is quantified through deviation of the recombinant F_2 and F_6 generations from the nonrecombinant composite generation. Under a model of fitness that only incorporates additive and dominant gene effects, recombination should have no effect on fitness components and hence no difference should be observed between composite, F_2 and F_6 generations. It is important to note that the regime of random mating used to generate each generation maintained equivalent allele frequencies between the composite and recombinant F_2 and F_6 generations. In this sense, the composite represents a null model of no epistatic contribution to fitness. If the F_2 or F_6 significantly differ from the composite, then epistasis must be invoked to explain the deviation. Differences between the F_2 and F_6 generations would be due to different linkage arrangements among interacting loci. We assume there was no selection throughout the course of cultivation of the hybrids. We note that cultivation in a benign greenhouse environment in conjunction with the large numbers of maternal families employed, a regime of outcrossing and suppression of fertility differences among lines should greatly reduce the power of natural selection to change allele frequencies that would affect fitness in natural habitats.

Statistical Analysis

Analysis of variance (ANOVA) was used to quantify differences in performance among the generations for all measured traits. We report here the effects of recombination for three traits: germination, biomass, and lifetime fitness. Because survivorship was high (>91%) for all generations, we did not examine the effect of generation on survivorship. To meet the assumptions of ANOVA, block means were used within each site. For each block, we obtained a mean value for each cross type, Kansas, Maryland, F_1, F_2, and F_6. We used SAS (proc GLM; SAS Institute 1996) to perform a mixed model ANOVA, with generation as a fixed effect and block, year, and state as random effects. No evidence of cytonuclear disequilibrium was observed; consequently, Maryland and Kansas cytotypes were pooled for each generation. All main effects and interactions among main effects were investigated for each trait examined. When a significant ($P \leq 0.05$) interaction between main effects was observed, the data were partitioned and the model adjusted accordingly to remove the interaction. Nonsignificant interactions involving random variables that had an *F*-value less than 2 were pooled with the error term.

To compare the F_2 and F_6 generations with the parental and F_1 generations, we conducted ANOVAs for each year and site and used Tukey's contrasts to determine significant differences between the parental, F_1, F_2, and F_6 generations (Figs. 1 and 2). We conducted these comparisons using all generations, in which we had no a priori expectations, in order to document local adaptation (relative parental performances) and heterosis (F_1 performance relative to the parents) and to measure the performance of the recombinant F_2 and F_6 generations (e.g., hybrid breakdown) relative to the parents and to the F_1. This latter examination does not by itself quantify the contribution of epistasis but it does allow us to consider the consequence of epistasis scaled to parental and F_1 performance. Since the contribution of epistasis to population differentiation is measured by a priori contrasts of the recombinant generations with the composite, orthogonal linear contrast statements were used to test for significant differences between a subset of the individual pairs of generations (composite vs. F_2 vs. F_6). The standard error of the contribution of epistasis was constructed by linear combination of the appropriately weighted variance estimates of the generation means that contribute to the estimate. Comparison of F_2 fitness is then based upon the weighted variance of the composite generation and the F_2, whereas the comparison of F_6 is based upon the weighted variance of the composite generation and F_6.

Germination

We observed no significant differences in germination among the different generations in both 2001 (Fig. 2) and 2002 (Fig. 3) based on ANOVA and Tukey's contrasts. However, the highest rates of germination were observed in the

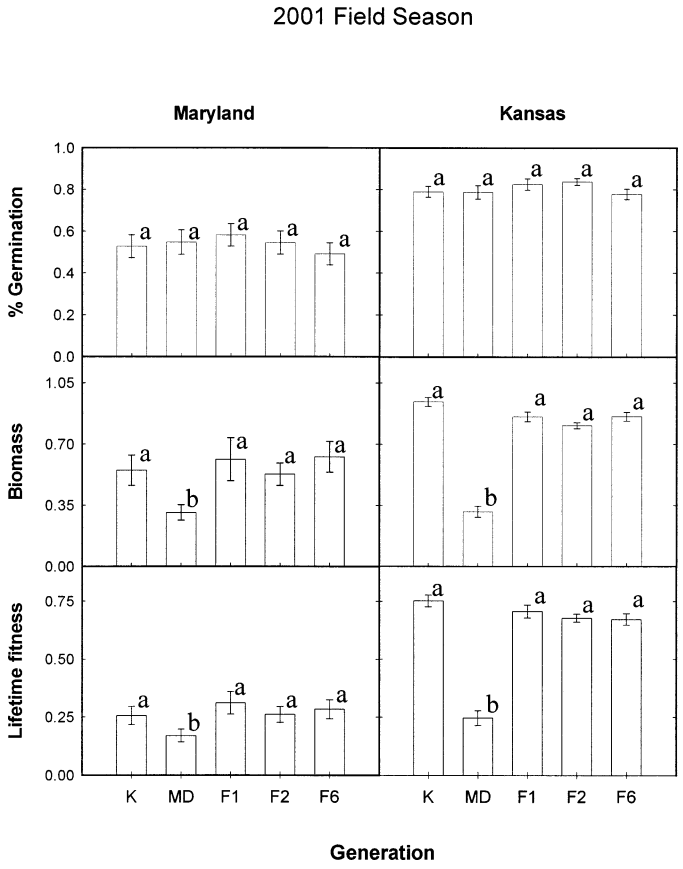

FIG. 2. Results for each generation planted for the 2001 field season are plotted. Data for all three fitness characters are shown displaying the differences among parental and F_1 performance as well as the proportional representation of those generations that constitute the ''composite'' generation. Bars denoted with different superscript lettering are significantly different ($\alpha \leq 0.05$) based on ANOVA with Tukey's contrast.

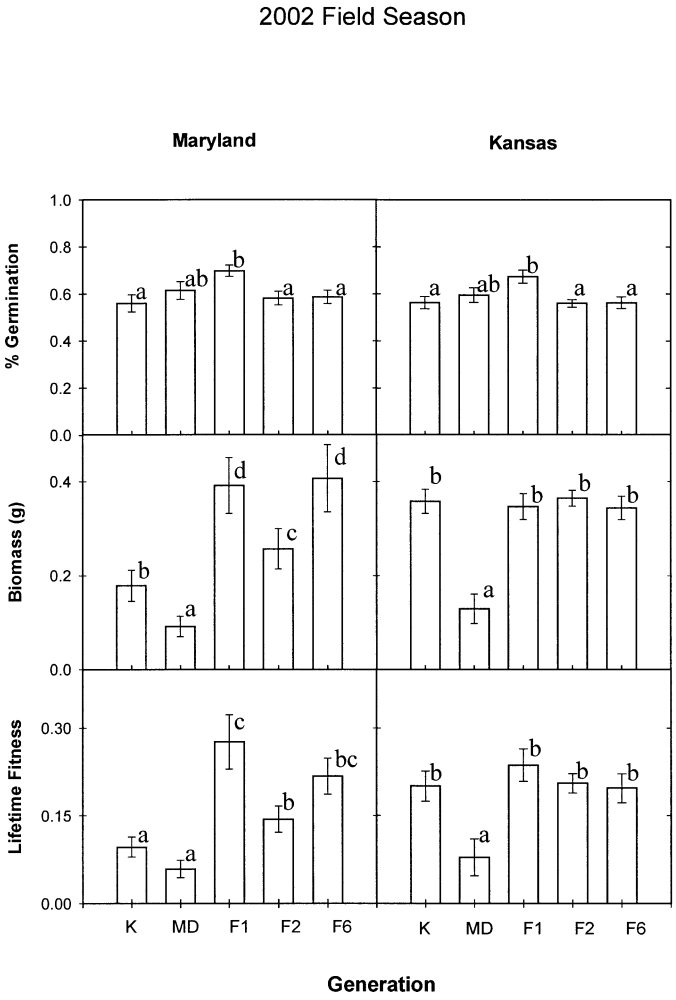

FIG. 3. Results for each generation planted for the 2002 field season are plotted. Data for all three fitness characters are shown, displaying the differences among parental and F_1 performance as well as the proportional representation of those generations that constitute the ''composite'' generation. Bars denoted with different superscript lettering are significantly different ($\alpha \leq 0.05$) based on ANOVA with Tukey's contrast.

F_1 generation, with the parental genotypes intermediate and the recombinant F_2 and F_6 generations lowest.

To more explicitly quantify the role of epistasis, we used linear contrasts between the two recombinants and the nonrecombinant composite. This three-generation contrast revealed significant differences in germination between the three generations. There was a significant interaction between year and generation ($F_{2,156} = 5.47$, $P < 0.006$), so all main effects were analyzed separately for 2001 and 2002. In both 2001 and 2002, the effect of generation ($F_{2,78} = 6.5$, $P < 0.0025$; $F_{2,78} = 12.0$, $P < 0.0001$ for 2001 and 2002, respectively) was significant, with the F_6 performing significantly below that of the composite (Table 1, Fig. 4) in both years. In 2002, the germination rate for the F_6 was significantly lower than both the F_2 ($F_{1,78} = 10.9$, $P = 0.0001$) and the composite ($F_{1,78} = 8.4$, $P = 0.005$). However, the F_2 and the composite were not significantly different from each other ($F_{1,78} = 0.17$, $P = 0.684$). The year-by-generation effect is largely attributable to variation in the F_2. In 2001, the F_2 generation exhibited the lowest rates of germination, whereas in 2002 it had the highest rate of germination, although it was not significantly higher than the composite.

TABLE 1. Germination rate among generations of a cross between Maryland and Kansas populations of *Chamaecrista fasciculata* using proc GLM with independent linear contrasts.

Source	df	2001 *F*	2002 *F*
Generation	2	6.5**	12.0**
State	1	25.9**	0.4
Block (state)	39	12.5**	8.4**
Error	78		
Contrast			
F_2 vs. composite	1	0.17	19.1**
F_6 vs. composite	1	8.4**	17.7**
F_2 vs. F_6	1	10.9**	0.08

** Significant at 0.01.

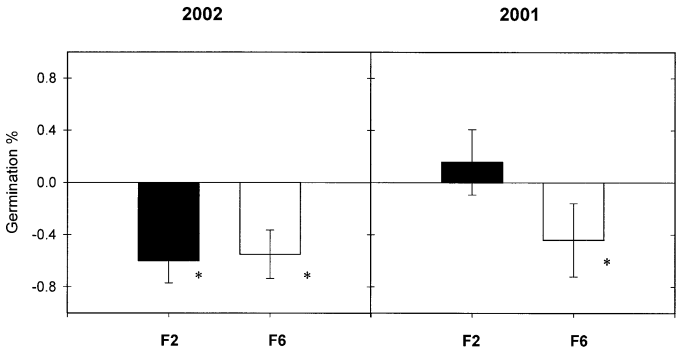

FIG. 4. Differences in germination percentage were tested for the F_2, F_6, and composite in *Chamaecrista fasciculata* are presented. Data are divided by year, 2001 and 2002, due to interaction between year and generation. The line at zero along the y-axis represents the expected value defined by the nonrecombinant composite generation. The asterisk denotes a significant difference from the expected value of zero (no epistasis). Bars are one standard error.

Biomass

The effect of recombination on biomass was very different than that for germination. First, there were significant differences observed between some of the planted generations as determined via ANOVA and Tukey's contrast. Specifically, the Maryland parental genotype performed poorly in all year/environment trials, and was significantly worse than the other genotypes when tested in the Kansas environment for both 2001 (Fig. 2) and 2002 (Fig. 3). The F_1 generation performed well in all years and sites. Similarly, the recombinant F_2 and F_6 generations performed well in all year/environment trials, and were not significantly different from the F_1 (save Maryland 2002, in which the F_2 exhibited significantly lower biomass; Fig. 2). In the three-generation contrast to quantify the contribution of epistasis to biomass, there were no significant interactions of generation with either state ($F_{2,2} = 6.54$, $P = 0.133$) or year ($F_{2,2} = 7.29$, $P = 0.121$); therefore, we combined results across years and states for analysis. The effect of generation was highly significant. Independent linear contrasts revealed the F_6 significantly outperformed both the F_2 ($F_{1,156} = 7.89$, $P = 0.006$) and composite ($F_{1,156} = 17.98$, $P < 0.0001$), whereas the F_2 and composite were not significantly different from each other ($F_{1,156} = 1.09$, $P = 0.298$; Table 2; Fig. 5).

Lifetime Fitness (Percent Survivorship × Biomass)

There was a consistent difference in the performance of the two parental genotypes, with Kansas outperforming Maryland in three of the four year/environment trials (Figs. 2 and 3). The Maryland parental genotype consistently exhibited the lowest lifetime fitness, and in only one year/environment trial did it not have significantly lower lifetime fitness than all other generations that were planted (Maryland 2002; Fig. 3). The F_1 generation exhibited high fitness in both environments for both years (Figs. 2 and 3). However, the F_1 did not exhibit significantly higher fitness than the two recombinant generations and was only significantly better than the Kansas parental genotype in one of four year/environment trials (Maryland 2002; Fig. 3). The F_6 generation exhibited high lifetime fitness in all year/environment trials, and was equivalent to the F_1 in all cases. The F_2 generation was more variable than the F_6, although it generally exhibited high fitness, only dropping significantly below the F_1 in one year/environment trial (Maryland 2001; Fig. 2).

TABLE 2. Data for biomass and lifetime fitness for *Chamaecrista fasciculata*, pooled over both years and states due to consistent effect of generation throughout. The effect of generation was highly significant for biomass production, but was nonsignificant for lifetime fitness.

	Biomass		Lifetime fitness	
Source	df	*F*	df	*F*
Generation	2	9.31**	2	2.85
Year	1	12.27	1	3.06
State	1	2.00	1	1.61
Block (year × state)	76	5.14**	76	6.23**
Error	156		156	
Contrast				
F_2 vs. composite	1	2.05	1	0.39
F_6 vs. composite	1	17.9**	1	5.32*
F_2 vs. F_6	1	7.89**	1	2.83

* Significant at 0.05; ** significant at 0.01.

In the three-generation contrast, there was no interaction between either year or state; therefore, we pooled data from across state and year. The F_6 performed significantly better than the composite generation ($F = 8.38$, $P > 0.005$), and also performed significantly better than the F_2 ($F = 10.9$, $P = 0.001$, Table 2). The substantial gain in fitness realized through biomass was more than enough to offset the loss of fitness observed through lower germination in the F_6, such that recombination among differentially adapted parental populations led to a gain in fitness (Fig. 5). Linkage among interacting loci was implicated through differences between the F_2 and F_6 generations, in which increased recombination resulted in a gain in fitness.

DISCUSSION

We used a large-scale, field-based experiment to examine the role of hybridization and recombination in mediating

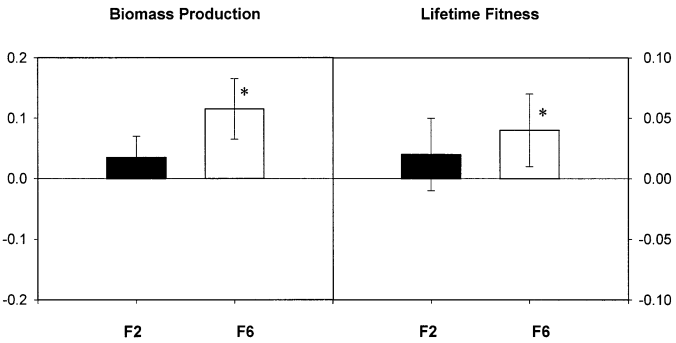

FIG. 5. Results for linear contrast among the composite, F_2, and F_6 for biomass production and lifetime fitness (percent germination × biomass) for *Chamaecrista fasciculata*. The graphs show untransformed data, while significance was tested using a square-root transformation on the data. The line at zero along the y-axis represents the expected value defined by the nonrecombinant composite generation. The asterisk denotes a significant difference from the expected value of zero (no epistasis). Bars are one standard error.

changes in fitness to natural populations of the legume *C. fasciculata*. Because we field-tested genotypes in native habitats, the results we observed reflect adaptively relevant changes to genetic architecture. This contrasts to greenhouse or laboratory experiments in which the fitness effects of experimental treatments are not tested under natural selective regimes. We specifically examined the role of epistasis in contributing to fitness in the field following hybridization and recombination between differentiated populations. We anticipated a decrease in fitness with increasing recombination, with the F_6 performing below the F_2 and composite. This expectation was derived from a prior study of the same populations of *C. fasciculata* in which a large decline in the fitness of a recombinant F_3 generation was observed relative to an F_2 and nonrecombinant composite (Fenster and Galloway 2000a) due to disruption of positive epistasis. However, there is an alternate null hypothesis regarding the outcome of hybridization based upon the creative power of sexual recombination and increased genetic variation (Kaltz and Bell 2002). In this context, the advantage of hybridization can be viewed as consistent with Wright's shifting balance through the assembly of novel multilocus genotypes or in the origin of homoploid hybrid species. Our results support the latter model, with hybridization and recombination increasing fitness and with a significant contribution of epistasis to the process, as inferred through a generation means contrast. Comparison of the recombinant F_2 and F_6 generations with the parental and F_1 generations (Figs. 2 and 3) demonstrates that the recovery of fitness in the F_6 produces genotypes with equal or greater fitness than either parent. As further evidence of the relative success of the F_6, under some situations the fitness of the F_6 approached that of the F_1 (Fig. 2, biomass production). Here the F_1 generation manifested consistently high fitness, indicative of heterosis and high genetic load in the parental lineages. Below, we discuss our observation of the contrasting effects of recombination on fitness at different life-history stages, the genetic mechanisms that can account for these results, and the evolutionary implications of our findings.

Consequences of Recombination on Different Life-History Stages

The F_6 generation exhibited a small but significantly decreased rate of germination in the field relative to the nonrecombinant composite (Fig. 4). This effect was consistent in all environments for both years. This result is consistent with the disruption of epistasis among coadapted gene complexes (Lynch and Walsh 1998). The disruption of beneficial epistasis is commonly observed in crosses between adaptively or geographically divergent populations (Templeton 1980; Orr and Coyne 1989; Hard et al. 1992; Parker 1992; Armbruster et al. 1997; Fenster and Galloway 2000a; Carroll et al. 2003), and may manifest itself as outbreeding depression (Price and Waser 1979; Fenster and Galloway 2000a) or as Dobzhansky-Muller incompatibilities (Palopoli and Wu 1994; Orr 1995; Orr and Irving 2001). The Dobzhansky-Muller model of speciation makes implicit assumptions regarding the central role of epistasis in differentiating the genome, such that epistasis serves as a mechanism that bridges the microevolutionary process with macroevolutionary patterns where differentiated populations evolve into distinct species (Wallace 1953; Orr 1995; Whitlock et al. 1995; Fenster et al. 1997). Our results on germination suggest that the populations from Kansas and Maryland have acquired incompatibilities that reinforce population differentiation through decreased germination rates. Thus, our results for germination rate are consistent with models of evolution that invoke epistasis as a critical component of genetic architecture that contributes to population divergence and hybrid breakdown (reinforcement) upon secondary contact (Wright 1940; Whitlock et al. 1995; Fenster et al. 1997; Wolf et al. 2000).

However, in contrast to hybrid breakdown in germination, we observed that the F_6 generation consistently outperformed the composite generation for both biomass production and lifetime fitness (germination $\times$ biomass). The effect was only significant in a three-way contrast between F_6, F_2, and the composite, and was present but not significant in a model testing all generations simultaneously. Yet, the significant gain in fitness of the F_6 generation was consistent among sites and years under the three-generation contrast. The F_6 generation similarly outperformed the F_2 generation in all sites and years. These results are counterintuitive in light of our expectation that adaptively differentiated populations should reflect the evolution of optimal genotypes in their respective environments. However, as we discuss below, the evolution of populations depends on what genetic variation is present. Thus, it is possible our crossing design introduced novel genetic variation into the recombinant populations, which allowed for the evolution of novel, highly fit genotypes after sufficient rounds of recombination disrupted linkage disequilibria among loci.

Mechanisms of Fitness Gain

Our experiment focused on the role of epistasis in the genetic architecture that underlies fitness and adaptation. The generation means test we employed specifically considered the role of epistasis in comparisons among recombinant hybrids and their nonrecombinant progenitors, which collectively we term the composite generation. Through the use of random mating and large numbers of maternal lines, we generated recombinant hybrids that have the same allele frequencies and rates of heterozygosity as that the composite, meaning that the effect of additive and dominant gene action are the same for all generations. Hence, changes in fitness among generations cannot be ascribed to changes in additive or dominant gene effects; instead, changes in fitness are attributed to changes in genic combinations, or epistasis. The assumption of the generation means contrast is that there is no selection and no change in allele frequency among generations. Because selection can never be entirely eliminated as a mechanism for observed gains in fitness, we explicitly consider how both epistasis and selection may have contributed to the unexpectedly high fitness we observed in the recombinant F_6 hybrid generation.

Epistasis

Increased rounds of recombination may have increased the opportunity for the de novo synthesis of highly fit hybrids

and/or the disruption of negative epistasis. The synthesis of highly fit hybrids can have occurred through the serendipitous combination of the appropriate alleles brought about by recombination. Early experiments on recombination demonstrated that hybridization and recombination can improve population fitness and response to selection (Thoday et al. 1964), and recent experiments demonstrated that populations harbor substantial epistatic genetic variance that can contribute to selection response and that this epistatic genetic variance can be increased through hybridization among different lines (e.g., Bradshaw et al. 2005). The assembly of genotypes that exhibited increased fitness was attributed to increased genetic diversity and the generation of fit recombinant hybrids. These fit recombinants may be derived from de novo genic combinations. Alternatively, hybridization and recombination may disrupt negative epistasis and generate an increase in fitness among recombinant hybrids. That we observed a fitness increase in the F_6 versus the F_2 generation implies that the interacting loci are linked.

Thus, the synthesis of novel recombinants or the purging of deleterious epistatic combinations in the F_6 may arise due to changes in genotype frequency, as opposed to the purging of deleterious alleles, which changes allele frequency. Recombination changes genotype frequencies but does not change allele frequencies. Hence, allele frequencies and levels of heterozygosity in the F_6 and composite generations are expected to be the same, so that differences in fitness reflected in the generation means contrast may be attributable to the effect of recombination and reflect the contribution of epistasis to the genetic architecture of fitness and adaptation.

Selection

Selection cannot be discounted in explaining gains in fitness. The high F_1 hybrid fitness we observed demonstrates that the populations are likely inbred and harbored significant genetic load. Recombination between differentiated populations that harbor a high genetic load can allow for selection to purge deleterious alleles, increasing population fitness. However, most deleterious mutations maintained within populations are recessive (Lynch and Walsh 1998) and would be masked by heterozygosity on an interpopulation cross. The high fitness of the F_1 generation we observed in part reflects heterozygosity masking deleterious alleles that were fixed in the two populations. The F_2 and F_6 populations maintain half the heterozygosity gain of the F_1; thus, selection against deleterious recessive alleles in the recombinant generations would be limited due to high heterozygosity. In addition, we minimized selection against families carrying a high load of deleterious recessive alleles because each maternal line contributed equally to the next generation, eliminating the effect of fertility selection. Although we cannot exclude the potential for selective purging, we suggest that it is difficult to purge deleterious alleles under a mating design that uses random mating, equal representation of maternal lineages, and a benign cultivation environment such as the greenhouse. We note that deleterious alleles affecting embryonic development, germination, or pollen viability could be purged under our design. However, because we saw a decrease in germination with recombination, it does not appear that there has been selective purging at the level of germination. Although we cannot entirely reject the possibility of selective purging during cultivation of the F_6 generation, our experimental design minimized the opportunity for such selection, suggesting that much of the difference observed in the generation means contrast is attributable to epistasis and linkage. We note that evolution through selective purging is not mutually exclusive to evolution due to epistatic interactions following recombination. A combination of both selection and epistasis may have contributed to changes in fitness we observed.

Linkage and dominance effects in F_6 versus F_3

The high fitness in the F_6 hybrid generation we tested contrasts with the low fitness of an F_3 generation tested previously (Fenster and Galloway 2000a). In both experiments, we compared the more recombinant hybrid with a composite generation to examine for the contribution of epistasis and with a less recombinant F_2 hybrid to examine the degree of linkage between interacting loci. An important difference between the current experiment and the prior is that in the current study we constructed both the F_2 and F_6 hybrids through crossing pollen to a maternal plant (Fig. 1), which eliminated differences in maternal effects among hybrid generations. This was not done in the prior study, and maternal effects may have contributed to differences between the F_2 and F_3 generations. If maternal effects contributed to enhanced fitness of the F_2 generation (through the maternal transmission of heterosis from the F_1 generation) in the prior study, it may have affected the contrast between F_2 and F_3 generations. It is possible that the maternal transmission of heterosis from the F_1 contributed to the high fitness in both F_2 and F_6 recombinant generations. Anecdotally, we note that the F_5 sires were highly vigorous in the greenhouse, whereas in a simultaneous crossing program we observed the F_3 to be much less vigorous. These observations, combined with the high performance of the F_1, suggest that maternal effects made a limited contribution to progeny performance. However, to reiterate, by making both F_2 and F_6 on the F_1, we control for maternal effects in contributing to any differences between the two generations.

If epistasis and linkage contribute to the high performance of the F_6, then its fitness must reflect the genotypic composition of its chromosomes. The F_3 recombinants tested previously that demonstrated hybrid breakdown would have had equal rounds of recombination on all chromosomes, two bouts of recombination. In contrast, the F_6 in this study would have one chromosome contributed by the F_5 generation via pollen (representing four rounds of recombination) and one chromosome contributed by the F_1 generation (representing one round of recombination) via the ovule. This unbalanced chromosomal design could lead to both the maintenance of beneficial epistasis on the F_1 chromosome as well as purging of negative epistasis on the F_5 chromosome. Dominance could exaggerate this effect if positive epistasis in the F_1 chromosome balances any disruption on the F_5. Although we did not directly test for dominant $\times$ dominant epistasis, we suggest that, just as genetic drift can drive deleterious recessive alleles to fixation within populations, so it may fix

deleterious alleles that exhibit epistasis. The breakdown of such negative epistasis following hybridization and recombination may promote hybrid fitness and merits further study.

Evolutionary Consequences

Our results demonstrating a recovery of fitness in a recombinant F_6 population are potentially important in the context of the outcome of hybridization, particularly our understanding of the relevance of homoploid hybridization (Grant 1981; Rieseberg 1991; Arnold 1997; Rieseberg et al. 1999; Barton 2001). Species formation through hybridization among taxa with the same number of chromosomes, without a change in chromosome number in the new species, is termed homoploid hybrid speciation. A number of studies have examined the role of homoploid hybridization in species formation, specifically investigating the genetic basis of hybrid fertility and sterility barriers between sister species (Grant 1958, 1981; Rieseberg et al. 1995, 1996, 1999, 2003; Burke et al. 1998). When fertile hybrids are observed to arise, they often maintain discrete chromosomal blocks of genes that suggest a role of epistasis in the development of novel hybrid species (Kim and Rieseberg 2001). Indeed, a recent study by Rieseberg et al. (2003) demonstrated that fertile hybrids generated in the laboratory resembled a naturally occurring hybrid species with respect to the maintenance of blocks of genes from each parent, suggesting that, by maintaining sets of interacting loci on different chromosomes from each parent, hybrids may maintain or improve fitness particularly in novel environments. We also note that an apparent trade-off occurred between fitness gain in germination and biomass production. This trade-off may be attributable to some antagonism in which alleles that improve fitness for one character decrease fitness for the other. In its most extreme form, this antagonism may be attributable to pleiotropy, and can similarly be more rigorously tested via quantitative trait loci (QTL) methods.

Our ability to generate novel, highly fit hybrid genotypes, that approached F_1 hybrid fitness, suggests that natural populations are limited in their ability to respond to natural selection due to limited genetic variation and that drift may reduce population fitness through the fixation of deleterious recessive alleles. These limits may be due to the burden of fixation of deleterious alleles or, as we argue, the lack of appropriate genetic variation needed to synthesize highly fit coadaptive genotypes. Although the exact mechanism of fitness gain may only be fully explored through more detailed genetic approaches (e.g. QTL approaches; Erickson et al. 2004), the gain in fitness observed is relevant to the evolutionary role of gene flow and hybridization in evolution. Species hybrids, when they do fare well, often do so only in novel environments (Schemske 2000). However, the gain in fitness we observed occurred in both parental environments over both years. We only observed the home parent to outperform the away parent in Kansas, but in previous studies we also observed a home fitness advantage in Maryland (Fenster and Galloway 2000b). Thus, because we tested fitness in the field, the gain in fitness of the F_6 reflects adaptively relevant changes in genotype due to hybridization and recombination among differentiated populations. Hybridization through range expansion, resulting in secondary contact between races or populations that have long been isolated from one another, may be a common phenomenon (Anderson 1949; Stebbins 1950; Mayr 1970; Wright 1977), especially considering the case of isolation among population in glacial refuges and their subsequent mixing (e.g., Lagercrantz and Ryman 1990; Heuertz et al. 2004). Thus, the artificial mixing of genotypes that we performed may parallel the dynamics of gene flow during the evolutionary history of a species as well as the making of a successful invasive following hybridization between differentiated colonists (Ellstrand and Schierenbeck 2000).

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