



The Effect of Nuclear and Cytoplasmic Genes on Fitness and Local Adaptation in an Annual Legume, *Chamaecrista fasciculata*

Laura F. Galloway; Charles B. Fenster

Evolution, Vol. 53, No. 6 (Dec., 1999), 1734-1743.

Stable URL:

<http://links.jstor.org/sici?sici=0014-3820%28199912%2953%3A6%3C1734%3ATEONAC%3E2.0.CO%3B2-6>

Evolution is currently published by Society for the Study of Evolution.

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/about/terms.html>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/journals/ssevol.html>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

For more information on JSTOR contact jstor-info@umich.edu.

©2003 JSTOR

THE EFFECT OF NUCLEAR AND CYTOPLASMIC GENES ON FITNESS AND LOCAL ADAPTATION IN AN ANNUAL LEGUME, *CHAMAECRISTA FASCICULATA*

LAURA F. GALLOWAY¹ AND CHARLES B. FENSTER²

¹Department of Biology, University of Virginia, Charlottesville, Virginia 22903-2477

E-mail: lg8b@virginia.edu

²Department of Biology, University of Maryland, College Park, Maryland 20742

E-mail: cf25@umail.umd.edu

Abstract.—The role of nuclear genes in local adaptation has been well documented. However, the role of maternally inherited cytoplasmic genes to the evolution of natural populations has been relatively unstudied. To evaluate the contribution of cytoplasmic and nuclear genomes and their interactions to local adaptation we created second-generation backcross hybrids between a Maryland and an Illinois population of the annual legume *Chamaecrista fasciculata*. Backcross progeny were planted in the sites native to each population for two years and we quantified germination, survivorship, fruit production, vegetative biomass, and cumulative fitness. We found limited evidence for the contribution of either cytoplasmic or nuclear genes to local adaptation. In Maryland plants had greater survivorship, biomass, fruit production, and cumulative fitness if their nuclear genome was composed predominately of native Maryland genes; cytoplasmic genes did not affect fitness. In Illinois local cytoplasm marginally enhanced fitness, whereas Maryland nuclear genes outperformed local nuclear genes. Interactions between cytoplasmic and nuclear genes influenced seed weight, vegetative biomass, and fitness and therefore may affect evolution of these characters. Genetic effects were stronger acting through seed size than directly on characters. However, seed size differences between the two populations were largely genetic and therefore selection on fitness components is likely to result in evolutionary change. The contribution of nuclear and cytoplasmic genes to fitness components varied across sites and years, suggesting that experiments should be replicated and conducted under natural conditions to understand the influence of these genomes and their interactions to population differentiation.

Key words.—Cytonuclear interactions, cytoplasmic DNA, nuclear-cytoplasmic interaction.

Received November 13, 1998. Accepted May 12, 1999.

In plants both mitochondrial and chloroplast DNA jointly code with nuclear DNA for gene products involved in major metabolic functions (Grant 1975; Gillham 1978). Given that plants have two cytoplasmic genomes, it seems likely that they have the greatest potential of any taxonomic group for expressing cytoplasmic and nuclear-cytoplasmic genetic effects. Indeed, non-Mendelian and cytoplasmic maternal inheritance was first demonstrated by Correns (1909, cited in Grant 1975) with the trait leaf variegation in *Mirabilis jalapa* (Nyctagenaceae). Since Correns's early observations, cytoplasmic inheritance has been demonstrated to affect a number of plant traits, including quantitative traits associated with yield (Robertson and Frey 1984; reviewed in Roach and Wulff 1987), the expression of heterosis (Rao and Fleming 1978; Beavis and Frey 1987; reviewed in Jan 1992), and male sterility (Couvret et al. 1990). In most cases the expression of cytoplasmic variation depends on the nuclear background. The influence of the nuclear background on cytoplasmic effects is also demonstrated in crosses between related taxa where reciprocal effects increase with genetic divergence (reviewed in Wright 1968).

To date, most studies of cytoplasmic effects have been confined to crops or traits measured in controlled conditions (but see Burke et al. 1998). Studies with wild organisms from the natural environment have focused on the linkage disequilibrium between cytoplasmic and nuclear genes that is expected to arise under conditions of nonrandom mating or epistatic selection for favorable combinations of cytoplasmic and nuclear genomes (Basten and Asmussen 1997 and references therein). However, these studies are unable to distinguish the role of selection versus nonrandom mating in the evolution of nuclear-cytoplasmic disequilibrium. Which

of these mechanisms underlies nuclear-cytoplasmic disequilibrium can be determined empirically by evaluating whether interactions between the genomes enhance fitness.

The influence of cytoplasmic genes and their interactions with nuclear genes for fitness has been the object of a number of studies. In *Drosophila*, nuclear-cytoplasmic interactions for fitness were found when nuclear genes of populations from six geographic regions were expressed in the cytoplasmic background of each population (Clark and Lyckegaard 1988). However, no cytonuclear interaction was found in a comparable experiment involving nuclear and cytoplasmic genes from a number of lines derived from a single population (Clark and Lyckegaard 1988). These results imply that nuclear-cytoplasmic interactions are adaptive and that mtDNA variants that enhance fitness in a population are rapidly fixed. Hutter and Rand (1995) also found a fitness advantage of matching nuclear and cytoplasmic genomes using interspecific combinations, but only in one direction of a reciprocal backcross. Other *Drosophila* population-cage studies have found that selection influences mtDNA variation and that the outcome typically depends on the nuclear background (MacRae and Anderson 1988; Fos et al. 1990), but these results may have other explanations (Singh and Hale 1990; Nigro and Prout 1990). Alternatively, changes in the frequency of mtDNA variants can be accounted for by genetic drift (Kambhampati et al. 1992) and by hitchhiking on nuclear based fitness variation (Kilpatrick and Rand 1995).

Given the potential for cytoplasmic genes and nuclear-cytoplasmic interactions to enhance fitness and influence response to selection (Naylor 1964; Kirkpatrick and Lande 1989), it is of interest to determine if they contribute to adaptive differentiation among populations. Local adaptive dif-

ferentiation and the concept of an ecotype was first fully explored and documented in plants (reviewed by Briggs and Walters 1997) and is common at a broad range of spatial scales (Linhart and Grant 1996). Given the prevalence of local adaptation and the potential for the expression of cytoplasmic inheritance through both chloroplast and mitochondrial genomes, plants provide excellent systems to study the contribution of cytoplasmic genomes to adaptive evolution. Using a backcrossing design, we quantify the contribution of nuclear, cytoplasmic, and nuclear-cytoplasmic effects to local adaptation in the annual native North American pea *Chamaecrista fasciculata*. Our design controls for maternal environment and endosperm nuclear effects, which, in addition to cytoplasmically inherited genes, may be expressed as maternal effects (Roach and Wulff 1987). The experiments were conducted in the natural environment at two sites and replicated across two years.

MATERIALS AND METHODS

Two geographically isolated *Chamaecrista fasciculata* (Fabaceae) populations were chosen to evaluate the effects of nuclear genes, cytoplasmic genes, and their interactions on local adaptation. One population was located at the margin of an agricultural field at the U.S. Department of Agriculture site at Beltsville, Maryland. The other was located in Goose-lake Prairie, a tall grass prairie near Morris, Illinois. Morphological and phenological characters differed between the populations when grown together in the greenhouse, thus revealing genetic divergence for traits likely to contribute to fitness (L. F. Galloway and C. B. Fenster, unpubl. data).

Crosses were performed to create seeds that differed in their combination of cytoplasmic and nuclear genes. Field-collected seeds from both sites were grown in the greenhouse. Reciprocal F_1 hybrids were formed by using Maryland individuals as pollen donors to Illinois plants and individuals from Illinois as pollen donors to Maryland plants. We assume that cytoplasmic genes are maternally inherited in *C. fasciculata* (maternal inheritance of plastid DNA is found in closely related *Cassia* [= *Senna*] *marylandica*; Corriveau and Coleman 1988); therefore, the F_1 generation will have the cytoplasmic genes of the maternal population. Two types of crosses were conducted using the F_1 hybrids as maternal parents and the parental populations as pollen donors. (1) F_1 individuals were backcrossed to their paternal population for two generations, resulting in seeds with cytoplasmic genes from their maternal population and, on average, 87.5% of their nuclear genes from the other population; and (2) F_1 individuals were backcrossed to their maternal population for two generations, resulting in seeds with cytoplasmic genes and, on average, 87.5% of their nuclear genes from the maternal population. This crossing design resulted in four types of second-generation backcross seed (BC2, where lower case letters refer to cytoplasm type and upper case to nuclear type): Maryland cytoplasm and nuclear genes (mM), Maryland cytoplasm and Illinois nuclear genes (mI), Illinois cytoplasm and Maryland nuclear genes (iM), and Illinois cytoplasm and nuclear genes (iI). At least 10 maternal families were used each generation and single pollen donors were randomly chosen for each cross to minimize the loss of genetic variation

when creating the experimental seed. In addition, variation in maternal environmental effects among the seeds was minimized by three generations of growth in the greenhouse. The BC2 generation was created from first-generation backcross (BC1) seed separately for each year of the experiment, resulting in a slightly different seed pool each year.

The four types of seed were planted into the sites native to the parental populations for two years. In 1996, 10 mM, 10 iM, 8 iI, and 6 mI seeds were planted into the natural vegetation in each of 22 fenced blocks in Maryland and in Illinois in a randomized block design. In 1997, aboveground biomass was partially removed prior to planting 24 seeds of each seed type in each of 36 fenced blocks per site. In total 5720 seeds were planted. Several weeks before natural *C. fasciculata* germination, scarified seeds were planted at 5-cm intervals 1 cm below the soil surface either directly in front of or behind (depending on the year) a plastic swizzle stick. Based on proximity to the swizzle stick and the presence of slight scarring on the cotyledon from scarification, we were able to differentiate experimental seedlings from naturally occurring ones. Seeds were censused for germination and early postgermination survivorship approximately six weeks after planting (referred to as "germination"). Survivorship from seed to fruit production was recorded. Most mortality occurred prior to flowering in 1996; only 2.4% of plants died between flowering and fruit production. When flowering was complete, plants were harvested, developing fruits removed and counted, and aboveground vegetative biomass weighed. Our estimate of fitness is the number of fruit produced per seed planted of a given seed type in a block. This fitness estimate is cumulative in that it includes both survivorship and fecundity. Note that all seeds planted contribute to the estimate of fitness, but only surviving individuals are included in vegetative biomass and fruit production measurements.

Statistical Analysis

All characters were analyzed using analysis of variance (ANOVA). Analyses were conducted on percent germination and percent survivorship of each seed type in each block, and block means for each cytonuclear treatment of seed weight, vegetative biomass, fruit production, and fitness. Block means minimized heteroscedasticity of residuals. All variables except seed weight were analyzed with a nested, mixed-model ANOVA in which nuclear genes (IL or MD), cytoplasmic genes (il or md), planting location (Maryland or Illinois), and year (1996 or 1997) were fixed effects and block, nested within planting location, was a random effect. To meet the assumptions of ANOVA, survivorship was arcsine transformed, biomass was square-root transformed, and fruit production and fitness were natural log transformed. To prevent the loss of data following transformations, the value one was added to all fruit production values and 0.1 to all fitness estimates. For all postgermination variables there were significant two-way or higher-order interactions involving planting location and year (analyses not presented). Because we were interested in the genetic contribution to local adaptation in each planting location, we performed separate analyses for each location. If there were significant interac-

tions between the genetic factors and year within a location, thus revealing that genetic effects differed between years, the two years were analyzed separately. Seed weight was analyzed using a three-way ANOVA with nuclear genes, cytoplasmic genes, and year as fixed effects.

Nuclear and cytoplasmic genes may affect fitness components either directly or through seed size. Genetic effects on a character acting through seed size may be estimated by statistically removing seed size variation through analysis of covariance. Although an analysis of phenotypic variation provides an understanding of the genetic underpinnings of characters that selection may act on, removing seed size effects provides a more mechanistic understanding of the source of genetic effects. Legume seeds consist of seed coat and embryo; all endosperm is digested during seed development. Therefore, seed weight reflects maternal environment and maternal genetic effects on the seed coat and seed provisioning. To remove seed size effects from later life characters, analyses were also performed with weight of the seeds planted as a covariate. Interactions with the covariate were dropped when not significant ($P > 0.05$).

RESULTS

Seed Weight.—Seeds with MD nuclear genes were heavier than those with IL nuclear genes, whereas the effect of cytoplasmic genes on seed weight varied with year and nuclear genes. Seed weight was analyzed separately for each year because a three-way interaction indicated that cytoplasmic-by-nuclear genetic interactions differed between years ($F_{1,455} = 14.09$, $P < 0.0002$, analysis not shown). This was due in part to a change in the weight of seeds with md cytoplasmic and IL nuclear genes relative to those with both Illinois nuclear and cytoplasmic genes between years (Fig. 1a,b). In 1996 there was a significant interaction between cytoplasmic and nuclear genes (Table 1A). Seeds with IL nuclear genes were heavier if they also had il cytoplasmic genes ($F_{1,86} = 26.07$, $P < 0.001$); however, seeds with MD nuclear genes were the same size regardless of cytoplasm type ($F_{1,85} = 0.03$, $P = 0.85$; Fig. 1a). In 1997, there was a significant main effect of both nuclear and cytoplasmic genes (Table 1B). Seeds having MD nuclear genes or md cytoplasmic genes were heavier than those with IL nuclear or il cytoplasmic genes (Fig. 1b).

Germination.—Germination rates in Maryland were twice that of Illinois, whereas cytoplasmic and nuclear genetic effects remained consistent across sites. Overall, 62% of seeds germinated in Maryland while only 30% germinated in Illinois (Table 1). The effect of the cytoplasmic genes on germination differed across years ($F_{1,378} = 8.08$, $P < 0.005$), therefore, analyses were conducted separately for each year. In 1996, only cytoplasmic genes influenced germination: seeds with md cytoplasmic genes had marginally greater germination when acting through seed weight (Table 1A, Fig. 1c) and significantly greater germination rates with seed weight effects removed (ANCOVA $F_{1,124} = 5.16$, $P < 0.02$, analysis not shown) than seeds with il cytoplasm. Seed weight was positively associated with germination ($F_{1,124} = 5.45$, $P < 0.02$). In 1997, seeds with il cytoplasmic genes and MD nuclear genes had the greatest rates of germination in both

Maryland and Illinois (Table 1B, Fig. 1d). This pattern is unchanged when the genetic effects do not act through seed weight (ANCOVA; cytoplasm: $F_{1,209} = 5.76$, $P < 0.02$; nuclear: $F_{1,209} = 10.21$, $P < 0.002$, analysis not shown) largely because the relationship between seed weight and germination was not significant ($F_{1,209} = 3.23$, $P < 0.07$). Nuclear effects differ in magnitude across the sites in 1997 (Table 1B). The germination advantage of seeds with MD nuclear genes relative to those with IL nuclear genes is 24% greater in Illinois than in Maryland.

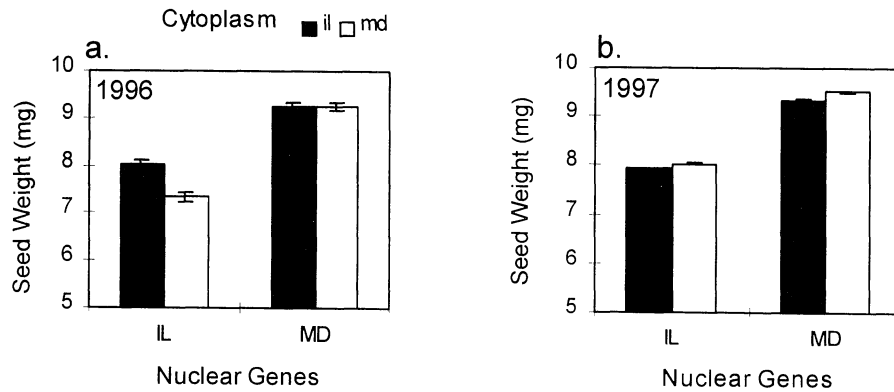
Survivorship.—Individuals with MD nuclear genes had greater survivorship regardless of planting location. Nuclear genes affected survival in the same way at both sites (nuclear by location: $F_{1,378} = 0.62$, $P = 0.43$; Table 2). In Illinois, 27% of individuals with MD nuclear genes survived, whereas only 21% of those with IL nuclear genes did. The pattern is slightly stronger in Maryland, 33% of individuals with MD nuclear genes survived, in contrast to 24% of those with IL genes. These nuclear genetic effects on survivorship act through seed weight. When seed weight is included as a covariate, nuclear effects are no longer significant for survivorship (Table 3).

Vegetative Biomass.—Genetic effects on vegetative biomass were only found in the Maryland planting site (Table 2B). Nuclear genes influenced final plant size in Maryland and their effect differed across years (Table 2B). In 1996, when overall plant size was larger, MD nuclear genes increased biomass almost threefold over IL nuclear genes (means: MD = 1.17 g, IL = 0.41 g), whereas in 1997 they increased biomass by only 1.5 times (means: MD = 0.37 g, IL = 0.20 g). Nuclear genetic effects in Maryland were also found when genes do not act through seed weight (Table 3B). However, with seed weight effects removed, cytoplasmic genes and a cytoplasm-by-nuclear interaction also influence biomass in Maryland (Table 3B). Plants with “matching” nuclear and cytoplasmic genes tend to be larger than those with “mismatched” nuclear and cytoplasmic genes within each nuclear type ($P < 0.07$; Fig. 2). Similarly, a cytoplasm-by-nuclear interaction for biomass is found when both the Maryland and Illinois planting locations are included in the ANCOVA ($F_{1,319} = 4.13$, $P < 0.05$, analysis not shown).

Fruit Production.—Like biomass, genetic effects on fruit production were only found in the Maryland planting site (Table 2B). Individuals with MD nuclear genes produced more fruit than those with IL nuclear genes when planted in Maryland (means: MD = 11.3, IL = 4.6; Table 2B). This difference is about 61% greater in 1996 than 1997, thus creating a nuclear-by-year interaction (Table 2B). Seed weight has little influence on fruit production; therefore, genetic effects remain the same regardless of whether they act through seed weight (Table 3B).

Fitness (Fruit Production/Seed Planted).—Both cytoplasmic and nuclear genes enhance fitness, but not necessarily local adaptation, in Illinois. In Illinois there was a significant three-way interaction revealing that specific combinations of cytoplasmic and nuclear genes enhance fitness and those combinations differ between years (Table 2A). To understand this interaction, analysis of the Illinois site was conducted separately by year. There was a significant cytoplasm-by-nuclear interaction in 1996 in Illinois (Table 4). Individuals

Seed Weight



Germination

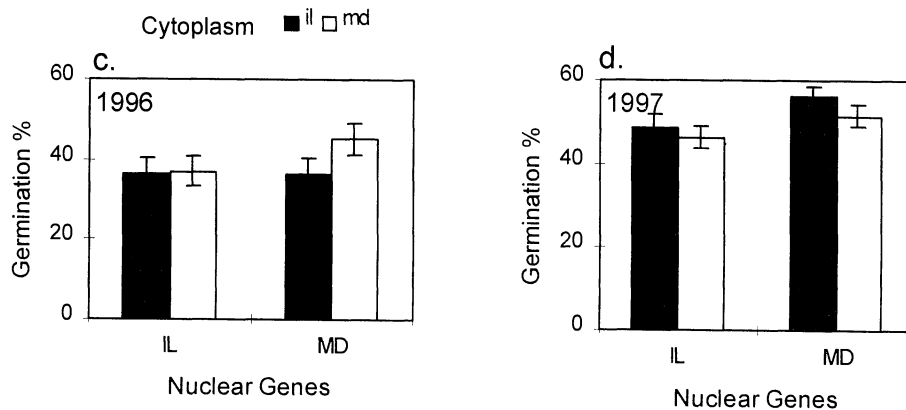


FIG. 1. Mean (\pm SE) weight and percent germination of seeds with either Illinois or Maryland cytoplasmic genes and 87% Illinois or Maryland nuclear genes planted in Maryland and Illinois over two years. See text for statistical analysis.

with il cytoplasm perform equally well with both IL and MD nuclear genes, whereas those with md cytoplasm outperform all other seed types when with MD nuclear genes and perform the most poorly when combined with IL nuclear genes (Fig. 3a). In 1997 in Illinois, plants with MD nuclear genes significantly outperformed those with IL nuclear genes, and there was a tendency ($P < 0.08$) for individuals with il cytoplasm to outperform those with md cytoplasm (Table 4, Fig. 3b). Genetic effects in Illinois are not significant when seed weight variation is removed, although there is a near significant ($P < 0.07$) interaction between cytoplasmic genes and year due to changes in the rank fitness between il and md cytoplasm across years (Table 3A).

Local nuclear genes enhance fitness in Maryland. In Maryland, individuals with MD nuclear genes perform better than those with IL nuclear genes, regardless of cytoplasm type (Table 2B; Fig. 3c,d). This pattern is unchanged when the effects of seed weight are removed (Table 3B).

DISCUSSION

The Contribution of Cytoplasmic and Nuclear Genes to Local Adaptation

Both cytoplasmic and nuclear genes in *C. fasciculata* contribute to fitness, although evidence for local adaptation is

weak (Table 5). In Maryland, survivorship, biomass, fruit production, and cumulative fitness were greater in plants whose nuclear genome was composed predominately of local genes. Surprisingly, Maryland genes also enhanced fitness in Illinois. In 1996 the most fit seed type had both Maryland nuclear genes and Maryland cytoplasmic genes. In 1997, fitness of individuals with Maryland nuclear genes was greater than those with local Illinois nuclear genes. In Illinois there was weak evidence for local adaptation. Local cytoplasmic genes enhanced fitness in 1997, but this result was not significant ($P < 0.08$).

Lack of a home-site advantage for Illinois genotypes was also found in 1996 in experiments to document the genetic architecture of fitness (L. F. Galloway and C. B. Fenster, unpubl. ms.). Despite the evidence of no home-site advantage in Illinois, we have documented genetic differences in phenology and morphology between the Maryland and Illinois populations that seem to reflect the action of local selective forces (L. F. Galloway and C. B. Fenster, unpubl. data). For example, Illinois has a shorter growing season than Maryland, and in a common environment Illinois plants flower and set seed significantly earlier than Maryland plants. These apparently adaptive differences between the populations suggest that absence of local adaptation in Illinois is not due to

TABLE 1. Analysis of variance of nuclear and cytoplasmic genetic effects on seed mass and percent germination in *Chamaecrista fasciculata* for two years. Planting location and block nested within planting location were added as factors for germination. *F*-statistics and *P*-values are reported for each factor. See text for transformations.

Source	df ¹	Seed weight	% Germination
A. 1996			
Cytoplasm	1	11.46***	3.09†
Nuclear	1	250.03***	2.17
Cyto × nuc	1	13.35***	2.50
Location	1	—	140.53***
Cyto × loc	1	—	0.01
Nuc × loc	1	—	0.81
Cyto × nuc × loc	1	—	0.13
Block (loc)	42	—	2.25***
B. 1997			
Cytoplasm	1	33.27***	10.44**
Nuclear	1	33344.00***	32.49***
Cyto × nuc	1	3.82†	0.85
Location	1	—	812.20***
Cyto × loc	1	—	0.94
Nuc × loc	1	—	5.71*
Cyto × nuc × loc	1	—	0.12
Block (loc)	70	—	9.43***

¹ 1996 *df*_{error}: seed weight = 171, % germination = 126; 1997 *df*_{error}: seed weight = 284, % germination = 210.

*** *P* < 0.001; ** *P* < 0.01; * *P* < 0.05; † *P* < 0.1.

insufficient time to respond to selection. Rather, the lack of a fitness advantage of Illinois plants over Maryland plants in Illinois may simply reflect year-to-year environmental variation. Presumably, selection is episodic and had we replicated the experiments over more years we would have detected an Illinois home-site advantage. Alternatively, greater fitness of Maryland nuclear genes in Illinois disappears when seed size is accounted for, suggesting the larger size of Maryland seeds underlies the fitness advantage of Maryland nuclear genes. Although seed size may be under selection to

increase in Illinois, it is also possible that by hand-scarifying the seeds and planting just prior to germination, we may have inadvertently changed the selection dynamics and reduced disadvantageous aspects of larger seeds in the Illinois site.

In studies of local adaptation, field-collected seeds are typically reciprocally transplanted between populations (e.g., Rice and Mack 1991; Sork et al. 1993; Bennington and McGraw 1995; Kindell et al. 1996; Nagy and Rice 1997; Stanton and Galen 1997). In this design, both genetic and maternal environmental effects may contribute to the estimation of local adaptation. Although having the strongest influence on seed and seedling characters, maternal environments commonly affect adult characters (e.g., Stratton 1989; Miao et al. 1991; Galloway 1995; Lacey 1996). We grew plants in a common environment for three generations, minimizing differences in maternal environmental effects among seed types; therefore, our test of local adaptation reflects genetically based differences between populations.

No other studies have explicitly explored the contribution of nuclear and cytoplasmic genes to local adaptation. Local adaptation is common at the spatial scale of 1000 km used in this study (reviewed in Linhart and Grant 1996); indeed it is often found at smaller scales (e.g., Antonovics and Bradshaw 1970; Galen et al. 1991; Jordan 1992; Sork et al. 1993; Bennington and McGraw 1995). Because cytoplasmic genes typically have uniparental inheritance (i.e., maternal) they are dispersed only through seeds. In contrast, nuclear genes are inherited biparentally, and therefore may be dispersed through both seeds and pollen. As a consequence of these different modes of dispersal, cytoplasmic genes typically show genetic structure at smaller spatial scales than nuclear genes (Lloyd 1982; McCauley 1995, 1998). Spatial structure is likely to influence local adaptation, resulting in finer scale local adaptation for cytoplasmic genes than nuclear genes. We did not explore local adaptation with enough spatial resolution to test this hypothesis. However, we found limited

TABLE 2. Analysis of variance of nuclear and cytoplasmic genetic effects on percent survivorship, vegetative biomass, fruit production, and cumulative fitness (fruit/seed planted) in *Chamaecrista fasciculata* planted in two locations for two years. *F*-statistics and *P*-values are reported for each factor. See text for transformations.

Source	df ¹	Survivorship	Biomass	Fruit production	Fitness
A. Illinois site					
Cytoplasm	1	0.52	2.30	0.92	0.07
Nuclear	1	14.97***	0.27	0.83	11.54***
Cyto × nuc	1	0.41	0.00	0.06	1.85
Year	1	51.22***	200.28***	161.64***	191.92***
Cyto × year	1	1.50	0.93	1.17	1.47
Nuc × year	1	0.10	0.02	0.17	0.29
Cyto × nuc × year	1	1.15	0.00	0.46	4.35*
Block	35	3.30***	3.92***	6.36***	5.59***
B. Maryland site					
Cytoplasm	1	0.00	0.08	0.04	0.00
Nuclear	1	19.53***	117.62***	94.32***	82.63***
Cyto × nuc	1	0.13	0.05	0.13	0.43
Year	1	90.82***	120.38***	86.47**	119.92***
Cyto × year	1	0.52	0.49	0.73	0.03
Nuc × year	1	0.09	25.50***	4.58*	2.99†
Cyto × nuc × year	1	0.71	0.03	2.11	2.46
Block	35	5.23***	1.66*	1.10	3.46***

¹ *df*_{error}: survivorship and fitness = 189, biomass and fruit production = 160.

*** *P* < 0.001; ** *P* < 0.01; * *P* < 0.05; † *P* < 0.1.

TABLE 3. Analysis of covariance of nuclear and cytoplasmic genetic effects on percent survivorship, vegetative biomass, fruit production, and cumulative fitness (fruit/seed planted) in *Chamaecrista fasciculata* planted in two locations for two years. Seed mass is included as a covariate. *F*-statistics and *P*-values are reported for each factor. See text for transformations and Table 2 for significance levels and df_{error} .

Source	df	Survivorship	Biomass	Fruit production	Fitness
A. Illinois site					
Cytoplasm	1	0.32	2.70	1.18	0.00
Nuclear	1	0.55	1.56	0.31	0.15
Cyto × nuc	1	0.03	0.13	0.01	0.24
Year	1	45.21***	185.22***	148.91***	173.31***
Cyto × year	1	2.32	1.56	1.85	3.33†
Nuc × year	1	0.26	0.00	0.29	0.80
Cyto × nuc × year	1	0.50	0.06	0.15	2.00
Block	35	3.33***	3.94***	6.36***	5.86***
Seed weight	1	1.47	1.30	1.28	5.08*
B. Maryland site					
Cytoplasm	1	0.02	4.01*	0.01	0.05
Nuclear	1	0.15	24.06***	16.16***	8.33**
Cyto × nuc	1	0.00	3.45†	0.11	0.18
Year	1	98.36***	98.66***	81.93***	120.93***
Cyto × year	1	1.30	1.17	0.33	0.38
Nuc × year	1	0.01	26.47**	4.38*	3.19†
Cyto × nuc × year	1	0.26	0.05	1.95	1.87
Block	35	5.43***	1.58*	1.10	3.50***
Seed weight	1	4.23*	0.27 ¹	0.63	2.80†

¹ Seed weight × cyto *F* = 3.95*.

evidence for local adaptation at the scale of 1000 km for both genomes, suggesting that differences in genetic structure do not influence local adaptation at large spatial scales in this species. Our finding of a lack of local adaptation at 1000 km is unusual. Although the contribution of cytoplasmic genes to local adaptation has not been previously reported, they have been found to enhance yield in agricultural settings and fitness under laboratory conditions, often through interactions with nuclear genes (e.g., Clark 1985; Beavis and Frey 1987; Clark and Lyckegaard 1988; Pollak 1991; Hutter and Rand 1995).

Cytoplasmic genetic variation in animal studies is almost certainly due to mtDNA (Nigro 1994), but in plants may be attributed to variation in either cpDNA or mtDNA. Sequences

of cpDNA and plant mtDNA are both more conserved than animal mtDNA (Gilham 1994). Structural rearrangements in plant mtDNA are common (Gilham 1994) and have been implicated in cytoplasmic male sterility (Saumitou-LaPrade et al. 1994), one of the most thoroughly investigated cytonuclear interactions. Mitochondrial DNA is also the source of cytonuclear interactions in tobacco, where plants with recombined mtDNA from two tobacco species suffered reduced fitness (Pollack 1991). These studies suggest that mtDNA differentiation between populations underlies observed cytoplasmic effects in *C. fasciculata*.

Coadaptation between Cytoplasmic and Nuclear Genes

Cytoplasmic and nuclear genomes in *C. fasciculata* are coadapted for seed weight, vegetative biomass, and fitness. In Illinois in 1996, individuals with Maryland nuclear genes had greater fitness if they also had Maryland cytoplasmic genes. Similarly, individuals with Illinois nuclear genes had

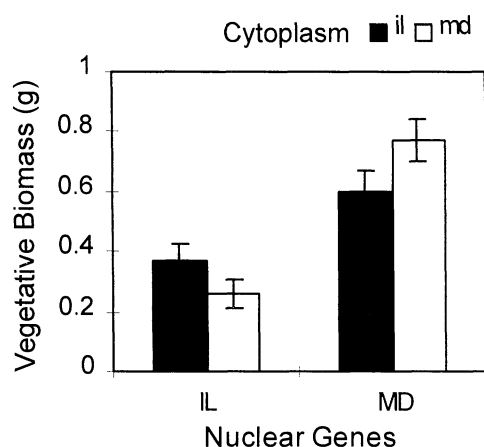


FIG. 2. Mean (\pm SE) vegetative biomass of plants with either Illinois or Maryland cytoplasmic genes and 87% Illinois or Maryland nuclear genes planted in Maryland. Least-square means adjusted for seed weight are shown. See Table 3 for statistical analysis.

TABLE 4. Analysis of variance of nuclear and cytoplasmic genetic effects on cumulative fitness (fruit/seed planted) in *Chamaecrista fasciculata* planted in Illinois for two years. *F*-statistics and *P*-values are reported for each year. See text for transformation.

	Source	df ¹	Fitness
1996	Cytoplasm	1	0.38
	Nuclear	1	3.43†
	Cyto × nuc	1	4.99*
	Block	21	3.94***
1997	Cytoplasm	1	3.19†
	Nuclear	1	22.75***
	Cyto × nuc	1	0.77
	Block	35	12.59***

¹ df_{error} : 1996 = 63, 1997 = 105.

*** *P* < 0.001; * *P* < 0.05; † *P* < 0.1.

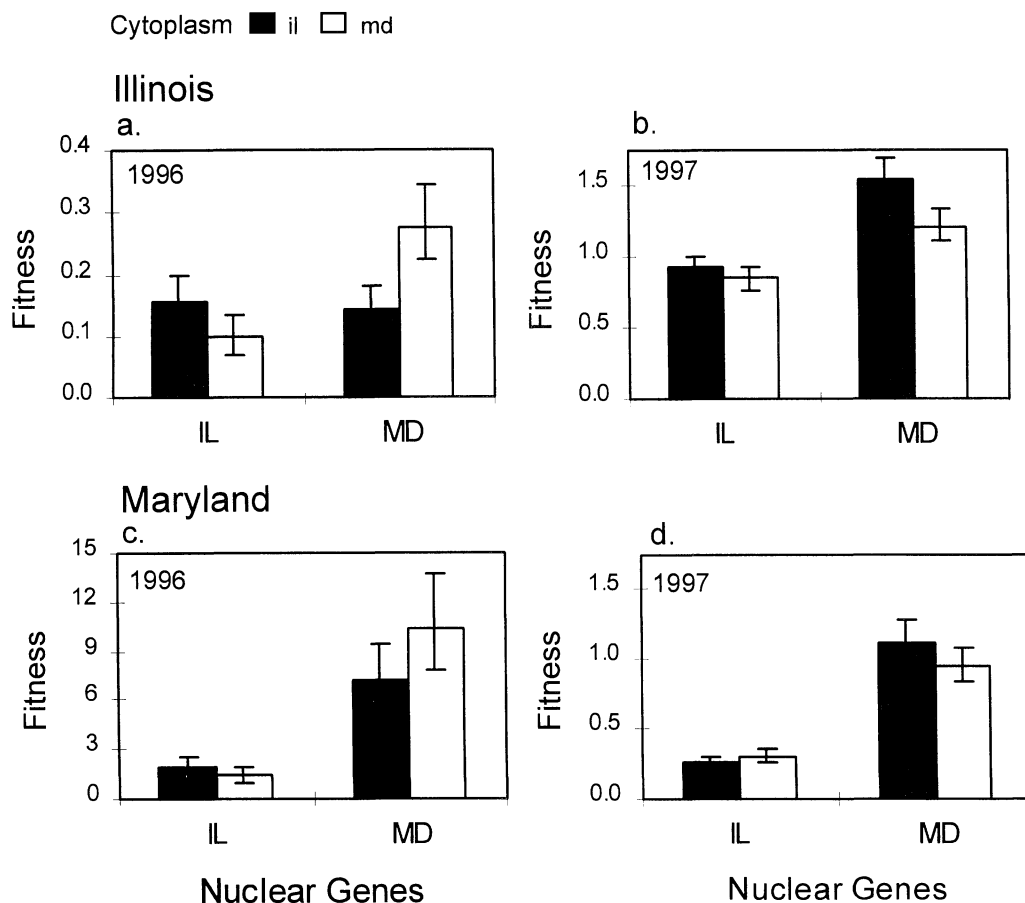


FIG. 3. Mean (\pm SE) cumulative fitness (fruit/seed planted) of plants with either Illinois or Maryland cytoplasmic genes and 87% Illinois or Maryland nuclear genes grown in Maryland and Illinois in two years. Note that scales on y-axes vary. See text for statistical analysis.

greater fitness when paired with Illinois cytoplasmic genes. This pattern is due to equivalent performance of individuals with Illinois cytoplasmic genes with both nuclear types, whereas individuals with Maryland cytoplasmic genes performed poorly when combined with Illinois nuclear genes and well when combined with Maryland nuclear genes. These results reveal coadaptation for fitness between Maryland nuclear and cytoplasmic genes for this year in Illinois, but not

between Illinois nuclear and cytoplasmic genomes. A clearer demonstration of coadaptation between nuclear and cytoplasmic genomes may have to incorporate more generations of backcrosses to increase the uniformity of the nuclear genetic background upon which cytoplasmic genes are tested.

A number of studies have demonstrated interactions between cytoplasmic and nuclear genes by creating individuals with mismatched genomes within a species (Clark and Lyckegaard 1988; MacRae and Anderson 1988; Fos et al. 1990; Nigro 1994; Palmer and Minor 1994) and between species (Grant 1975; Robertson and Frey 1984; Beavis and Frey 1987; Pollak 1991; Jan 1992; Inai et al. 1993; Breeuwer and Werren 1995; Hutter and Rand 1995). In animals there is no evidence for cytonuclear interactions in within population comparisons (Clark and Lyckegaard 1988), perhaps reflecting a lack of variation in the cytoplasmic genome at this scale. However, a number of studies on plants, particularly those investigating cytoplasmic male sterility, have documented cytoplasmic genetic variation (e.g., Belhassen et al. 1993; Saumitou-Laprade et al. 1993; McCauley 1995; Ronfort et al. 1995) and cytonuclear interactions within populations (Belhassen et al. 1991).

Our results, combined with the above cited examples of cytoplasmic effects and nuclear-cytoplasmic interactions,

TABLE 5. Summary of nuclear and cytoplasmic genetic effects on fitness components and cumulative fitness in *Chamaecrista fasciculata* planted in two locations for two years. NS, not significant; S, significant at $P < 0.05$; †, significant at $0.10 > P > 0.05$.

Character	Type of effect		
	Nuclear	Cytoplasmic	Nuclear \times cytoplasmic
Seed weight	S	S	S
% Germination	S ^{1,2}	S ^{1,2}	NS
% Survivorship	S ¹	NS	NS
Vegetative biomass	S ^{1,2}	S ²	† ²
Fruit production	S ^{1,2}	NS	NS
Fitness (fruit/seed planted)	S ^{1,2}	† ^{1,2}	S ¹

¹ Effect due to genes acting through seed mass.
² Effect due to genes acting after seed mass.

suggest that assumptions of neutrality of cytoplasmic markers that are completely linked to the rest of the cytoplasmic genome should be scrutinized. Cytoplasmically inherited markers have been used to infer maternal or paternal (in the case of paternal inheritance of cytoplasm genomes) patterns of dispersal (McCauley 1993, 1994, 1995; Avise 1994), coalescent times (e.g., human populations, Cann et al. 1987; Rogers and Harpending 1992) and hybrid zone dynamics (reviewed in Arnold 1993; Rieseberg 1995), particularly cytoplasmic "capture" via unidirectional patterns of gene flow (reviewed in Avise 1994). In these uses of cytoplasmic markers, nonneutrality, either through main effects or through interaction with nuclear background, compromises inferences of the evolutionary processes underlying patterns of molecular variation. Our results suggest that joint nuclear-cytoplasm genomes will have to be surveyed when using DNA level variation to infer evolutionary processes.

Seed Size Effects on the Genetic Basis of Characters

Nuclear and cytoplasmic genes may affect characters directly or indirectly through seed size. For all characters in this study, the degree to which phenotypic variation was genetically based was greater when genetic effects acted both directly on characters and through seed size. When seed size effects were statistically removed, the genetic factors explained less variation. In particular, for survivorship (Illinois and Maryland) and fitness (Illinois) all genetic variation acted through seed size. Because direct genetic variation for characters was limited, it might appear that response to selection is also limited. However, seed size differences between Illinois and Maryland populations are largely genetically determined (see below); therefore selection, in favoring specific nuclear genes or nuclear-cytoplasmic combinations, effectively acts on seed size variation. Seed size effects may also mask the contribution of cytoplasmic genes and nuclear-cytoplasmic interactions to characters, for example, vegetative biomass in Maryland. In this case, selection acting on the expressed phenotype will not alter biomass because cytoplasmic and nuclear-cytoplasmic genetic contributions are not expressed in the phenotype. Seed characters have frequently been found to both create and mask genetic variation (Schmitt and Antonovics 1986; Stratton 1989; Schmitt et al. 1992; Galloway 1995). Here we know seed weight is genetically determined at the interpopulation level and therefore can predict the evolutionary consequences of seed weight effects on phenotypic variation.

Environmental Variation in the Expression of Nuclear and Cytoplasmic Genetic Effects

Genetic contributions to seed size and germination differed between the two years of the experiment. In 1996, genetic factors explained 62% of the variation in seed weight, whereas in 1997 they explained 92%. These genetic factors include cytoplasmic genes, nuclear genes, and their interaction. The relative contribution of the genomes differs between years. Nuclear effects were greater in 1997, whereas nuclear-cytoplasm interactions were stronger in 1996. The BC2 seeds were created separately each year and therefore differences in the genetic composition between years could reflect either

genetic or maternal environment factors. Because the same pool of BC1 and parental seed was used in creating the BC2 seed each year, it seems unlikely that the seed pools for the two years differ in their genetic constitution. In contrast, the BC1 individuals were larger and more robust the second year that seed were created (pers. obs.). As a consequence, maternal environmental effects are likely to differ between the seeds created in the two years, potentially affecting seed size, germination patterns, and the expression of genetic variation.

The contribution of nuclear and cytoplasmic genes to fitness components varies across sites and years. Significant interactions between genetic effects and year for all characters reveals temporal variation in the degree to which phenotypic variation in a character is genetically determined. Year effects are due to environmental differences between years (which typically differ between sites) and differences in the seeds used each year. Planting site and block represent spatial measures of environmental variation. It is not possible to quantify variation in the expression of genetic effects at a small spatial scale (among blocks) because analyses were conducted on block means. However, expression of genetic effects differed across large spatial scales (planting site, analyses not shown; see Materials and Methods) for all characters. Environmental effects on the proportion of phenotypic variation of quantitative characters that is genetically based has been frequently demonstrated (e.g., Barton and Turelli 1989; Mazer and Schick 1991; Shaw and Platenkamp 1993; Merilä 1997; also cytoplasmic Rao and Fleming 1978). Because the contribution of genetic variation to fitness and fitness-related traits is environment dependent, evidence for local adaptation often varies temporally and spatially (e.g., Rice and Mack 1991; Jordan 1992; Kindell et al. 1996; Nagy and Rice 1997). Indeed, it is difficult to generalize the contribution of each genome and their interaction to adaptation because these contributions to the phenotype depend on the environment. As a consequence experiments in natural habitats will provide important information on cyto-nuclear contributions to population differentiation.

ACKNOWLEDGMENTS

We thank J. Crow for initial discussions; J. A. Hughes for help in conducting the experiments; and M. Dudash, D. McCauley, and two anonymous reviewers for comments on a previous version of the manuscript. The help of staff at USDA Beltsville, Gooselake Prairie, The Illinois Department of Natural Resources, and the University of Maryland greenhouse and a number of undergraduate and graduate students is gratefully appreciated. This work was supported by National Science Foundation grant DEB-9312067 to CBF and National Science Foundation grant DEB-9752947 to LFG.

LITERATURE CITED

- Antonovics, J., and A. D. Bradshaw. 1970. Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity* 25:349-362.
- Arnold, J. 1993. Cytonuclear disequilibria in hybrid zones. *Annu. Rev. Ecol. Syst.* 24:521-554.
- Avise, J. C. 1994. *Molecular markers, natural history, and evolution*. Chapman and Hall, New York.
- Barton, N. H., and M. Turelli. 1989. Evolutionary quantitative

- genetics: how little do we know? *Annu. Rev. Genet.* 23:337–370.
- Basten, C. J., and M. A. Asmussen. 1997. The exact test for cytonuclear disequilibrium selection. *Genetics* 146:1165–1171.
- Beavis, W. D., and K. J. Frey. 1987. Expression of nuclear-cytoplasmic interactions and heterosis in quantitative traits of oats (*Avena* spp.) *Euphytica* 36:877–886.
- Belhassen, E., B. Dommee, A. Atlan, P. H. Gouyon, D. Pomente, M. W. Assouad, and D. Couvet. 1991. Complex determination of male sterility in *Thymus vulgaris* L.: genetic and molecular analysis. *Theor. Appl. Genet.* 82:137–143.
- Belhassen, E., A. Atlan, D. Couvet, P.-H. Gouyon, and F. Quetier. 1993. Mitochondrial genome of *Thymus vulgaris* L. (Labiatae) is highly polymorphic between and among natural populations. *Heredity* 71:462–472.
- Bennington, C. C., and J. B. McGraw. 1995. Natural selection and ecotypic variation in *Impatiens pallida*. *Ecol. Monogr.* 65:303–323.
- Breuer, J. A. J., and J. H. Werren. 1995. Hybrid breakdown between two haplodiploid species: the role of nuclear and cytoplasmic genes. *Evolution* 49:705–717.
- Briggs, D., and S. M. Walters. 1997. Plant variation and evolution. Cambridge Univ. Press, Cambridge, U.K.
- Burke, J. M., T. J. Voss, and M. L. Arnold. 1998. Genetic interactions and natural selection in Louisiana iris hybrids. *Evolution* 52:1304–1310.
- Cann, R. L., M. Stoneking, and A. C. Wilson. 1987. Mitochondrial DNA and human evolution. *Nature* 325:31–36.
- Clark, A. G. 1985. Natural selection with nuclear and cytoplasmic transmission. II. Tests with *Drosophila* from diverse populations. *Genetics* 111:97–112.
- Clark, A. G., and E. M. S. Lyckegaard. 1988. Natural selection with nuclear and cytoplasmic transmission. III. Joint analysis of segregation and mtDNA in *Drosophila melanogaster*. *Genetics* 118:471–481.
- Corriveau, J. L., and A. W. Coleman. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *Am. J. Bot.* 75:1443–1458.
- Couvet, D., A. Atlan, E. Belhassen, C. Gliddon, P. H. Gouyon, and F. Kjellberg. 1990. Co-evolution between two symbionts: the case of cytoplasmic male-sterility in higher plants. Pp. 225–247 in D. Futuyma and J. Antonovics, eds. *Oxford surveys in evolutionary biology*. Vol. 7. Oxford Univ. Press, Oxford, U.K.
- Fos, M., M. A. Dominguez, A. Latorre, and A. Moya. 1990. Mitochondrial DNA evolution in experimental populations of *Drosophila subobscura*. *Proc. Natl. Acad. Sci. USA* 87:4198–4201.
- Galen, C., J. S. Shore, and H. Deyoe. 1991. Ecotypic divergence in alpine *Polemonium viscosum*: genetic structure, quantitative variation, and local adaptation. *Evolution* 45:1218–1228.
- Galloway, L. F. 1995. Response to natural environmental heterogeneity: maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. *Evolution* 49:1095–1107.
- Gillham, N. W. 1978. *Organelle heredity*. Raven Press, New York.
- . 1994. *Organelle genes and genomes*. Oxford Univ. Press, Oxford, U.K.
- Grant, V. 1975. *Genetics of flowering plants*. Columbia Univ. Press, New York.
- Hey, J. 1997. Mitochondrial and nuclear genes present conflicting portraits of human origins. *Mol. Biol. Evol.* 14:166–172.
- Hutter, C. M., and D. M. Rand. 1995. Competition between mitochondrial haplotypes in distinct nuclear genetic environments: *Drosophila pseudoobscura* vs. *D. persimilis*. *Genetics* 140:537–548.
- Inai, S., K. Ishikawa, O. Nunomura, H. Ikehashi. 1993. Genetic analysis of stunted growth by nuclear-cytoplasmic interaction in interspecific hybrids of *Capsicum* by using RAPD markers. *Theor. Appl. Genet.* 87:416–422.
- Jan, C. C. 1992. Cytoplasmic-nuclear gene interaction for plant vigor in *Helianthus* species. *Crop Sci.* 32:320–323.
- Jordan, N. 1992. Path analysis of local adaptation in two ecotypes of the annual plant *Diodia teres* Walt. (Rubiaceae). *Am. Nat.* 140:149–165.
- Kambhampati, S., R. Rai, and D. Verleye. 1992. Frequencies of mitochondrial DNA haplotypes in laboratory cage populations of the mosquito, *Aedes albopictus*. *Genetics* 132:205–209.
- Kilpatrick, S. T., and D. M. Rand. 1995. Conditional hitchhiking of mitochondrial DNA: frequency shifts of *Drosophila melanogaster* variants depend on nuclear genetic background. *Genetics* 141:1113–1124.
- Kindell, C. E., A. A. Winn, and T. E. Miller. 1996. The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. *J. Ecol.* 84:745–754.
- Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. *Evolution* 43:485–503.
- Lacey, E. P. 1996. Parental effects in *Plantago lanceolata*. I. A growth chamber experiment to examine pre- and postzygotic temperature effects. *Evolution* 50:865–878.
- Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Ecol. Syst.* 27:237–277.
- Lloyd, D. G. 1982. Selection of combined versus separate sexes in seed plants. *Am. Nat.* 120:571–585.
- MacRae, A. F., and W. W. Anderson. 1988. Evidence for non-neutrality of mitochondrial DNA haplotypes in *Drosophila pseudoobscura*. *Genetics* 120:485–494.
- Mazer, S. J., and C. T. Schick. 1991. Constancy of population parameters for life-history and floral traits in *Raphanus sativus* L. II. Effects of planting density on phenotype and heritability estimates. *Evolution* 45:1888–1907.
- McCaughey, D. E. 1993. Evolution in metapopulations with frequent local extinction and recolonization. Pp. 109–134 in D. Futuyma and J. Antonovics, eds. *Oxford surveys in evolutionary biology*. Vol. 9. Oxford Univ. Press, Oxford, U.K.
- . 1994. Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: implications for studies of gene flow in plants. *Proc. Natl. Acad. Sci. USA* 91:8127–8131.
- . 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends Ecol. Evol.* 10:198–202.
- . 1998. The genetic structure of a gynodioecious plant: nuclear and cytoplasmic genes. *Evolution* 52:255–260.
- Merilä, J. 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution* 51:526–536.
- Miao, S. L., F. A. Bazzaz, and R. B. Primack. 1991. Effects of maternal nutrient pulse on reproduction of the two colonizing *Plantago* species. *Ecology* 72:586–596.
- Nagy, E. S., and K. J. Rice. 1997. Local adaptation in two subspecies of an annual plant: implications for migration and gene flow. *Evolution* 51:1079–1089.
- Naylor, A. F. 1964. Natural selection through maternal influence. *Heredity* 19:509–511.
- Nigro, L. 1994. Nuclear background affects frequency dynamics of mitochondrial DNA variants in *Drosophila simulans*. *Heredity* 72:582–586.
- Nigro, L., and T. Prout. 1990. Is there selection on RFLP differences in mitochondrial DNA? *Genetics* 125:551–555.
- Palmer, R. G., and V. C. M. Minor. 1994. Nuclear-cytoplasmic interaction in chlorophyll-deficient soybean, *Glycine max* (Fabaceae). *Am. J. Bot.* 81:977–1003.
- Pollak, P. E. 1991. Cytoplasmic effects on components of fitness in tobacco cybrids. *Evolution* 45:785–790.
- Rao, A. P., and A. Fleming. 1978. Cytoplasmic-genotypic effects in the GT 112 maize inbred with four cytoplasm. *Crop Sci.* 18:935–937.
- Rice, K. J., and R. N. Mack. 1991. Ecological genetics of *Bromus tectorum*. III. The demography of reciprocally sown populations. *Oecologia* 88:91–101.
- Rieseberg, L. H. 1995. The role of hybridization in evolution: old wine in new skins. *Am. J. Bot.* 82:944–953.
- Roach, D. A., and R. D. Wulff. 1987. Maternal effects in plants. *Annu. Rev. Ecol. Syst.* 18:209–235.
- Robertson, L. D., and K. J. Frey. 1984. Cytoplasmic effects on

- plant traits in interspecific matings of *Avena*. *Crop Sci.* 24:200–204.
- Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evol.* 9:552–568.
- Ronfort, J., P. Saumitou-Laprade, J. Cuguen, and D. Couvet. 1995. Mitochondrial DNA diversity and male sterility in natural populations of *Daucus carota* ssp. *carota*. *Theor. Appl. Genet.* 91: 150–159.
- Saumitou-Laprade, P., G. J. A. Rouwendal, J. Cuguen, F. A. Krens, and G. Michaelis. 1993. Different CMS sources found in *Beta vulgaris* ssp. *maritima*: mitochondrial variability in wild populations revealed by a rapid screening procedure. *Theor. Appl. Genet.* 85:529–535.
- Saumitou-Laprade, P. J. Cuguen, and P. Vernet. 1994. Cytoplasmic male sterility in plants: molecular evidence and the nucleocytoplasmic conflict. *Trends Ecol. Evol.* 9:431–435.
- Schmitt, J., and J. Antonovics. 1986. Experimental studies of the evolutionary significance of sexual reproduction. III. Maternal and paternal effects during seedling establishment. *Evolution* 40:817–829.
- Schmitt, J., J. Niles, and R. D. Wulff. 1992. Norms of reaction of seed traits to maternal environments in *Plantago lanceolata*. *Am. Nat.* 139:451–466.
- Shaw, R. G., and G. A. J. Platenkamp. 1993. Quantitative genetics of response to competitors in *Nemophila menziesii*: a greenhouse study. *Evolution* 47:801–812.
- Singh, R. S., and L. R. Hale. 1990. Are mitochondrial DNA variants selectively non-neutral? *Genetics* 124:995–997.
- Sork, V. L., K. A. Stowe, and C. Hochwender. 1993. Evidence for local adaptation in closely adjacent subpopulations of northern red oak (*Quercus rubra* L.) expressed as resistance to leaf herbivores. *Am. Nat.* 142:928–936.
- Stanton, M. L., and C. Galen. 1997. Life on the edge: adaptation versus environmentally mediated gene flow in the snow buttercup, *Ranunculus adoneus*. *Am. Nat.* 150:143–178.
- Stratton, D. A. 1989. Competition prolongs expression of maternal effects in seedlings of *Erigeron annuus* (Asteraceae). *Am. J. Bot.* 76:1646–1653.
- Wright, S. 1968. *Evolution and the genetics of populations. Vol. 1, Genetic and biometric foundations.* Univ. of Chicago Press, Chicago.

Corresponding Editor: D. Piñero