



Effects of Differential Pollen-Tube Growth on Hybridization in the Louisiana Irises

Shanna E. Carney; Scott A. Hodges; Michael L. Arnold

Evolution, Vol. 50, No. 5. (Oct., 1996), pp. 1871-1878.

Stable URL:

<http://links.jstor.org/sici?sici=0014-3820%28199610%2950%3A5%3C1871%3AEODPGO%3E2.0.CO%3B2-Z>

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.

JSTOR is an independent not-for-profit organization dedicated to and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact support@jstor.org.

EFFECTS OF DIFFERENTIAL POLLEN-TUBE GROWTH ON HYBRIDIZATION IN THE LOUISIANA IRISES

SHANNA E. CARNEY,¹ SCOTT A. HODGES,² AND MICHAEL L. ARNOLD³
Department of Genetics, University of Georgia, Athens, Georgia 30602

Abstract.—To elucidate the importance of hybridization in evolution, it is necessary to understand the processes that affect hybridization frequency in nature. Here we focus on postpollination, prefertilization isolating mechanisms using two hybridizing species of Louisiana iris as a study system. We compared the effects of differential pollen-tube growth on the frequency of F_1 hybrid formation in experimental crosses between *Iris fulva* and *Iris hexagona*. Analyses of seed production in fruits from pure conspecific and heterospecific pollinations revealed that more seeds were produced in the top half than the bottom half of fruits for all four crosses. Heterospecific pollen was applied to flowers of each species at zero to 24 h prior to conspecific pollen, thereby giving a head start to the foreign pollen. Using diagnostic isozyme markers, the frequency of hybrid progeny was examined at the level of the whole fruit and separately for the top and bottom halves of fruits. In both species, the proportion of hybrid seeds per fruit increased significantly with increasing head starts, suggesting that differences in pollen-tube growth rates affect the frequency of hybridization. In *I. fulva* fruits, the increase in hybrid seeds occurred in both halves of the fruits, but in *I. hexagona* an increase was only detected in the top half of fruits. These findings are consistent with a model that assumes attrition of pollen tubes due to the greater length of *I. hexagona* styles. While pollen-tube growth rate appears to be the most important factor affecting hybridization frequency in *I. fulva*, both pollen-tube growth rate and pollen-tube attrition appear to be important in *I. hexagona*.

Key words.—Hybridization, *Iris*, pollen competition, pollen-tube attrition, reproductive isolation.

Received August 10, 1995. Accepted March 19, 1996.

Hybridization is an important mechanism in the evolution of both plants and animals (Anderson 1949; Stebbins 1959; Lewontin and Birch 1966; Grant 1981; Arnold 1992; Grant and Grant 1992; Dowling and DeMarais 1993; Rieseberg and Wendel 1993; Masterson 1994) and can lead to a wide range of outcomes. These include the merging of hybridizing taxa (Grant 1963), hybrid speciation (Randolph 1966; Arnold et al. 1990; Rieseberg et al. 1990; DeMarais et al. 1992), reinforcement of reproductive isolation (Dobzhansky 1970; Howard 1986), genetic assimilation of one of the hybridizing taxa (Heusmann 1974; Hinton 1975; Rieseberg 1991), maintenance of a stable hybrid zone (Barton and Hewitt 1985), and the invasion and utilization of novel habitats by individuals with introgressed genotypes (Lewontin and Birch 1966; Harrison 1990, 1993; Cruzan and Arnold 1993).

Because hybridization is widespread in both plants (Stebbins 1959; Grant 1981; Ehrlich and Wilson 1991; Whitham et al. 1991; Masterson 1994) and some groups of animals (e.g., Howard 1986; Szymura and Barton 1986; Rand and Harrison 1989; DeMarais et al. 1992; Grant and Grant 1992, 1994), it is important to understand the processes that affect hybridization frequency in nature. Many aspects of reproduction must coincide for hybridization to occur. In most animals, potential heterospecific (or heterosubspecific) mates must occur in the same habitat, have coincidental mating times, recognize each other's mating behavior or other attraction characteristics, have compatible genitalia, and have gametes that are attracted to each other or are viable in in-

dividuals of the other taxon (Dobzhansky 1970). If fertilization occurs, the production of hybrid offspring may still be prevented by postzygotic mechanisms (Dobzhansky 1970). In plants, prepollination factors such as overlap in flowering time and heterospecific visits by pollinators are necessary for hybridization to occur (Levin 1978). Once heterospecific pollen has been transferred, it must be able to germinate, grow the length of the style, and fertilize ovules. Furthermore, postfertilization processes such as ovule abortion may affect the extent of hybrid seed formation (Levin 1978).

We have focused our efforts on postpollination, prefertilization isolating mechanisms in plants, using the Louisiana irises as a study system. In this species complex (series *Hexagonae* of *Iris*), initial F_1 hybrid formation is rare, despite the presence of many hybrid populations (Arnold 1993a,b; 1994). Few F_1 seeds have been found in a population of *Iris fulva* into which *Iris hexagona* plants were experimentally introduced (Arnold et al. 1993; M. L. Arnold and J. L. Hamrick, unpubl. data). Furthermore, adult plants with F_1 hybrid genotypes have never been found, though hundreds of individuals from several natural hybrid populations have been examined (Nason et al. 1992; Arnold 1993a,b). This rarity of F_1 hybrids suggests that barriers to initial heterospecific reproduction exist between these *Iris* species.

Heterospecific pollen transfer between the flowers of *I. fulva* and *I. hexagona* is probably frequent because the flowering times of the two species overlap broadly (Arnold et al. 1993; Cruzan et al. 1994) and the same pollinators (bees and hummingbirds) visit both species, though pollinator preference may still limit the frequency of heterospecific pollen transfer (S. A. Hodges, S. E. Carney, and M. B. Cruzan, unpubl. data). In addition to prepollination factors, pre- or postfertilization processes may also act to reproductively isolate the two species. Indeed, in two previous studies, both pre- and postfertilization processes (differential pollen-tube

¹ Present address: Department of Biology, Indiana University, Bloomington, Indiana 47402. E-mail: carney@dogwood.botany.uga.edu.

² Present address: Department of Biology, University of California, Santa Barbara, California 93106. E-mail: hodges@lifesci.lscf.ucsb.edu.

³E-mail: arnold@dogwood.botany.uga.edu.

growth and ovule abortion) were implicated in limiting hybrid seed formation (Arnold et al. 1993; Carney et al. 1994). While patterns of seed siring suggested that relative pollen-tube growth rates affect hybridization frequency, pollen-tube growth rates estimated from measurements of pollen-tube length at a single time point did not predict the observed patterns of hybrid seed formation (Carney et al. 1994). Therefore, more accurate methods of inferring the effect of differential pollen-tube growth on fertilization success are necessary.

Three questions are addressed in this study: (1) What is the spatial pattern of seed set following pure conspecific and heterospecific pollinations? (2) When pollen from two species competes, how do pollen-tube growth rates affect hybridization frequency? and (3) What role does pollen-tube attrition (or prefertilization growth failure; Cruzan 1989) play in determining the frequency of hybridization? To address these questions, we document spatial patterns of seed set from pure conspecific and heterospecific pollinations in each species. Next, we examine flowers of both species pollinated with heterospecific pollen followed by conspecific pollen after time intervals of several lengths, thus giving heterospecific pollen various "head starts." Relative growth rates of conspecific and heterospecific pollen tubes can then be inferred from the proportions of conspecific and hybrid progeny produced. To test for attrition of pollen tubes, patterns of hybrid seed formation were examined on the level of whole fruits and independently in the top and bottom halves of fruits. These position data provide information about the distance to which pollen tubes can grow in different stylar environments.

When heterospecific pollen is given a head start relative to conspecific pollen, three patterns of resulting progeny are possible. First, there may be an increase in the number of hybrid seeds produced with longer intervals between pollinations. This would suggest that the growth rates of pollen tubes are a limiting factor in determining the frequency of hybridization. Providing heterospecific pollen with a head start would compensate for slower tube growth, increasing its chance of fertilization. Second, the proportion of hybrid seeds may be constant across all interval treatments, indicating heterospecific pollen-tube attrition, an inability to fertilize ovules, or abortion of hybrid seeds. Despite a temporal advantage, only a limited number of tubes would reach the ovary and/or be able to fertilize ovules. Finally, there may be a decrease in the number of hybrids as heterospecific pollen receives greater head starts. This would suggest that the presence of conspecific pollen has a beneficial effect on the growth and/or fertilization ability of heterospecific pollen tubes (i.e., the mentor effect; Visser and Verhaegh 1980). When conspecific pollen is not present (or when it arrives much later), heterospecific pollen-tube growth would be inhibited or prevented.

MATERIALS AND METHODS

Pollinations

Flowering plants used in this study were located 7.3 km west of Labadieville in Assumption Parish, Louisiana. This is the same population of *I. fulva* and *I. hexagona* used for

previous conspecific and heterospecific pollen-tube growth experiments (Carney et al. 1994). Flowers of both species were tagged upon opening from 25 March to 9 April 1994. Anthers and sepals were removed to prevent self-pollination and to inhibit legitimate visitation by pollinators. Bumble bees and hummingbirds do not come in contact with the stigma during visitation if sepals are removed (Mertzweiller 1988).

Pollinations were performed when the stigmas had become receptive to pollination (the day after opening for *I. hexagona* flowers and two days after opening for *I. fulva*; Carney et al. 1994). Pollen from newly dehisced anthers of several individuals was mixed for each species. By pollinating with mixtures, we prevented biasing our results due to (1) variation in seed siring ability among different conspecific individuals; and (2) interactions between specific pollen and style genotypes. In addition, since pollen carry-over is common in many species and is often high (Schaal 1980; Thomson and Plowright 1980; Levin 1981; Price and Waser 1982; Waser and Price 1984), it is likely that mixed pollen loads are deposited in natural pollinations. Therefore, pollen mixtures may mimic the composition of natural pollen loads.

Pure conspecific and heterospecific pollinations were performed on flowers of each species. In addition, separate flowers were used for pollination interval (or head start) treatments. Heterospecific pollen was applied with a piece of fishing line to one stigmatic lobe on each of the three stigmas in each flower. The second lobe of each stigma was pollinated with conspecific pollen after an interval of 0, 1, 3, 6, or 24 h. Pollen tubes from the two lobes on a stigma grow down opposite walls of the stylar canal for the majority of the way to the ovary (at least to the point at which the stylar arms fuse; S. E. Carney, pers. obs.). This made it possible to use separate lobes for pollen from different sources to prevent clogging of the stigma and style by pollen grains and tubes from the first application (heterospecific).

Pollen loads contained as many as 6000+ pollen grains per flower, far more grains than necessary to fertilize all ovules (a mean of 78 ovules in *I. fulva*, 114 in *I. hexagona*; S. E. Carney, unpubl. data). Thus, the possibility of pollen limitation was minimized.

Position-Specific Seed Formation

Fruits were collected at maturity. The locations of seeds were recorded (counting from the stylar to the basal end) for 10 fruits from the pure conspecific and heterospecific pollinations. These data provide information about the distance to which pollen tubes can grow in different stylar environments. Identification of ovule position is possible in *Iris* because unfertilized and aborted ovules are still visible in the ovary. *Iris* fruits have three locules, each with two rows of seeds, so there are six ovules in each numbered position.

Each fruit was divided into top (stylar) and bottom (penduncular) halves by dividing in half the number of ovules in the longest row. To test for an association between pollen source (conspecific or heterospecific) and the proportion of mature seeds in the top and bottom halves of fruits, the Cochran-Mantel-Haenszel statistic of general association (CMH; FREQ procedure of SAS, SAS Institute 1988) was used. The

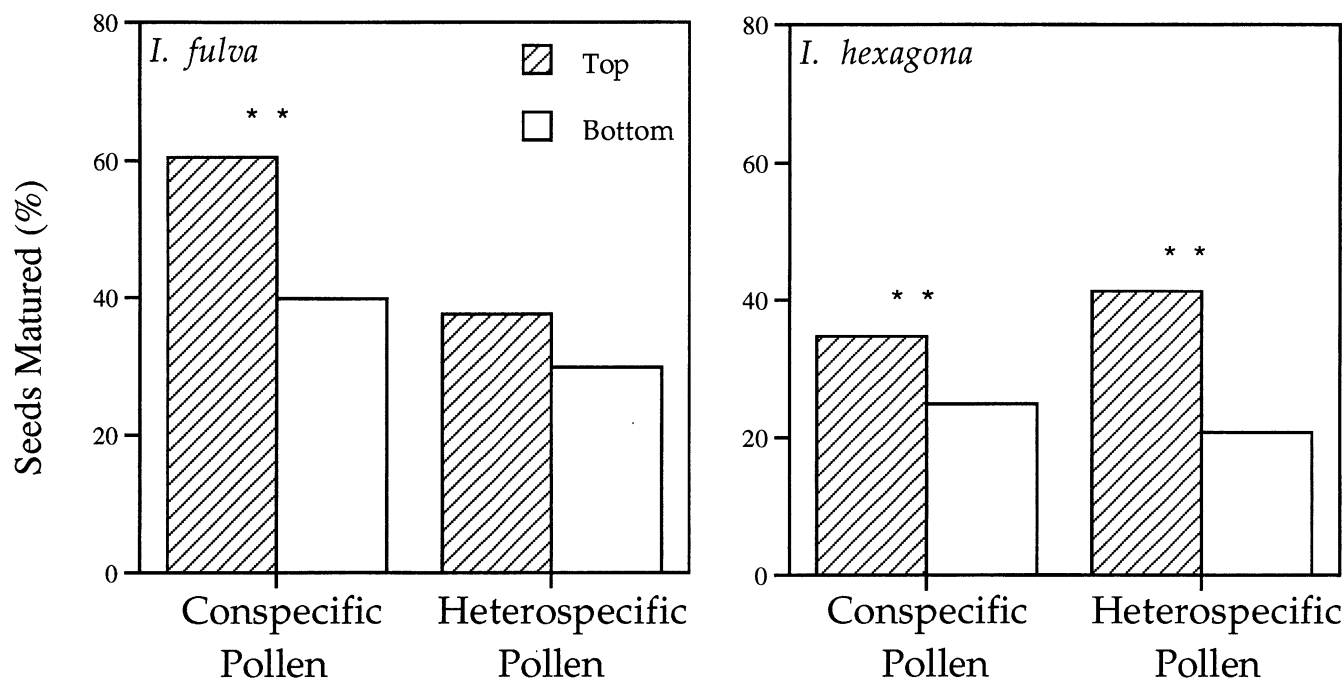


FIG. 1. The mean percentage of ovules that matured into seeds in the top and bottom halves of *Iris fulva* and *Iris hexagona* fruits following pure conspecific and heterospecific pollinations. Asterisks indicate significant differences in the percentage of mature seeds between top and bottom halves of the fruit after protecting for multiple tests: ** $P < 0.01$ (CMH test, FREQ procedure of SAS; sequential Bonferroni, Holm 1979; Rice 1989).

CMH statistic is equivalent to a χ^2 or G -test, but it corrects for the fact that data were pooled across plants. A posteriori comparisons of the CMH statistics were performed using the sequential Bonferroni test (Holm 1979; Rice 1989), which protects significance levels for multiple tests. We also used PROC CATMOD (SAS Institute 1988) to test the effect of pollen type, position in fruit, and their interaction as sources of variance in the number of mature seeds.

Pollen-Tube Growth Rate Effects

Five seeds from each of 20 fruits were selected randomly for each of the five pollination interval treatments. Protein electrophoresis of *Pgi-3* (phosphoglucosomerase; conditions described in Carney et al. 1994) unequivocally identifies hybrid seeds due to fixed allelic differences between *I. fulva* and *I. hexagona* (Arnold et al. 1990).

The data were analyzed using ANOVA to determine whether the proportion of hybrid seeds per fruit varied among treatments (pollination interval), flowering order within inflorescence, and date of flowering. All high order interaction terms that were nonsignificant ($P > 0.4$ in all cases) were removed from the model. A posteriori comparisons were made using the Tukey's Studentized range test. Analyses were performed using PROC GLM of SAS (SAS Institute 1988).

Positional Analyses

To further investigate the effect of differential pollen-tube growth on hybrid seed formation, another five fruits were selected for all pollination interval treatments. This was possible in all treatments except the *I. hexagona* 24-h treatment

in which only one fruit remained and the *I. fulva* 6-h pollination interval, in which three fruits remained. The *I. hexagona* 24-h pollination interval treatment was removed from all analyses due to a lack of replicate fruits.

For each fruit, the position of each seed within the ovary was marked. Of these seeds, approximately 20 per fruit were selected for analysis. In the majority of fruits, seeds were selected from both the top and bottom halves, and they were chosen to sample approximately equal numbers of seeds at each position in the combined sample. Fruits were divided into top and bottom halves as described above, and the same statistical analyses and a posteriori tests were performed. In addition, comparisons of the frequency of hybrids in the top and bottom halves of fruits for each species and pollination interval combination were protected for multiple comparisons using the sequential Bonferroni a posteriori test (Holm 1979; Rice 1989).

RESULTS

Position-Specific Seed Formation

In *I. fulva*, conspecific pollen sired significantly more seeds than heterospecific pollen, but there was no difference in *I. hexagona* (Fig. 1, Table 1). In both species, significantly more seeds were found in the top half of fruits than the bottom (Fig. 1, Table 1). Seed set in the two halves of fruits differed significantly between fruits from conspecific and heterospecific pollinations in both species (pollen type \times position interaction; Table 1). When fruits resulting from different species \times pollination-type combinations were examined separately and the sequential Bonferroni test was performed on

TABLE 1. Maximum-likelihood ANOVA of seed frequency in *I. fulva* and *I. hexagona* fruits produced from pure conspecific and heterospecific pollinations (CATMOD procedure of SAS; SAS Institute 1988).

Source	df	Chi-square	P
<i>I. fulva</i>			
Intercept	1	30.90	< 0.0001
Pollen type	1	31.66	< 0.0001
Position	1	23.71	< 0.0001
Pollen type × position	1	4.06	0.0438
<i>I. hexagona</i>			
Intercept	1	332.91	0.0001
Pollen type	1	0.04	0.8455
Position	1	60.60	0.0001
Pollen type × position	1	7.54	0.0060

the resulting CMH statistics, there were significantly more seeds in the top halves of fruits than the bottom for all but *I. fulva* flowers pollinated with heterospecific pollen (Fig. 1).

Pollen-Tube Growth Rate Effects

Hybrid seed formation increased with pollination interval in *I. fulva* and *I. hexagona*, but the pattern of increase differed significantly between the two species (Table 2; Fig. 2a,b). In *I. fulva*, the 0-, 1-, 3-, and 6-h pollination interval treatments did not differ significantly in the proportion of hybrid seeds formed, but each of these had significantly fewer hybrids than the 24-h delay treatment. Many more hybrids were formed in *I. hexagona* fruits. The proportion of hybrids formed with simultaneous pollen applications (0-h interval treatment) was significantly less than all other treatments, which were not significantly different from each other.

Positional Analyses

The pattern of hybrid seed formation within fruits across treatments was similar in the two species. With only a small pollination interval, the frequency of hybrid seeds in the bottom and top half of fruits was similar. After longer pollination intervals, the majority of hybrids was found in the top half of fruits (Fig. 2c,d). For *I. hexagona*, after performing the sequential Bonferroni test on the CMH statistics, significant differences in the frequency of hybrid seeds in top and bottom halves of fruits were found for the 3- and 6-h interval treatments ($P < 0.01$ for both treatments; Fig. 2d). The frequency of hybrids in the other pollination interval treatments did not differ significantly between ovule positions at the 0.05 significance level (Fig. 2c,d).

In *I. hexagona*, the proportion of hybrids in the two halves of fruits differed significantly across treatments (pollination interval × position interaction; Table 3). Because this interaction term was significant, we also examined the proportion of hybrids for the top and bottom halves of fruits separately. In *I. fulva*, the proportion of hybrids differed significantly among treatments in both the top and bottom halves of the fruits ($P < 0.0001$ in both cases), increasing with longer pollination intervals. In *I. hexagona*, there was a significant difference in the fraction of hybrids in the top half of fruits across treatments ($P < 0.0001$), with hybridization increasing

TABLE 2. ANOVA of the proportion of hybrid seeds in *I. fulva* and *I. hexagona* fruits after different pollination intervals. Fruit refers to the order of pollination on each plant (first, second, third, fourth, or fifth flower) and date is the date the flower opened.

Source	df	Type III SS	F	P > F
Species	1	161.1165	87.58	0.0001
Pollination interval	4	59.0342	8.02	0.0001
Species × pollination interval	4	26.0148	3.54	0.0085
Fruit	4	4.7669	0.65	0.6292
Date	17	46.5936	1.49	0.1034
Error	169	310.9107		

with increases in pollination interval, but there was no difference across pollination interval treatments in the bottom half ($P > 0.05$) (Fig. 2d).

DISCUSSION

This study shows that relative pollen-tube growth rates can be a major factor in determining the frequency of hybrids produced between two species. In *I. fulva* and *I. hexagona*, seeds are more likely to mature in the top half of fruits than in the bottom half. This position-specific seed formation suggests that either pollen competition for ovules is intense in the top half but declines in the bottom half, perhaps due to pollen-tube attrition, or that ovule abortion is greater in the bottom half of fruits. Our data from sequential pollinations suggest that pollen-tube growth rate and attrition are major factors contributing to the pattern of seed maturation. Though our data cannot directly address whether ovule abortion also contributes to the pattern of seed maturation, there is the potential for it to be an important factor. This possibility is suggested because the number of seeds produced is always much lower than the total number of ovules even when a large excess of pollen is applied to the stigmas (S. E. Carney, pers. obs.).

The increase in hybrid seed formation with longer pollination intervals suggests that pollen-tube growth rate is an important mechanism in determining the frequency of hybridization between *I. fulva* and *I. hexagona*. When heterospecific pollen and conspecific pollen were applied simultaneously and in equal proportions to stigmas, the frequency of hybrid seed formation was lower than the expected 50% in both species (5% in *I. fulva*, 31% in *I. hexagona*; Fig. 2a,b; Arnold et al. 1993; Carney et al. 1994). However, as the length of the head start increased, the proportion of hybrid seeds sired also increased (Fig. 2a,b). These results clearly indicate that pollen-tube growth rate affects fertilization success of heterospecific pollen when it is in competition with conspecific pollen in these species.

Heterospecific pollen competition may be a common isolating mechanism between hybridizing taxa. For instance, in section *Isopappus* of the genus *Haplopappus*, two species were judged to be "competitively equal" when an interval between heterospecific and conspecific pollination resulted in the production of 50% hybrid seeds (Smith 1968, 1970). Heterospecific pollen tubes grew more slowly than conspecific pollen tubes in all of the pairs of crosses, with one species pair failing to produce 50% hybrids after 100 min, the maximum pollination interval used in the study.

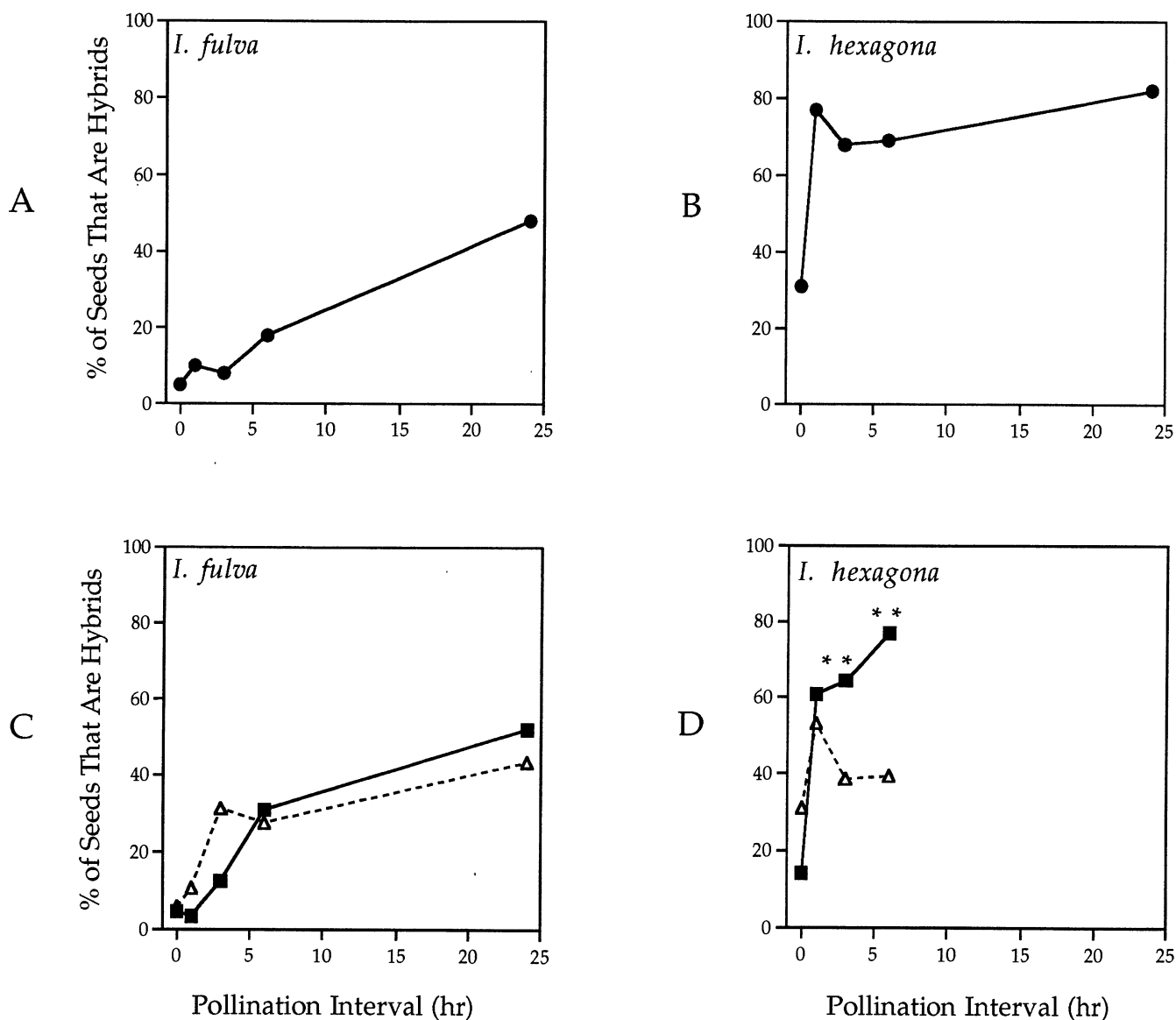


FIG. 2. The mean percentage of hybrid seeds in whole fruits (a and b) and in the top and bottom half of fruits (c and d) of *Iris fulva* and *Iris hexagona* after five pollination intervals. In c and d, solid lines = top half of fruits, dashed lines = bottom half. Asterisks indicate significant differences in the percentage of hybrid seeds between top and bottom halves of the fruit after protecting for multiple tests: ** $P < 0.001$ (CMH test, FREQ procedure of SAS; sequential Bonferroni, Holm 1979; Rice 1989). Statistical comparisons between top and bottom halves of fruits were not made for the 0- and 1-h intervals for *I. fulva* because the number of hybrids recorded was too small.

In contrast with the results for *Iris* described here and those of Smith (1968, 1970), Rieseberg et al. (1995) did not find an increase (from 0%) in hybrid seed formation when *Helianthus annuus* pollen was given a 15 or 30 min temporal advantage over *H. petiolaris* pollen on *H. petiolaris* flowers. However, pollinations with only heterospecific pollen led to achene set that was not significantly different from that following conspecific pollinations (Rieseberg et al. 1995), and therefore, longer pollination intervals than those used in their experiment may be needed to provide *H. annuus* pollen tubes with a growth advantage over *H. petiolaris* tubes.

This phenomenon may be common among closely related species, but additional studies are needed before any con-

clusions about its frequency can be made. However, of the three studies that used pollination intervals to assess pollen-tube growth as a barrier to hybridization, interspecific pollen competition has been shown to be important among two groups and is equivocal in the third (Smith 1968, 1970; Rieseberg et al. 1995; this study). In fact, Darwin suggested such an isolating mechanism:

The simplest and best known case of prepotent action in pollen . . . is that of a plant's own pollen over that from a distinct species. If pollen from a distinct species be placed on the stigma of a castrated flower, and then after the interval of several hours, pollen from the same

TABLE 3. Maximum-likelihood ANOVA of the frequency of hybrid seeds in *I. fulva* and *I. hexagona* fruits (CATMOD procedure of SAS; SAS Institute 1988). The 24 h pollination interval treatment of *I. hexagona* was removed from the analysis due to a lack of replicate fruits.

Source	df	Chi-square	P
<i>I. fulva</i>			
Intercept	1	123.33	< 0.0001
Pollination interval	4	64.91	< 0.0001
Position	1	2.17	0.1406
Pollination interval \times position	4	8.08	0.0885
<i>I. hexagona</i>			
Intercept	1	2.13	0.1442
Pollination interval	3	41.04	< 0.0001
Position	1	6.87	0.0088
Pollination interval \times position	3	22.52	0.0001

species be placed on the stigma, the effects of the former are wholly obliterated, excepting in some rare cases (Darwin 1876).

Two mechanisms could account for heterospecific pollen competition. In *Iris*, it appears that differential pollen tube growth rate is the major factor, but that pollen-tube attrition contributes to the pattern when *I. hexagona* is the maternal plant. Hybrid seed formation increased with pollination interval in both halves of *I. fulva* fruits (Table 3, Fig. 2c). Pollen-tube growth rate appears to be the most important factor determining hybridization frequency in this species. Similarly, in the top half of *I. hexagona* fruits, the fraction of seeds that are hybrids increased significantly with increasing pollination intervals (Table 3, Fig. 2d). This trend suggests that fertilization success in the top half of the fruit is largely determined by pollen-tube growth rate. However, the bottom half of fruits did not exhibit an increasing pattern of hybrid seed formation with increased pollination interval, suggesting that pollen-tube growth rate alone is not responsible for determining the frequency of hybrid seed formation there. It is likely that pollen-tube attrition, ovule abortion, or some combination of the two affect the pattern of hybrid seed formation in the bottom half of *I. hexagona* fruits.

Model Of Pollen-Tube Growth

The model we propose to explain the pattern of hybrid seed formation in *I. hexagona* and *I. fulva* incorporates a combination of differential pollen-tube growth rate and attrition. Pollen-pistil interactions cause conspecific pollen tubes to grow faster than heterospecific tubes in both species. Heterospecific tubes are able to sire more seeds when given head starts relative to conspecific pollen. *Iris hexagona* flowers have longer pistils than *I. fulva* flowers (distance from stigma to base of ovary is approximately 9.5 cm for *I. hexagona* and 6.5 cm for *I. fulva*), so pollen attrition reduces competition in the bottom half of *I. hexagona* ovaries. This is because *I. fulva* pollen is required to grow longer distances when growing on *I. hexagona* flowers than it is accustomed to growing (and presumably adapted to growing) on *I. fulva* flowers. This results in more hybrid seeds in the top half of the ovary than the bottom half. Attrition of *I. hexagona* pollen

tubes does not occur in *I. fulva* pistils because of their shorter length. Without the head start afforded to heterospecific pollen in this study, pollen competition limits the formation of hybrid seeds.

The proposed model is diagrammed in Figure 3. In *I. hexagona* flowers, following simultaneous pollinations conspecific pollen tubes reach the ovary and begin fertilization prior to heterospecific pollen tubes. Pollen-tube attrition occurs toward the base of the ovary, and fertilization is no longer competitive in this region (Fig. 3a). When heterospecific pollen is given a 24-h head start, its pollen tubes reach the ovary and fertilize ovules prior to conspecific pollen (Fig. 3b). Because pollen-tube attrition affects seed siring in the basal end of *I. hexagona* ovaries, a similar proportion of hybrid seeds is seen in the bottom of these fruits regardless of the pollination interval (Fig. 3a,b). In *I. fulva*, conspecific pollen tubes reach the ovary first and sire the majority of seeds following simultaneous pollinations (Fig. 3c). In contrast, after a 24-h head start, heterospecific pollen tubes reach the ovary and begin fertilization, followed by conspecific tubes (Fig. 3d). Attrition of pollen tubes does not occur in *I. fulva*, so the frequency of hybrids in both the bottom and top half of fruits increases from the simultaneous pollinations to the 24-h pollination interval (Fig. 3c,d).

A similar model of pollen-tube growth was proposed to explain differences in the location of hybrid seeds in the ovaries of domesticated and free-living varieties of *Cucurbita pepo* ssp. *ovifera* following mixed pollinations (Wilson and Payne 1994). In the domesticated zucchini, which has pistils approximately four times as long as the free-living *texana* variety, more hybrids were found in the stylar end of fruits than in the peduncular end. In the smaller, free-living variety, the reverse was seen. The authors suggest that the spatial pattern of hybrids results from a combination of relative pollen-tube growth rates and adaptation of pollen tubes to growth in styles of specific lengths (Wilson and Payne 1994).

The results of this study suggest that differential pollen-tube growth acts as a strong prezygotic barrier to heterospecific reproduction when mixed pollen loads are deposited on the stigmas of *Iris* flowers. The magnitude of the reproductive barrier produced by differential pollen-tube growth can be determined by examining the proportion of hybrid seeds resulting from pure heterospecific versus simultaneous (0-h treatment) pollinations. The frequency of hybrid seeds resulting from competitive pollinations was approximately 70% less than from pure pollinations in *I. hexagona* and 95% less in *I. fulva*.

These findings also suggest a directionality to hybridization, favoring *I. hexagona* as the seed parent. While *I. hexagona* exhibited a significant increase in the frequency of hybrid seeds with a pollination interval of only 1 h, *I. fulva* required a 24-h pollination interval before the frequency of hybrid seeds increased significantly from the simultaneous pollination treatment (Fig. 2a,b). This trend is also suggested by patterns of pollen-tube growth and hybrid seed formation resulting from previous experimental and natural pollinations (Arnold et al. 1993; Carney et al. 1994; M. L. Arnold and J. L. Hamrick, unpubl. data). Evidence of the restrictive nature of *I. fulva* in forming hybrids is also found in its reproductive

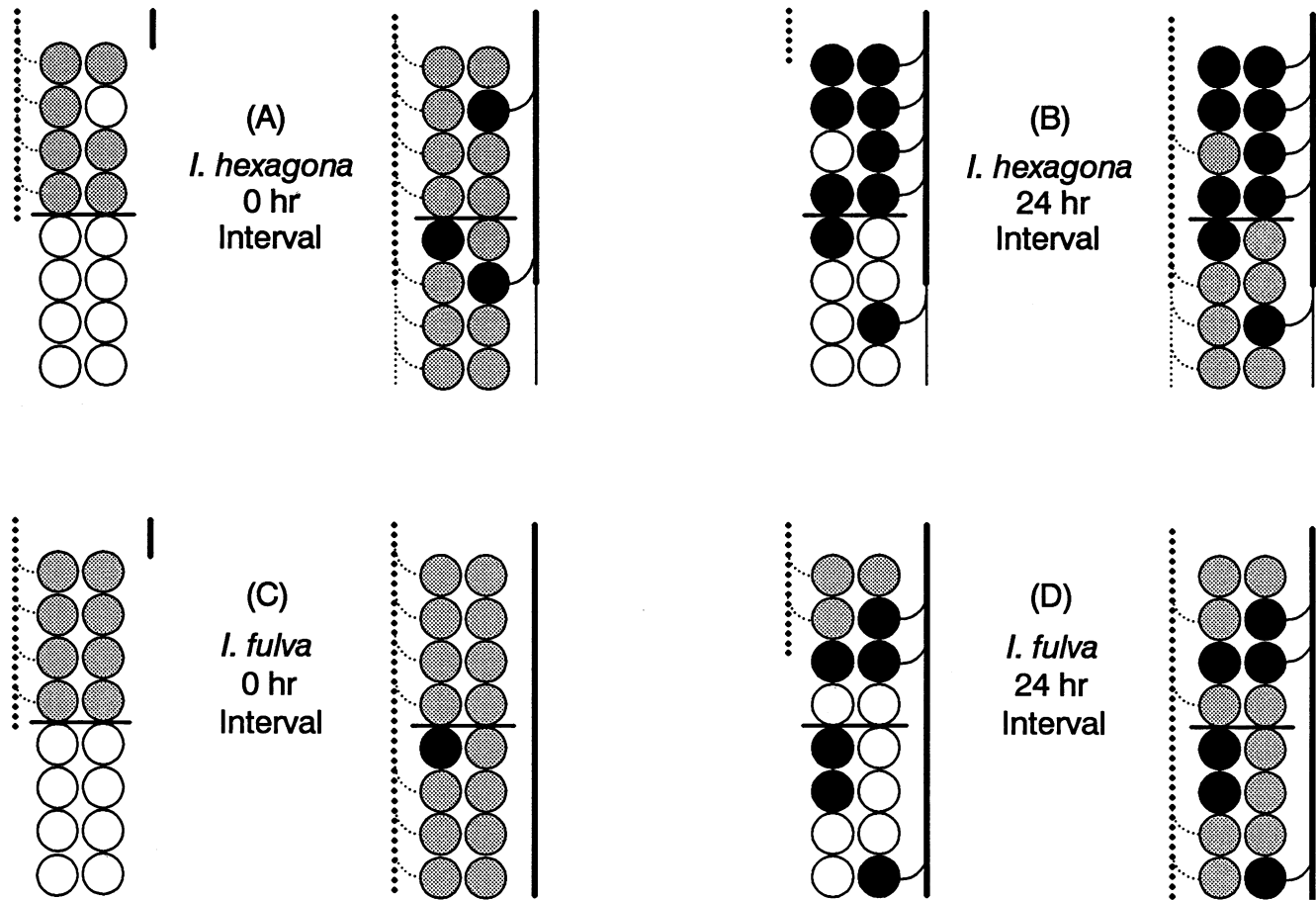


FIG. 3. A model of pollen tube growth and fertilization in *Iris fulva* and *Iris hexagona* flowers. Circles represent ovules: open = unfertilized; stippled = conspecific; black = hybrid. Vertical lines represent pollen tubes: dashed = conspecific; solid = heterospecific. Line width is indicative of the number of pollen tubes reaching a given section of the ovary (i.e., a narrow line signifies that attrition has occurred). A horizontal line through each ovary divides it into top and bottom halves. For each panel, the diagram on the left is an intermediate point between pollination and completion of fertilization, and the diagram on the right is after fertilization has been completed.

interactions with a third species in the Louisiana iris species complex, *I. brevicaulis* (Cruzan and Arnold 1994).

It is apparent from the results presented here that differential pollen-tube growth affects the rate of hybridization in the Louisiana irises in nature. Because of broadly overlapping flowering phenologies (Arnold et al. 1993; Cruzan et al. 1994), and the fact that heterospecific visits by bees and hummingbirds are seen in nature (S. A. Hodges, S. E. Carney, and M. B. Cruzan, unpubl. data), it is likely that pollen is deposited on stigmas by pollinators that have visited flowers of both species in succession. In addition, it is likely that a large amount of pollen is deposited with each pollinator visit. This suggests that conspecific and heterospecific pollen grains probably compete for the ability to fertilize ovules in natural mixed populations of *Iris*. Thus, the effect of differential pollen-tube growth on hybridization frequency that we have demonstrated with controlled crosses has the opportunity to act as a heterospecific isolating mechanism in nature.

ACKNOWLEDGMENTS

We thank J. Burke, S. Emms, D. Geiser, M. Hare, C. Schlichting, D. Shoemaker, and J. Williams for comments on

the manuscript; S. Emms for statistical advice; G. Hill, Y. Lay, M. J. Godt, and J. Hamrick for technical assistance; and the family of L. Talbot for the use of their property. This research was supported by National Science Foundation Grant DEB 9317654 to MLA, National Science Foundation/United States Department of Agriculture/Department of Energy Grant BIR 9220329, an American Iris Society Foundation grant to MLA, and an American Iris Society scholarship to SEC. SEC was supported by National Institutes of Health Genetics Training Grant GM07103.

LITERATURE CITED

- ANDERSON, E. 1949. *Introgressive hybridization*. Wiley, New York.
- ARNOLD, M. L. 1992. Natural hybridization as an evolutionary process. *Ann. Rev. Ecol. Syst.* 23:237-261.
- . 1993a. *Iris nelsonii* (Iridaceae): Origin and genetic composition of a homoploid hybrid species. *Am. J. Bot.* 80:577-583.
- . 1993b. Rarity of hybrid formation and introgression in Louisiana irises. *Plant Genet. Newsl.* 9:14-17.
- . 1994. Natural hybridization and Louisiana irises. *Bioscience* 44:141-147.
- ARNOLD, M. L., J. L. HAMRICK, AND B. D. BENNETT. 1990. Allo-

- zyme variation in Louisiana irises: A test for introgression and hybrid speciation. *Heredity* 65:297–306.
- . 1993. Interspecific pollen competition and reproductive isolation in *Iris*. *J Hered* 84:13–16.
- BARTON, N. H., AND G. M. HEWITT. 1985. Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.* 16:113–48.
- CARNEY, S. E., M. B. CRUZAN, AND M. L. ARNOLD. 1994. Reproductive interactions between hybridizing irises: Analyses of pollen-tube growth and fertilization success. *Am. J. Bot.* 81:1169–1175.
- CRUZAN, M. B. 1989. Pollen tube attrition in *Erythronium grandiflorum*. *Am. J. Bot.* 76:562–570.
- CRUZAN, M. B., AND M. L. ARNOLD. 1993. Ecological and genetic associations in an *Iris* hybrid zone. *Evolution* 47:1432–1445.
- . 1994. Assortative mating and natural selection in an *Iris* hybrid zone. *Evolution* 48:1946–1958.
- CRUZAN, M. B., J. L. HAMRICK, M. L. ARNOLD, AND B. D. BENNETT. 1994. Mating system variation in hybridizing irises: Effects of phenology and floral densities on family outcrossing rates. *Heredity* 72:95–105.
- DARWIN, C. 1876. The effects of cross and self fertilisation in the vegetable kingdom. 1900. Reprint. John Murray, London.
- DEMARAIS, B. D., T. E. DOWLING, M. E. DOUGLAS, W. L. MINCKLEY, AND P. C. MARSH. 1992. Origin of *Gila seminuda* (Teleostei: Cyprinidae) through introgressive hybridization: Implications for evolution and conservation. *Proc. Nat. Acad. Sci. USA* 89:2747–2751.
- DOBZHANSKY, TH. 1970. Genetics of the evolutionary process. Columbia Univ. Press, New York.
- DOWLING, T. E., AND B. D. DEMARAIS. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. *Nature (Lond.)* 362:444–446.
- EHRlich, P. R., AND E. O. WILSON. 1991. Biodiversity studies: Science and policy. *Science (Wash., DC)* 253:758–762.
- GRANT, P. R., AND B. R. GRANT. 1992. Hybridization of bird species. *Science (Wash., DC)* 256:193–197.
- . 1994. Phenotypic and genetic effects of hybridization in Darwin's finches. *Evolution* 48:297–316.
- GRANT, V. 1963. The origin of adaptations. Columbia Univ. Press, New York.
- . 1981. Plant speciation. Columbia Univ. Press, New York.
- HARRISON, R. G. 1990. Hybrid zones: Windows on evolutionary process. *Oxf. Surv. Evol. Biol.* 7:69–128.
- . 1993. Hybrids and hybrid zones: Historical perspective. Pp. 3–12 in R. G. Harrison, ed. *Hybrid zones and the evolutionary process*. Oxford Univ. Press, New York.
- HEUSMANN, H. W. 1974. Mallard-Black Duck relationships in the northeast. *Wildl. Soc. Bull.* 2:171–177.
- HINTON, W. F. 1975. Natural hybridization and extinction of a population of *Physalis virginiana* (Solanaceae). *Am. J. Bot.* 62:198–202.
- HOLM, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.* 6:65–70.
- HOWARD, D. J. 1986. A zone of overlap and hybridization between two ground cricket species. *Evolution* 40:34–43.
- LEVIN, D. A. 1978. The origin of isolating mechanisms in flowering plants. Pp. 185–317 in M. W. Hecht, W. C. Steere, and B. Wallace, eds. *Evolutionary biology*. Vol. 11. Appleton Century Crofts, New York.
- . 1981. Dispersal versus gene flow in plants. *Ann. Missouri Bot. Gard.* 68:233–253.
- LEWONTIN, R. C., AND L. C. BIRCH. 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution* 20:315–336.
- MASTERTSON, J. 1994. Stomatal size in fossil plants: Evidence for polyploidy in majority of angiosperms. *Science (Wash., DC)* 264:421–423.
- MERTZWEILLER, J. K. 1988. Propagation of Louisiana irises. Pp. 69–80 in M. Caillet and J. K. Mertzweiller, eds. *The Louisiana iris: The history and culture of five native American species and their hybrids*. Texas Gardener Press, Waco, TX.
- NASON, J. D., N. C. ELLSTRAND, AND M. L. ARNOLD. 1992. Patterns of hybridization and introgression in populations of oaks, manzanitas and irises. *Am. J. Bot.* 79:101–111.
- PRICE, V. M. AND N. M. WASER. 1982. Experimental studies of pollen carryover: Hummingbirds and *Ipomopsis aggregata*. *Oecologia (Berl.)* 54:353–358.
- RAND, D. M. AND R. G. HARRISON. 1989. Ecological genetics of a mosaic hybrid zone: Mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* 43:432–449.
- RANDOLPH, L. F. 1966. *Iris nelsonii*, a new species of Louisiana iris of hybrid origin. *Baileya* 14:143–169.
- RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- RIESEBERG, L. H. 1991. Hybridization in rare plants: Insights from case studies in *Cercocarpus* and *Helianthus*. Pp. 171–181 in D. A. Falk and K. E. Holsinger, eds. *Genetics and conservation of rare plants*. Oxford Univ. Press, New York.
- RIESEBERG, L. H., AND J. F. WENDEL. 1993. Introgression and its consequences in plants. Pp. 70–109 in R. G. Harrison, ed. *Hybrid zones and the evolutionary process*. Oxford Univ. Press, New York.
- RIESEBERG, L. H., R. CARTER, AND S. ZONA. 1990. Molecular tests of the hypothesized hybrid origin of two diploid *Helianthus* species (Asteraceae). *Evolution* 44:1498–1511.
- RIESEBERG, L. H., A. M. DESROCHERS, AND S. J. YOUNG. 1995. Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *Am. J. Bot.* 82:515–519.
- SAS INSTITUTE. 1988. SAS user's guide: Statistics. SAS Institute, Inc., Cary, NC.
- SCHAAL, B. A. 1980. Measurement of gene flow in *Lupinus texensis*. *Nature (Lond.)* 284:450–451.
- SMITH, E. B. 1968. Pollen competition and relatedness in *Haplopappus* section *Isopappus*. *Bot. Gaz.* 129:371–373.
- . 1970. Pollen competition and relatedness in *Haplopappus* section *Isopappus* (Compositae). II. *Am. J. Bot.* 57:874–880.
- STEBBINS, G. L. 1959. The role of hybridization in evolution. *Proc. Am. Phil. Soc.* 103:231–251.
- SZYMURA, J. M., AND N. H. BARTON. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *B. variegata*, near Cracow in southern Poland. *Evolution* 40:1141–1159.
- THOMSON, J. D., AND R. C. PLOWRIGHT. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. *Oecologia (Berl.)* 46:68–74.
- VISSEr, T., AND J. J. VERHAEGH. 1980. Pollen and pollination experiments. II. The influence of the first pollination on the effectiveness of the second one in apple. *Euphytica* 29:385–390.
- WASER, N. M., AND M. V. PRICE. 1984. Experimental studies of pollen carryover: Effects of floral variability in *Ipomopsis aggregata*. *Oecologia (Berl.)* 62:262–268.
- WHITHAM, T. G., P. A. MORROW, AND B. M. POTTS. 1991. Conservation of hybrid plants. *Science (Wash., DC)* 254:779–780.
- WILSON, H. D., AND J. S. PAYNE. 1994. Crop/weed microgametophyte competition in *Cucurbita pepo* (Cucurbitaceae). *Am. J. Bot.* 81:1531–1537.