BOAG, P. T., AND A. VAN NOORDWIJK. 1987. Quantitative genetics. Pp. 45–78 *in* F. Cooke, and P. A. Buckley, eds. Avian genetics: A population and ecological approach. Academic Press, New York.
BOYCE, M. S. 1979. Seasonality and patterns of natural selection for life histories. Am. Nat. 114:569–583.
FRETWELL, S. D. 1972. Populations in a seasonal environment. Princeton Univ. Press, Princeton, NJ.
GOMULKIEWICZ, R., AND M KIRKPATRICK. 1992. Quantitative genetics and the evolution of reaction norms. Evolution 46:390–411.
GRANT, B. R., AND P. R. GRANT. 1989. Evolutionary dynamics of a natural population: The large cactus finch of the Galápagos. Univ. of Chicago Press, Chicago.
———. 1993. Evolution of Darwin's finches caused by a rare climatic event. Proc. R. Soc. Lond. B Biol. Sci. 251:111–117.
GRANT, P. R. 1986. Ecology and evolution of Darwin's finches. Princeton Univ. Press, Princeton, NJ.
GREENBERG, R. 1979. Body size, breeding habitat, and winter exploitation systems in *Dendroica*. Auk 96:756–766.
———. 1981. Dissimilar bill shapes in New World tropical versus temperate forest foliage-gleaning birds. Oecologia 49:143–147.
GROTH, J. G. 1993. Evolutionary differentiation in morphology, vocalizations, and allozymes among nomadic sibling species in the North American red crossbill (*Loxia curvirostra*) complex. Univ. Calif. Publ. Zool., Berkeley.
LANDE, R., AND S. J. ARNOLD. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.
LEMON, W. C. 1991. Fitness consequences of foraging behaviour in the zebra finch. Nature 352:153–155.
———. 1993. The energetics of lifetime reproductive success in the zebra finch *Taeniopygia guttata*. Physiol. Zool. 66:946–963.
LEVINS, R. 1968. Evolution in changing environments. Princeton Univ. Press, Princeton, NJ.
LIEM, K. F., AND L. S. KAUFMAN. 1984. Intraspecific macroevolution: Functional biology of the polymorphic cichlid species *Cichlasoma minckleyi*. Pp. 203–215 *in* A. A. Echelle, and I. Kornfield, eds. Evolution of fish species flocks. Univ. of Maine Press, Orono.
MCKAYE, K. R., AND A. MARSH. 1983. Food switching by two specialized algae-scrapping cichlid fishes in Lake Malawi, Africa. Oecologia 56:245–248.
MITCHELL-OLDS, T., AND R. G. SHAW. 1987. Regression analysis of natural selection: Statistical inference and biological interpretation. Evolution 41:1149–1161.
NEWTON, I. 1967. The adaptive radiation and feeding ecology of some British finches. Ibis 109:33–98.
———. 1972. Finches. Collins, London.
PRICE, T. D., AND P. R. GRANT. 1984. Life history traits and natural selection for small body size in a population of Darwin's finches. Evolution 38:483–494.
PRICE, T. D., P. R. GRANT, H. L. GIBBS, AND P. T. BOAG. 1984. Recurrent patterns of natural selection in a population of Darwin's finches. Nature 309:787–789.
SCHLUTER, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 42:849–861.
———. 1995. Adaptive radiation in sticklebacks: Trade-offs in feeding performance and growth. Ecology 76:82–90.
SCHLUTER, D., AND P. R. GRANT. 1984. Determinants of morphological patterns in communities of Darwin's finches. Am. Nat. 123:175–196.
SCHLUTER, D., AND J.N.M. SMITH. 1986. Natural selection on beak and body size in the song sparrow. Evolution 40:221–231.
SCHOPMEYER, C. S. 1974. Seeds of woody plants in the United States. Agricultural handbook number 450. U.S. Dept. Agric., Washington, DC.
SIMMS, E. L. 1990. Examining selection on the multivariate phenotype: Plant resistance to herbivores. Evolution 44:1177–1188.
SMITH, T. B. 1990. Resource use by bill morphs of an African finch: Evidence for intraspecific competition. Ecology 71:1246–1257.
———. 1991. Inter- and intra-specific diet overlap during lean times between *Quelea erythrops* and bill morphs of *Pyrenestes ostrinus*. Oikos 60:76–82.
———. 1993. Disruptive selection and the genetic basis of bill size polymorphism in the African finch *Pyrenestes*. Nature 363: 618–620.
STEPHENS, D. W., AND J. R. KREBS. 1986. Foraging theory. Princeton Univ. Press, Princeton, NJ.
SUDWORTH, G. B. 1967. Forest trees of the Pacific slope. Dover, New York.
WALSBERG, G. E. 1983. Avian ecological energetics. Pp. 161–220 *in* D. S. Farner, J. R. King, and K. C. Parkes, eds. Avian biology. Vol. 7. Academic Press, New York.
WIENS, J. A. 1989. The ecology of bird communities. Vol. 1. Cambridge Univ. Press, New York.

Corresponding Editor: A. Larson

Evolution, 50(6), 1996, pp. 2504–2509

NATURAL FORMATION OF IRIS HYBRIDS: EXPERIMENTAL EVIDENCE ON THE ESTABLISHMENT OF HYBRID ZONES

SCOTT A. HODGES,[1] JOHN M. BURKE, AND MICHAEL L. ARNOLD[2]
Department of Genetics, University of Georgia, Athens, Georgia 30602
[2]*E-mail: arnold@dogwood.botany.uga.edu*

Key words.—Gene flow, introgression, reproductive isolation, secondary contact.

Received July 11, 1995. Accepted May 14, 1996.

Studies of animal and plant hybrid zones have been used to examine the early stages of speciation and mechanisms of reproductive isolation, as well as the interactions between genetic and ecological features of differentiated populations (Barton and Hewitt 1989; Arnold 1992; Harrison 1993). Despite the widespread use of hybrid zones for studies of evolutionary processes, little is actually known about the early

[1] Present address: Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106.

stages in hybrid zone formation. Two scenarios have been proposed. First, hybrid zones may form as the result of secondary contact between populations that have differentiated in allopatry (Mayr 1942). Second, hybrid zones could arise from primary intergradation due to differential selection along an environmental gradient (Endler 1977). These two scenarios are extremely difficult to distinguish once a hybrid zone has been established because both can result in identical patterns of variation (Endler 1977). These opposing views, notwithstanding, little experimental work has been performed to define the initial stages of either of these scenarios. The relative ease of experimental manipulation of plants affords the opportunity to study the initial stages of secondary contact between species through transplantation, monitoring of particular genotypes, and measuring parameters such as gene flow and natural selection (Cruzan and Arnold 1993).

The Louisiana irises are a well-studied group of hybridizing species (Viosca 1935; Riley 1938; Anderson 1949) that include three species with widespread distributions overlapping in southern Louisiana (*Iris fulva*, *I. hexagona*, and *I. brevicaulis*) and the rare, endemic species *I. nelsonii*. The widespread distributions of *I. fulva*, *I. hexagona*, and *I. brevicaulis* suggest that they may have previously been allopatric and then, through range expansion, come into secondary contact (Viosca 1935). Presently, numerous hybrid populations occur in the area of overlap (Riley 1938; Anderson 1949; Arnold et al. 1990a, 1991) and therefore it is surprising that the formation of natural F_1 hybrids appears to be a rare event (Arnold 1993b). For instance, though numerous hybrid populations between *I. fulva* and *I. hexagona* have been examined genetically, no identifiable adult F_1 hybrid plants have been found (Arnold et al. 1990b, 1991; Arnold 1993b). Furthermore, when 200 *I. hexagona* rhizomes were planted into an *I. fulva* population, very low rates of F_1 hybrid formation were detected over several years of flowering (Arnold et al. 1993). These observations have led to the suggestion that the formation of F_1 hybrids between these two species, though a rare event in itself, subsequently results in a much greater frequency of advanced generation hybrid production (Arnold 1993b).

To test the hypothesis that the rate of advanced generation hybrid production is greater than the rate of initial F_1 hybrid formation, we planted small patches of F_1 hybrids near *I. fulva* and *I. hexagona* plants to simulate the earliest stage of hybrid zone formation. We then monitored flowering and collected fruits produced by both the F_1 plants and the nearby parental plants. By genotyping seeds of these fruits for two allozyme loci with fixed differences between the species, we determined the frequency of conspecific, F_1, F_2, and backcross progeny produced.

Materials and Methods

F_1 plants were produced by controlled hand pollinations between *I. fulva* and *I. hexagona* in the Botany Department greenhouses at the University of Georgia. Rhizomes of F_1 individuals were planted in the fall of 1993 into the Talbot population (Arnold et al. 1993) that contained both pure *I. fulva* and *I. hexagona* individuals. This population was originally a natural population of *I. fulva* individuals into which a single block of 200 *I. hexagona* rhizomes was planted in 1989 (Arnold et al. 1993). Molecular genetic evidence suggests that initial hybridization does not occur due to seed dispersal of one species into the habitat of the other followed by hybridization, but rather that F_1 hybrids are formed due to pollen dispersal from one species into a population of the other (Arnold et al. 1993). Thus, five blocks, each consisting of 20 F_1 rhizomes, were planted into the population in the following manner: one block was placed near each of three separate patches of *I. fulva* individuals and two blocks were placed on opposite sides of the large *I. hexagona* patch (Fig. 1).

Throughout the 1994 flowering season, each flower produced on F_1 plants was tagged and the date of anthesis noted. Over 1500 flowers were also tagged on the *I. fulva* and *I. hexagona* individuals near the F_1 patches. Natural pollination was allowed to occur and mature fruits were collected in June before the fruits dehisced.

Two allozyme loci (PGI-3 and ADH) have fixed allelic differences that are diagnostic for *I. fulva* and *I. hexagona* and were used to genotype seeds. Electrophoretic conditions can be found elsewhere (Arnold et al. 1990b). All seeds from F_1 fruits were genotyped. Some 13–14 fruits from *I. fulva* and *I. hexagona* plants were sampled near each of the F_1 patches for a total of 41 *I. fulva* fruits and 27 *I. hexagona* fruits. Ten seeds from each of these fruits were genotyped. Parental fruits were chosen for sampling if the flowers that produced them were in their female phase when nearby F_1 flowers were in male phase. Fruits from the parental species were also chosen to span the blooming times of nearby F_1 plants.

Nine two-locus genotypes at PGI-3 and ADH were possible and the expected frequencies of these genotypes for specific crosses (backcross to either *I. fulva* [B_f] or *I. hexagona* [B_h], and F_2; Table 1) were calculated. Because expected frequencies for some of the nine genotypes were low (e.g., for a cross producing F_2 individuals FF/HH = 0.0625), the genotypes were grouped into four classes. The expected frequencies of genotypes due to different second generation crosses were then used to distinguish the types of crosses that contributed to seeds on both F_1 and parental plants. Hybrid seeds from *I. fulva* and *I. hexagona* fruits could only be produced from crosses between the species (producing F_1 seeds) or from backcrossing with F_1 individuals.

Because seeds produced on F_1 maternal plants could belong to all four genotypic classes (A–D, Table 1), a maximum-likelihood model was used to estimate the percentage of seeds produced on F_1 plants that were B_f, F_2, or B_h. By iterating through different combinations of crosses, the model estimated the expected seed genotype frequencies using the frequencies in Table 1. Then the program computed the likelihood ratio given the observed data. By maximizing this ratio, the program estimated the combination of crosses that most likely produced the observed genotypes.

Results

Either three or four rhizomes produced a flowering stalk within each F_1 patch (17 flowering stalks total). These flowering stalks produced a total of 34 flowers (5–9 flowers per

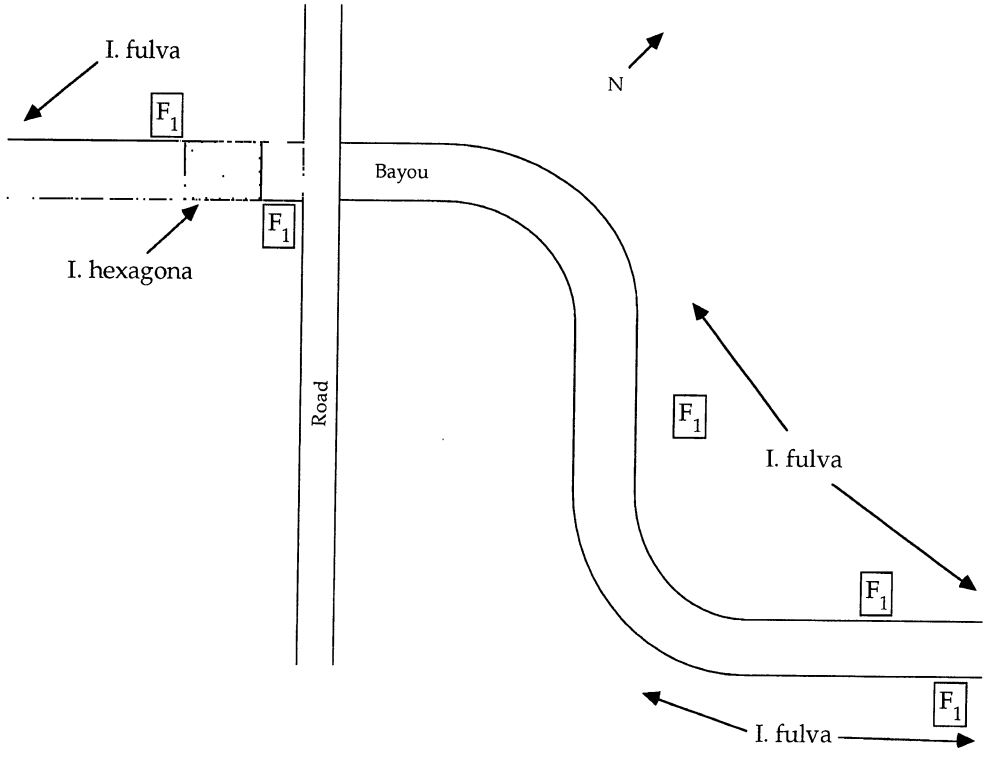

FIG. 1. Map of the Talbot iris population. *Iris fulva* grows along the bayou and a block of *I. hexagona* was planted into the bayou in 1989. The relative positions of F_1 plants are indicated.

patch) over 18 d (24 March to 10 April 1994). Only four pairs of flowers had completely coincidental flowering within any one patch. Flowers of both *I. fulva* and *I. hexagona* completely overlapped the flowering of the F_1 plants. Thirteen fruits were produced on the F_1 plants (1–5 fruits per patch). Two fruits, both from one patch (near *I. fulva*) were lost, leaving seven fruits from two plots near *I. fulva* and four fruits from two plots near *I. hexagona* for analysis.

F_1 Plants.—The distribution of seeds in each genotypic class was determined separately for seeds from F_1 plants near *I. fulva* and *I. hexagona* (Table 2). These distributions were significantly different from each other (Table 2) indicating that distinct types of crosses occurred on F_1 plants when they were near *I. fulva* or *I. hexagona*. The distribution of seeds from F_1 plants that were near *I. fulva* were not significantly different from the expected distribution for pure F_2 progeny though nearly so ($\chi^2 = 7.52$, $P < 0.10$). This nearly significant result was caused by fewer than expected class C seeds (15 versus 26 expected) and an excess of class D seeds (42 versus 32.5 expected). All second generation crosses are expected to produce the same frequency of class C seeds, but only backcrossing to *I. hexagona* is expected to produce high levels of class D seeds (Table 1). The relative contributions of F_2, B_h, and B_f crosses were calculated to be 90%, 10%, and 0%, respectively by the maximum-likelihood model (Table 3). In contrast, seeds from F_1 plants that were near *I. hexagona* were not significantly different from the expected distribution of B_h fruits ($\chi^2 = 0.17$, ns: tested using only seeds that were classified in genotypic classes C and D; 56 of the 58 seeds; Table 2). The remaining two seeds in class A could only have been produced from a B_f or F_2 cross (Table 2). The relative contributions of F_2, B_h, and B_f crosses were

TABLE 1. Expected genotype and genotypic class frequencies of seeds for crosses that would produce pure backcross-*Iris fulva* (B_f), F_2, or backcross-*I. hexagona* (B_h) fruits.

Genotype		Expected frequencies			Geno-typic class	Expected frequencies		
Locus 1	Locus 2	B_f	F_2	B_h		B_f	F_2	B_h
FF	FF	0.25	0.0625					
FF	FH	0.25	0.125		A	0.75	0.3125	
FH	FF	0.25	0.125					
FF	HH		0.0625		B		0.125	
HH	FF		0.0625					
FH	FH	0.25	0.25	0.25	C	0.25	0.25	0.25
FH	HH		0.125	0.25				
HH	FH		0.125	0.25	D		0.3125	0.75
HH	HH		0.0625	0.25				

TABLE 2. Number of seeds produced in each genotypic class by F_1 plants that were physically near either *Iris fulva* or *I. hexagona*.

Genotypic class	F_1 plants near	
	I. fulva	*I. hexagona*
A	33	2
B	14	0
C	15	13
D	42	43

$X^2 = 31.0$, $P \ll 0.001$.

TABLE 3. Percentage of seeds that were attributed to specific types of crosses based on their genotype. Values for *Iris hexagona* and *I. fulva* maternal plants are direct calculations, while those for F_1 maternal plants are estimated from the maximum-likelihood model.

	Maternal parent			
			F_1 plants near	
Paternal parent	*I. hexagona*	*I. fulva*	(*I. hexagona*)	(*I. fulva*)
I. hexagona	93.1	0.03*	95	10
I. fulva	0.74*	98.3	5	0
F_1	6.9	1.70	0	90

* Arnold et al. 1993; J. Hamrick and M. Arnold unpubl. data.

calculated to be 0%, 95%, and 5%, respectively by the maximum-likelihood model (Table 3).

Parental Plants.—Only five fruits from parental plants had seeds with genotypes distinguishable as hybrids: two of the 41 fruits sampled from *I. fulva* and three of the 27 fruits sampled from *I. hexagona*. *Iris hexagona* produced significantly more hybrid seeds than *I. fulva* (Table 4). The 19 seeds sampled from the two *I. fulva* fruits that contained detectable hybrid seeds had a significantly different genotypic class distribution than expected for pure B_f fruits ($\chi^2 = 3.95$, $P < 0.05$) due to an excess of parental-like seeds. Thus, these seeds were likely a result of fertilization by a mixture of *I. fulva* and F_1 pollen. In contrast, the 29 seeds sampled from the three *I. hexagona* fruits that contained detectable hybrid seeds did not have a significantly different genotypic class distribution than expected for pure B_h fruits ($\chi^2 = 1.39$, ns).

Seeds produced on either of the parental species with double heterozygote genotypes (class C) could have been produced either by crosses between the two species (100% expected frequency) or by backcrossing with F_1 plants (25% expected frequency, Table 1). None of the fruits that contained hybrid seeds in either species had only class C seeds. Furthermore, each of these fruits also contained seeds that could only be produced through backcrossing with F_1 individuals. Thus it is likely that the class C seeds in these fruits were produced through backcrossing.

DISCUSSION

This study demonstrates how the formation of F_1 hybrids, even if a rare event, can nonetheless be a catalyst for higher rates of advanced generation hybrid production and also that the evolutionary pathway of these hybrids is context dependent. The initial formation of F_1 hybrids between *I. fulva* and *I. hexagona* appears to be a rare event (Arnold 1993b) even though these hybrids can be produced with ease by controlled pollinations. Despite numerous genetic surveys of *Iris* populations, no adult F_1 hybrids have yet been identified (Arnold et al. 1990b, 1991; Arnold 1993b). In addition, when a block of 200 *I. hexagona* rhizomes were introduced into the Talbot population of *I. fulva*, low rates of F_1 seed formation occurred over multiple years of flowering. In three years of monitoring, over 5000 seeds were genotyped and less than one percent were found to have an F_1 genotype (Table 3; Arnold et al. 1993; J. Hamrick and M. Arnold unpubl. data). Furthermore, a directionality to F_1 formation was detected with *I. hexagona* plants serving as maternal plants more often than *I. fulva* plants (Table 3; Arnold et al. 1993).

TABLE 4. Number of seeds that had hybrid or parental genotypes for all seeds sampled from *Iris fulva* and *I. hexagona*.

	Parental plants	
Genotype	*I. fulva*	*I. hexagona*
hybrid	7	19
parental	407	258

$X^2 = 12.32$, $P < 0.01$.

Though the formation of natural F_1 hybrids between *I. fulva* and *I. hexagona* is a rare event, the parental species produce offspring with F_1 individuals at much higher rates. In *I. hexagona*, backcross-seed formation is nearly an order of magnitude greater than F_1 seed formation, and in *I. fulva*, backcross-seed formation is nearly 60-fold greater than F_1 seed formation (Table 3). In addition, the F_1 plants themselves produced F_2 and backcross progeny and thus the numbers of hybrid seeds and the range of genotypes produced in the population were vastly increased once F_1 hybrids were established. Therefore, when an F_1 hybrid does become established in a population, it serves as a bridge for the founding of advanced generation hybrids and possibly, new evolutionary lineages.

Even if F_1 hybrids suffer a reduction in fitness, the evolutionary consequences of hybridization may be significant (Anderson 1949; Stebbins 1950; Grant 1971). For instance, crosses between *Helianthus annuus* and *H. petiolaris* yield F_1 progeny that have extremely low pollen fertility and seed production (means of 14% and 2%, respectively, Heiser et al. 1969). However, molecular genetic analyses suggest that hybrids between these two species have resulted in at least three independent lineages (Rieseberg 1991). Furthermore, Grant (1966) showed that despite strong sterility barriers between *Gilia malior* and *G. modocensis*, some advanced generation hybrids regained their fertility and were reproductively isolated from their parental species without becoming polyploid. Arnold and Hodges (1995) found that among both animal and plant taxa F_1 hybrids tend to be less fit than parental-like hybrids. Thus, it may be common that the initial formation of F_1 hybrids is rare with subsequent hybrid generations forming at greater frequency.

Several factors may limit the formation of natural F_1 hybrids between *I. fulva* and *I. hexagona* despite the ease of F_1 formation in controlled crosses. Though the two species have coincident flowering phenologies (Arnold et al. 1993), they have different major pollinators: hummingbirds visit *I. fulva* and bumblebees visit *I. hexagona* (Viosca 1935). However, these pollinator classes do make interspecific movements that may result in interspecific pollen transfer (Carney, Cruzan, and Hodges, pers. obs.). Even when interspecific pollen transfer occurs, pollen competition experiments suggest a reproductive barrier that may explain differences in seed formation among parental and hybrid classes (Arnold et al. 1993; Carney et al. 1994, 1996). First, when 50:50 mixtures of *I. fulva* and *I. hexagona* pollen are used to pollinate *I. fulva* or *I. hexagona* flowers, F_1 seeds are produced at a lower rate than expected (Arnold et al. 1993; Carney et al. 1994, 1996). This

barrier to F_1 formation apparently is stronger for *I. fulva* plants than *I. hexagona* plants (Carney et al. 1994, 1996) and therefore may explain the more than 20-fold difference in F_1 seed formation between *I. fulva* and *I. hexagona* plants in nature (Table 3). If *I. fulva* plants can also discriminate between *I. fulva* and F_1 pollen tubes to a greater extent than can *I. hexagona* plants, then pollen-tube growth rates may also explain the greater extent of backcrossing into *I. hexagona* (Table 3). Furthermore, *I. fulva* pollen tubes grow more slowly than either *I. hexagona* or F_1 pollen tubes when placed on F_1 stigmas (Carney et al. 1994). Thus, pollen competition may also explain the rarity of backcross-seed formation on F_1 plants near *I. fulva* (Table 3).

The apparent difficulty in establishing initial F_1 progeny indicates that these hybrids may act as a genetic bottleneck for hybrid populations, particularly when near *I. fulva*. As such, the combination of genes in an initial F_1 plant and its subsequent hybrid offspring will be some fraction of all possible combinations between the parental species. As with founder events (Mayr 1942), this scenario would allow certain gene combinations that may be advantageous to become established in a population. For instance, the F_1 plants near *I. fulva* apparently did not backcross with *I. fulva*, but instead produced predominately F_2 progeny. If the pollen-tube growth data described above contributes to this pattern of mating, then the F_2 generation may also form few backcrosses with *I. fulva* because *I. fulva* pollen produce significantly shorter pollen tubes than F_2 pollen on F_2 flowers (Carney et al. 1994). Thus, the initial advanced-generation hybrids formed near *I. fulva* plants may be largely reproductively isolated, which could, in turn, allow the establishment of advantageous gene combinations and a new evolutionary lineage (Arnold and Hodges 1995).

The above model suggests that new lineages may be more likely to form when *I. fulva* is the dominant plant in the vicinity of the initial hybridization. It is therefore significant to note that *I. fulva* has played a major role in two examples of hybrid differentiation. First, *I. nelsonii*, appears to be a diploid level hybrid derivative from three *Iris* species (*I. fulva*, *I. hexagona*, and *I. brevicaulis*; Arnold 1993a). While this species contains genetic markers from all three of these *Iris* species, both the chloroplast DNA marker and the vast majority of nuclear DNA markers are indicative of *I. fulva* (Arnold 1993a). Additionally, Cruzan and Arnold (1994) concluded that *I. fulva* likely discriminates against *I. brevicaulis* pollen in an *I. fulva/I. brevicaulis* hybrid zone. Cruzan and Arnold (1993) also found that *I. fulva*-like hybrids in the *I. fulva/I. brevicaulis* hybrid zone had ecological affinities that were different from either of the parental species or *I. brevicaulis*-like hybrids. Again, this indicates the ability of *I. fulva*-like hybrids to become differentiated relative to their parents.

In contrast to the formation of hybrids near *I. fulva*, when F_1 hybrids were placed near *I. hexagona,* predominately B_h progeny were produced (Tables 1,3). The production of B_h progeny on both *I. hexagona* and F_1 plants suggest that when initial F_1 establishment occurs near *I. hexagona*, introgression will occur into the *I. hexagona* genome. Again, these results are consistent with pollen-tube growth experiments that show that *I. hexagona* plants are less discriminating than *I. fulva* plants among pollen genotypes (Carney et al. 1994). Additionally, when *I. hexagona* and F_1 pollen were placed on F_1 stigmas, no differences in pollen-tube growth rates were detected (Carney et al. 1994). Thus, as opposed to hybrid formation near *I. fulva* plants, differences in pollen-tube growth rates are unlikely to act as a barrier to backcrossing between *I. hexagona* and F_1 plants.

In summary, this study shows that the early events in the formation of a hybrid zone through secondary contact are context dependent. F_1 hybrid establishment near *I. fulva* and *I. hexagona* had very different outcomes in the types of hybrid progeny produced. This context dependent result may have strong implications for the direction of evolutionary change in a population. Furthermore, this study demonstrates that despite the difficulty in the establishment of an initial F_1 hybrid, these rare events can be a bridge to the rapid establishment of a hybrid zone.

Acknowledgments

We thank S. Emms, S. Carney, and M. Hare for comments on the manuscript, and M. Goodisman for constructing the maximum-likelihood model. This research was supported by the American Iris Society Foundation, National Science Foundation grant DEB 9317654 (MLA) and by NSF/USDA/DOE Plant Molecular Evolution training grant BIR-9220329.

Literature Cited

Anderson, E. 1949. Introgressive hybridization. Wiley, New York.

Arnold, M. L. 1992. Natural hybridization as an evolutionary process. Annu. Rev. Ecol. Syst. 23:237–262.

———. 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. News. 9:14–17.

Arnold, M. L., and S. A. Hodges. 1995. Are natural hybrids fit or unfit relative to their parents? Trends Ecol. Evol. 10:67–71.

Arnold, M. L., B. D. Bennett, and E. A. Zimmer. 1990a. Natural hybridization between *Iris fulva* and *Iris hexagona*: Pattern of ribosomal DNA variation. Evolution 44:1512–1521.

Arnold, M. L., C. M. Buckner, and J. J. Robinson. 1991. Pollen-mediated introgression and hybrid speciation in Louisiana irises. Proc. Nat. Acad. Sci. USA 88:1398–1402.

Arnold, M. L., J. L. Hamrick, and B. D. Bennett. 1990b. Allozyme 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. 1989. Adaptation, speciation and hybrid zones. Nature 341:497–503.

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.

Carney, S. E., S. A. Hodges, and M. L. Arnold. 1996. Effects of differential pollen-tube growth on hybridization in the Louisiana irises. Evolution 50:1871–1878.

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.

Endler, J. A. 1977. Geographic variation, speciation, and clines. Monographs in population biology. Princeton Univ. Press, Princeton, NJ.

Grant, V. 1966. The origin of a new species of *Gilia* in a hybridization experiment. Genetics 54:1189–1199.

———. 1971. Plant speciation. Columbia Univ. Press, New York.

HARRISON, R. G. 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.
HEISER, C. B., JR., D. M., SMITH, S. B. CLEVENGER, AND W. C., MARTIN JR. 1969. The North American Sunflowers (*Helianthus*). Mem. Torrey Bot. Club 22:1–218.
MAYR, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.
RIESEBERG, L. H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): Evidence from ribosomal genes. Am. J. Bot. 78:1218–1237.
RILEY, G. P. 1938. A character analysis of colonies of *Iris fulva*, *Iris hexagona* var. *giganticaerulea* and natural hybrids. Am. J. Bot. 25:727–738.
STEBBINS, G. L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York.
VIOSCA, P. J. 1935. The irises of southeastern Louisiana: A taxonomic and ecological interpretation. Bull. Am. Iris Soc. 57:3–56.

Corresponding Editor: D. Howard

Evolution, 50(6), 1996, pp. 2509–2515

TEMPORAL INSTABILITY OF GENETIC COMPONENTS OF FLORAL TRAIT VARIATION: MATERNAL FAMILY AND POPULATION EFFECTS IN *SPERGULARIA MARINA* (CARYOPHYLLACEAE)

SUSAN J. MAZER[1] AND VERONIQUE A. DELESALLE[2]
[1]*Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, California 93106*
E-mail: mazer@lifesci.lscf.ucsb.edu
[2]*Department of Biology, Gettysburg College, Gettysburg, Pennsylvania, 17325*
E-mail: delesall @ gettysburg.edu

Key words.—Floral traits, gamete production, gender, maternal effects, population differentiation, quantitative characters, sex allocation.

Received April 2, 1996. Accepted April 5, 1996.

Modular organs present a special problem for evolutionary geneticists aiming to measure the genetic component of their variation. If the phenotype of traits expressed by newly produced leaves, flowers, or fruits changes as an individual ages, then phenotypic measurements of these traits recorded from individuals, genotypes, and populations may confound ontogenetic and genetic sources of variation. A number of investigators have examined the effects of plant age or flowering sequence on flower size, gender expression, or gamete production, while others have measured genetic and external environmental (natural or experimentally imposed) sources of variation in floral traits (Lord 1980; Thomson and Barrett 1981; Bawa and Webb 1983; Holtsford 1985; Solomon 1985; Thomson 1985, 1989; Pellmyr 1987; Lee 1988; Thompson and Pellmyr 1989; Mazer and Schick 1991a,b; Ashman and Baker 1992; Emms 1993). There are few studies, however, that evaluate both ontogenetic and genetic sources of variation in floral traits (Mazer et al. 1986; Young and Stanton 1990; Armbruster 1991; Diggle 1993). These studies and the frequent discovery of temporal changes in seed and fruit traits (Cavers and Steel 1984; Harder et al. 1985; Holtsford 1985; Marshall et al. 1985; Thomson 1985, 1989; Lubbers and Christensen 1986; Mazer et al. 1986, 1989; Pellmyr 1987; Thomson et al. 1989; Byrne and Mazer 1990; Kang and Primack 1991, and references therein) raise concern that reproductive traits in particular may show strong ontogenetic changes. This, in turn, suggests that measures of genetic variation in floral traits may similarly be sensitive to the age at which individuals are sampled. The studies cited above, however, did not determine whether the magnitude of potential sources of genetic variation (maternal families or populations) depends upon the age of the individuals from which flowers are sampled.

The study described here is one part of a broad investigation of the causes and evolutionary significance of variation in sex allocation and gamete-packaging patterns within and among genotypes of *Spergularia marina* (L.) Griseb. (sandspurrey; Caryophyllaceae). Below, we show that temporal, age-related changes in floral phenotype contribute significantly to phenotypic variation and can mask underlying sources of genetic variation in floral traits at two levels: (a) among maternal families within populations; and (b) among natural populations. We detected strong week-to-week changes in the statistical significance of both intra- and interpopulation sources of phenotypic variation in several primary and secondary sexual traits. We also report variation among traits and among populations in the degree to which this temporal variation is expressed. This study differs from previous evaluations of the role of flowering time on floral traits in that we carried out in a controlled environment a hierarchical experimental design that permits the partitioning of variance in floral phenotype into geographic, maternal family, and ontogenetic components.

MATERIALS AND METHODS

Study Organism

Spergularia marina is a cosmopolitan, short-lived, annual herb of coastal saltmarshes and wetlands, described in detail