Ecological isolation and introgression:
biochemical confirmation of introgression
in an *Arctostaphylos* (Ericaceae) population

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ABSTRACT

Putative hybrid swarms are rarely the subject of combined physiological, ecological, and genetic analyses. Here, we present genetic evidence for hybridization and introgression in a putative *Arctostaphylos* hybrid swarm previously subject to physiological and ecological study (BALL *et al.*, 1983). Considerable introgression is evident in the study population when its isozyme phenotypes (zymotypes) are compared with those in nearby pure populations of *A. viscida* and *A. patula*. However, while plants with introgressant (backcross) zymotypes are frequent, those with hybrid or segregant zymotypes are very rare. Overall, zymotypes correlate with the species identity index calculated from vegetative characters. These data, combined with those of the prior ecological study, support ANDERSON's (1948, 1949) hypothesis that interspecific gene flow will occur via introgression when ecologically differentiated species meet at an ecotone.

KEY WORDS: Arctostaphylos - *Ericaceae* - *Introgression* - *Hybrid swarm*.

RÉSUMÉ

Les populations supposées hybrides ont rarement fait l'objet d'études combinant les approches physiologiques, écologiques et génétiques. Ce travail apporte une preuve génétique de l'hybridation et de l'introgression dans une population supposée hybride d'*Arctostaphylos* qui avait antérieurement fait l'objet d'une étude physiologique et génétique (BALL *et al.*, 1983). Une introgression considérable a pu être mise en évidence dans la population étudiée en comparant ses phénotypes isozymiques (zymotypes) avec ceux de populations presque pures d'*A. viscida* et d'*A. patula*. Toutefois, alors que les plantes à zymotypes introgressants sont fréquentes, celles à zymotypes hybrides ou segrégants sont très rares. Dans l'ensemble les zymotypes sont corrélés avec l'indice (species index) calculé à partir des caractères végétatifs. Ces données, combinées avec celles de l'étude écologique antérieure, corroborent l'hypothèse d'ANDERSON (1948, 1949) selon laquelle le flux génique passe par l'introgression lorsque des espèces écologiquement différenciées se rencontrent dans un écotone.

MOTS CLÉS : Arctostaphylos - *Ericaceae* - *Introgression* - *Population hybride*.

1. – INTRODUCTION

Interspecific hybridization commonly occurs in natural plant populations and is not uncommon for many animal species (LEVIN, 1979). However, while little is known of the role of hybridization and subsequent introgression as an avenue for interspecific gene flow, the potential seems to be present. In many plant species, congeneric mating frequently results in fertile hybrids (GRANT, 1981). But when congeners are sympatric, the opportunity for evolutionarily important interspecific gene flow is thought to be limited primarily by "ecological isolation" (GRANT, 1981).

The mechanism of ecological isolation was proposed by ANDERSON (1948). He suggested that environmental patchiness serves as a selective sieve. Each parent species is adapted to a different niche in which it outcompetes its congener as well as all hybrids (F_1s) and segregants (F_2s). Thus, hybrids are expected to be best adapted to the few intermediate niches that occur on the interface of their parents' ranges. But suitable microhabitats for most segregants are expected to be so rare that these individuals end up in environments to which they are poorly suited and are likely to suffer strong competition, either by the parent species or by the hybrids. If successful mating occurs in a hybrid swarm, it should occur primarily in the relatively common introgressants (B_1s), individuals representing backcrossing of the hybrids to one of the parent species, whose ecological requirements should allow them to grow in a niche similar to that of the backcrossed parent. Because interspecific gene flow will be largely mediated by matings involving introgressants, instead of hybrids and segregants, ecological isolation presents a barrier through which only a few genes would "trickle" from one species into another (ANDERSON, 1949).

If ecological isolation acts as an important barrier to interspecific gene flow, then a number of predictions concerning the structure of hybrid swarms follow from ANDERSON's model. In a long-standing swarm, if space is limiting, introgressants should be much more frequent than hybrids or segregants. The introgressants should be physiologically and morphologically similar to one of the parental types. The few hybrids should occur in intermediate microhabitats and be physiologically intermediate to the parental species. Segregants, if present at all, are expected to be very rare, occuring in "hybridized" niches (ANDERSON, 1948, 1949). Thus, a test of the evolutionary importance of ecological isolation in a hybrid swarm should include genetic, physiological, and ecological analyses of a hybrid swarm.

However, the studies of plant hybrid swarms going beyond morphological analyses usually involve only a genetic confirmation of hybridization, usually using cytogenetic analysis or codominant biochemical markers, such as phenolics or allozymes (e. g., ALSTON & HEMPEL, 1964; HAUBER & BLOOM, 1983; LEVIN, 1967 a, 1976 b, 1968, 1975; OLIVIERI, 1985; ROLLO et al., 1985). Other studies are primarily ecological, usually measuring the edaphic characteristics of microsites of putative hybrids to ask whether putative hybrids occur in an environment intermediate to their parents (e. g., MULLER, 1952; BRAYTON & MOONEY, 1966; HADLEY & LEVIN, 1967; CHASE & RAVEN, 1975; GRANT, 1976). But only a handful of studies have tested the physiological characteristics of putative hybrids (BALL et al., 1983; ROBICHAUX, 1984). For example, BALL et al. (1983) scored physiological and morphological characters of a putative hybrid swarm of *Arctostaphylos* and found that

while variation in the morphological characters correlated closely with microhabitat changes along a cline of increasing aridity, most individuals were physiologically like one or the other of the putative parental species. Because environmental modification of morphological traits may make accurate identification of hybrids, introgressants, and segregants difficult or impossible (ADAMS & TURNER, 1970; GOTTLIEB, 1972), ecological and physiological studies of putative hybrid swarms that rely only on morphological traits for classification of individuals are, at best, of limited value and, at worst, unreliable.

At least two studies have combined genetic evidence with ecological data. BELL & LESTER (1978) used allozymes to confirm introgression from *Sabatia arenicola* into a population of *S. formosa* along a sloping edaphic cline. They found introgression matching ecological parameters; namely, changes in both morphology and electromorph frequencies correlated with edaphic changes along the ecological gradient. Likewise, WELLS (1983) documents considerable hybridization and recombination of *Cirsium californicum* and *C. occidentale* in a series of populations along a road. He also found ecological correlates of introgression. Electrophoretic and morphological phenotypes match microsite community structure.

Despite the added information that genetic markers have provided for hybrid swarm analysis, they have yet to be employed to confirm the identity of putative natural hybrids in a physiological study. Therefore, we returned to the putative *Arctostaphylos* hybrid swarm analyzed physiologically by BALL *et al.* (1983); we electrophoretically analyzed this population to test for evidence of hybridization and introgression. Specifically, we addressed the following questions:

(1) Has any interspecific mating occurred in this population? If so, what are the relative frequencies of hybrids, segregants, and introgressants?

(2) How closely do genetic markers match morphological characters?

(3) Do the genetic, morphological, and physiological patterns in this population support Anderson's prediction that most interspecific gene flow of ecologically differentiated species is mediated by introgression?

2. – MATERIALS AND METHODS

2.1. THE TAXA

The two co-occuring taxa studied are generally discrete, both ecologically and morphologically. *Arctostaphylos viscida* Parry ssp. *mariposa* (Dudley) Wells is a glandular, glaucous, gray-leaved shrub of chaparral; *A. patula* Greene is a glabrous, green-leaved shrub of montane forest at higher elevations. The species are also somewhat reproductively isolated by phenology; *A. viscida* flowers earlier than *A. patula*, overlapping only in April (MUNZ, 1959). The two taxa have overlapping distributions at about 1,400 m in the Californian Sierra Nevada; *A. viscida* occurs in generally more open, xeric sites, and *A. patula*, in more shaded, mesic sites. Studies of morphological characters have implicated hybridization and introgression of these taxa in that zone of overlap (EPLING, 1947; DOBZHANSKY, 1952).

2.2. STUDY AREA

Our study sites involved one such putative hybrid swarm (Inspiration Point) as well as nearby isolated populations of *A. viscida* (lower in elevation than the swarm) and of *A. patula* (higher in elevation than the swarm) that show no evidence of introgression to serve as controls.

2.3. COLLECTION OF MATERIAL FOR ANALYSIS

All three sites were visited on October 23, 1982. At the Inspiration Point study site, we collected a branch from each of 56 plants to be scored for the species identity index. In some cases, we found plants marked with permanent tags from the original ecophysiological study of BALL *et al.* (1983); unfortunately, not all of these plants could be relocated. Plants were sampled from the full range of microhabitats that were available. To compare the morphological differences of *A. viscida* and *A. patula*, 9 specimens of the former and 15 of the latter were collected from nearly allopatric sites.

2.4. ANALYSIS OF *Arctostaphylos* MORPHOLOGY

We used the same eight morphological characters that made up the "vegetative component" of the species identity index in the previous ecophysiological study (BALL *et al.*, 1983): petiole length, leaf length, leaf width, leaf thickness, leaf margin, leaf color, leaf indument, and branch indument. Because plants were sampled in the fall, we did not attempt to score reproductive characters. The first four quantitative characters were normalized on a scale from 0 to 1, with "pure" *A. viscida* characters scored as 0. Similarly, the last four qualitative characters were measured on a meristic scale from 0 to 1. The species identity scored for each individual was computed by summing the eight normalized scores and dividing by eigth.

2.5. ANALYSIS OF *Arctostaphylos* ALLOZYMES

Collected branches were kept refrigerated at 4°C until leaves could be analyzed for their allozyme genotype. Starch gel electrophoresis was performed on extracts of mature leaves. A single mature leaf from each each branch was first frozen with nitrogen and then ground to a fine powder with a mortar and pestle. Enough extraction buffer (MITTON *et al.*, 1979) was added to the frozen powder to produce a slurry. The extract was absorbed onto 3×6 mm strips of chromatography paper (Whatman #3 MM) which were placed in a vertical slot cut in 12% electrostarch gels. Electrophoresis was performed horizontally with ice on the gels to prevent overheating and was terminated after about four hours when the discontinuous front had migrated anodally 5 cm. At this time, the gels were sliced horizontally to produce several gel sheets that could be stained separately.

Five enzyme systems were assayed in the gels of two buffer systems: Glutamate oxaloacetate transaminase (GOT), phosphoglucomutase (PGM), and triose phosphate isomerase (TPI) in GOTTLIEB's (1981 *a*) buffer system I, and alcohol dehydrogenase (ADH) and phosphoglucoisomerase (PGI) in the same buffer system with the gel buffer titrated to pH 7.3 with HCl. Stains for enzymes were standard (WENDEL & STUBER, 1984). Allozyme patterns were the typical zymograms for these particular enzymes whose inheritance has been well-documented (GOTTLIEB, 1981 *b*; TANKSLEY & ORTON, 1983); therefore, we interpret them as simple, co-dominant genetic markers.

3. — RESULTS

3.1. MORPHOLOGICAL CLASSIFICATION OF *Arctostaphylos* INDIVIDUALS

Species identity scores, calculated from the eight vegetative characters, separated the *A. viscida* and *A. patula* plants sampled in the allopatric stands (fig. 1 A). *Arctostaphylos viscida* plants from a low elevation stand were typically glaucous with whitish-gray leaves having acute apices; their species identity scores ranged from 0.20 to 0.34. *A. patula* plants from a high elevation stand were typically glabrous with green rounded leaves with species identity scores from 0.79 to 0.98 (fig. 1 B). The mixed Inspiration Point population, however, had scores which ranged from 0.03 to 0.79 (fig. 1 B). Some of these plants were very similar to *A. viscida*; others were similar to *A. patula*. However, many plants had species

identity scores between those of the two allopatric species. We refer to plants with species identity scores between 0.35 and 0.75 as morphologically intermediate; 32 of the 56 (57%) plants had such a score. The distributions of species identity scores calculated solely for the vegetative characters closely followed those previously calculated for these populations using a composite index involving both vegetative and reproductive characters (BALL et al., 1983).

3.2. ALLOZYME VARIATION IN THE THREE *Arctostaphylos* POPULATIONS

Allozyme analysis uncovered variation in all six enzyme loci, a total of 27 electromorphs. However, the electromorph frequencies varied considerably over the three populations (table I). Both control populations showed a considerable number of species-specific electromorphs, 15 in the case of *A. viscida*, 4 for *A. patula*. Only five alleles are shared by both species. Three electromorphs were found exclusively in the Inspiration Point population, but these are very rare (all <0.05).

Inspection of the allozyme genotype (zymotype) of individuals at the Inspiration Point population allows assigment of individuals to six different categories:

(1) "Viscida" (coded V), not distinguishable from *A. viscida*;

(2) "Patula" (coded P), not distinguishable from *A. patula*;

(3) "Hybrid" (coded H), not distinguishable from the expected pattern for an F_1;

(4) "Patula-introgressant" (coded PI) not distinguishable from *A. patula* except that the plant is *heterozygous* for one or two viscida-specific electromorphs;

(5) "Viscida-introgressant" (coded VI) not distinguishable from *A. viscida* except that the plant is *heterozygous* for one or two patula-specific electromorphs, and

(6) "Segregant" (coded S) at least one locus is homozygous for patula-specific electromorphs and at least one locus is homozygous for viscida-specific electromorphs.

Those rare electromorphs found only at Inspiration Point were scored as "species-neutral," as if they were shared by both parents. The above six categories embrace all of the zymotypes encountered; no other zymotypes were observed.

Our assignment method is biased towards assigning parental and hybrid types at the expense of introgressants and segregants. Because not every electromorph is diagnostic and because we have subsampled only a fraction of the total loci, the P and V types may include introgressants and segregants, the H types may include introgressants and segregants, and the PI and VI types may include segregants. But, if hybridization, introgression, and segregation are common, then these will be detected. Many of the individuals sampled had both patula-specific and viscida-specific electromorphs (table II); nonetheless, 64% of the individuals sampled could not be distinguished electrophoretically from the parental taxa.

3.3. RELATIONSHIP OF ZYMOTYPE TO SPECIES IDENTITY INDEX

The distribution of zymotypes versus species identity indices are displayed graphically in figure 2. The mean species identity index for each zymotype is given in table II. Not surprisingly, every plant with an H or S zymotype was
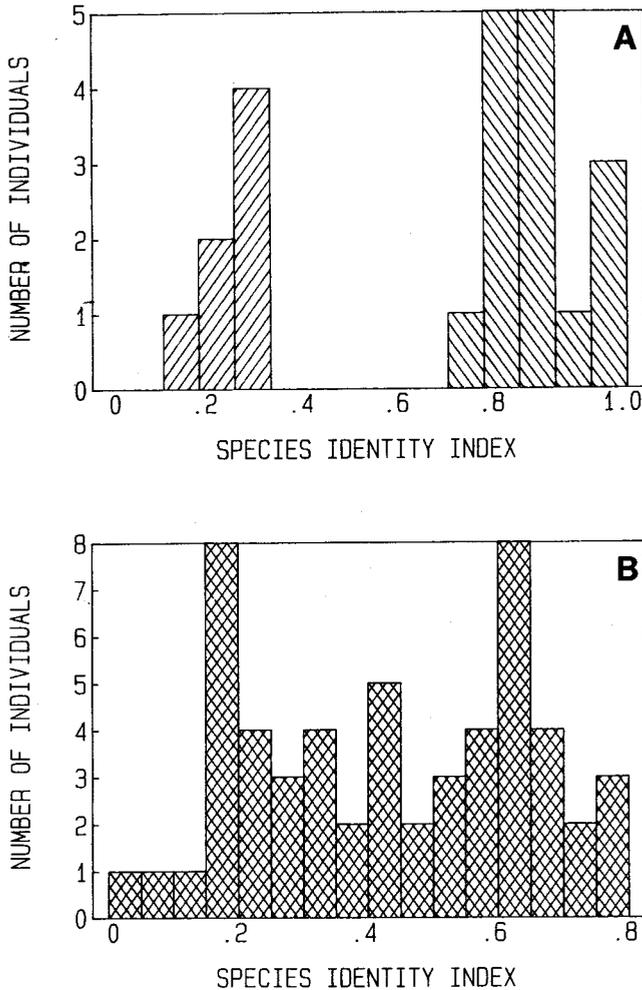
FIG. 1. — The species identity index is an index calculated from vegetative characters. (A) contrasts scores for specimens of *A. viscida* and *A. patula*. (B) is the frequency distribution for such scores at Inspiration Point.

morphologically intermediate (see above). Only four of the plants with V zymotypes (22%) had a morphologically intermediate score: two of those with VI zymotypes (40%) had such a score. Almost all of the non-intermediates bearing these zymotypes had a morphology more or less typical of *A. viscida*; only one plant was morphologically similar to *A. patula*. In contrast, most of the plants with P (77%) and PI (70%) had intermediate scores. Surprisingly, of the seven non-intermediates bearing these zymotypes, five were morphologically similar to *A. viscida*.

Are these zymotype classes morphologically different from each other? The V and P classes were found to have significantly different species identity indices

TABLE I. — *Electromorph frequencies in* 3 Arctostaphylos *populations.*

Locus	Electro-morph	A. viscida	A. patula	"Hybrid swarm"
ADH	1	–	0.27 ([b])	0.15
	2	–	0.73 ([b])	0.50
	3	0.11 ([a])	–	0.04
	4	0.78 ([a])	–	0.31
	5	0.06 ([a])	–	–
	6	0.06 ([a])	–	–
GOT	1	0.28 ([a])	–	0.07
	2	0.28	1.00	0.69
	3	0.22 ([a])	–	0.19
	4	0.22 ([a])	–	0.05
PGI	1	0.06 ([a])	–	–
	2	0.50 ([a])	–	0.22
	3	0.22	0.03	0.16
	4	0.17	0.97	0.60
	5	0.06 ([a])	–	0.02
PGM-1	1	–	0.43 ([b])	0.12
	2	0.44	0.57	0.51
	3	0.56 ([a])	–	0.36
	4	–	–	0.01 ([c])
PGM-2	1	1.00 ([a])	–	0.45
	2	–	1.00 ([b])	0.51
	3	–	–	0.04 ([c])
TPI	1	0.17	1.00	0.60
	2	0.28 ([a])	–	0.13
	3	0.11 ([a])	–	0.04
	4	0.44 ([a])	–	0.21
	5	–	–	0.02 ([c])

([a]) "Viscida-specific".
([b]) "Patula-specific".
([c]) Found only in hybrid swarm.

TABLE II. — *Zymotype frequencies and species identity indices
for* 56 Arctostaphylos *individuals at Inspiration Point.*

Zymotype ([a])	Code ([a])	Frequency (n)	Mean Species identity index
Patula	P	0.32(18)	0.53 ([b])
Viscida	V	0.32(18)	0.28 ([b])
Hybrid	H	0.05 (3)	0.47
Patula-introgressant	PI	0.18(10)	0.54
Viscida-introgressant	VI	0.09 (5)	0.33
Segregant	S	0.04 (2)	0.56

([a]) Defined in text.
([b]) Means of species identity indices of these groups are significantly different (P < 0.05; GT2 method of comparing pairs of means; SOKAL & ROHLF, 1981).
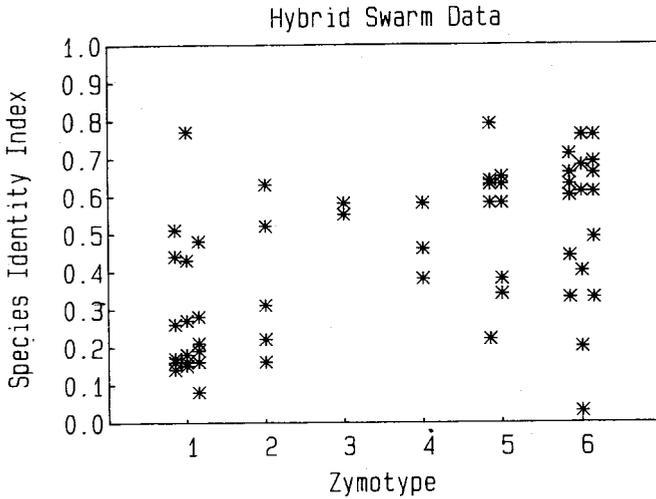
FIG. 2. — Species identity scores for plants of different zymotypes at Inspiration Point.
Zymotype codes: 1 = V, 2 = VI, 3 = S, 4 = H, 5 = PI, 6 = P.

(GT2-method; $P < 0.05$; see SOKAL & ROHLF, 1981; p. 248-250); no other differences were detected, possibly due to the very small sample sizes (GT2-method; $P > 0.05$). If morphologies are primarily genetically based, then species identity scores should increase from viscida-like zymotypes to patula-like zymotypes. We found that a significant increase in the index correlates with the following zymotype ranking: V < VI < H + S < PI < P (Spearman rank test; $r_s = 0.559$; $P < 0.01$; one-tailed test).

4. — DISCUSSION

Electrophoretic analysis of 56 *Arctostaphylos* individuals at Inspiration Point confirms the assertion, previously based on morphology, physiology, and ecology (EPLING, 1947; DOBZHANSKY, 1953; BALL *et al.*, 1983), that the population is a hybrid swarm. Many of the individuals in the population have species-specific electromorphs for both *A. patula* and *A. viscida*. Most of these individuals were indistinguishable from introgressants, those progeny produced by backcrossing of a hybrid to one of its parents. Surprisingly, only three plants had the zymotypes of true hybrids. This number may be an overestimate since certain introgressants and segregants could have the same zymotype at the few loci assayed.

The limited number of loci analyzed and the few patula-specific alleles makes detailed interpretation of our data more difficult. Individuals assigned to V or P may include some segregants and introgressants. Individuals assigned to VI or PI may include segregants. If we consider our two loci that are absolutely diagnostic, half of the first-generation introgressants would be misscored as V or P;

furthermore, half of the first-generation segregants would be misscored as introgressants, 25% as hybrids, and one-eigth as pure species. The fact that certain species-specific alleles occur at four other loci reduces the likelihood of misscores.

However, the relative abundance of introgressant zymotypes and the extreme dearth of hybrid and segregant zymotypes supports ANDERSON's (1948, 1949) contention that if interspecific gene flow is to occur among ecologically differentiated species, it will occur through successful introgressants rather than hybrids and segregants. Similar electrophoretic analyses of putative hybrid swarms have uncovered extensive introgression in *Sabatia* and *Cirsium* populations (BELL & LESTER, 1978; WELLS, 1983). On the other hand, genetic analyses of *Phlox, Carduus*, and *Quercus* populations have confirmed hybridization but demonstrated interspecific gene flow to be very limited (LEVIN, 1975; OLIVIERI, 1985; NASON, in preparation).

Our data demonstrate that interspecific gene flow has occurred at Inspiration Point and that some differences in plant morphology are at least partially genetically-based. BALL *et al.* (1983) measured the microhabitat and physiology of these plants. These two studies make up the first multidisciplinary examination of hybrid swarm capable of testing ANDERSON's (1948, 1949) hypothesis that interspecific gene flow among ecologically differentiated species meeting at an ecotone will be mediated primarily by introgressants ecophysiologically similar to the parental species, rather than by intermediate hybrids and segregants. BALL *et al.* (1983) showed that the environment was clearly ecotonal, from very xeric to very mesic sites. However, individuals of intermediate morphology sampled from north-facing slopes were physiologically very similar to *A. viscida*, while those sampled from protected areas were physiologically very similar to *A. patula*. Data from both studies confirms ANDERSON's hypothesis. Hybridization has occurred, followed by considerable introgression resulting in individuals of intermediate morphology that are physiologically very similar to one of the two parental types. The potential for interspecific gene flow in such a population will depend on how many successul introgressants are present. Apparently, the long-term opportunity for interspecific gene exchange at this site is considerable.

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