INCONGRUITY IN THE PROGENY OF INTERSPECIFIC CROSSES OF *VITIS*

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ABSTRACT

Three classes of hybrids involving *Vitis riparia* Michx. were evaluated for evidence of incongruity, a phenomenon reflecting passive evolutionary divergence between the genomes of the mating partners. Each class consisted of families derived from four maternal clones of *V. riparia*. The R x V class (*Vitis riparia* x *V. vinifera* L.) was the most divergent, and the least divergent was the intraspecific R x R class (*V. riparia* x *V. riparia*). The R x FH class (*V. riparia* x French Hybrid) was derived from multiple species and was highly intercrossed. Reproductive dysfunction was detected in the F1 generation. The R x R class displayed the highest percent berry set and the largest mean number of seeds per berry, while the R x V class manifested reduced berry set and reduced mean number of seeds per berry. The intercrossed R x FH class exhibited intermediate berry set and numbers of seeds per berry. Differences in percent berry set were found to be related to percent aborted flowers, but not to percent shot (aborted) berries. Germination was similar among the classes. Reproductive efficiency was calculated by multiplying mean number of seeds per berry by percent berry set, and differences among classes were maintained. The F2 generation was evaluated in the seedling stage for morphological aberrations. Abnormalities observed included dwarfing, achlorophyllic variegation, deformed leaves, yellow leaf mottling, sparse roots, and enation. Pooled results indicated incongruity was present at the greatest levels in the most divergent, R x V class, in the lowest levels in the R x R class, and at intermediate levels in the R X FH class. When incongruity was studied as a syndrome of multiple abnormalities expressed per individual, differences between classes were heightened. A congruity index, the product of reproductive efficiency times the frequency of F2 plants with 0-1 abnormalities, was calculated for several crosses, and proposed as a tool to compare breeding potential for specific mating partners.

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GENERAL INTRODUCTION

GENE EXCHANGE IN CROP IMPROVEMENT: INTROGRESSION AND INTERSPECIFIC HYBRIDIZATION

Successful gene exchange in crop improvement depends on recombination of traits expressed in the parental individuals. Commonly used methods of plant breeding rely on successful transfer of donor genes into progeny (Allard 1960, Fehr 1987). The transfer of genes from one entity to another is referred to as introgression (Anderson 1949). In intraspecific crosses, within adapted germplasm, it is often possible to recover an economically useful type in the progeny after recombining genes. The primary barrier to introgression in such crosses is linkage, and genetic gain is usually possible within a limited number of generations.

Interspecific hybridization has been used in many crops in an attempt to introgress genes from one species, sometimes a wild relative, into the background of another species, usually cultivated or more desirable. Problems involved in interspecific hybridization have limited its use in many crops, but in ornamental crops such as *Lilium*, interspecific hybridization has been useful in producing novel types (van Tuyl, et al. 1986). In some genera of fruit crops, such as *Rubus* and *Vaccinium*, interspecific hybridization has been used extensively, while in others, such as *Malus* and *Pyrus*, it has only recently become a prominent method for crop improvement (Scorza 1986).

CONCEPT OF SPECIES AND MODES OF REPRODUCTIVE ISOLATION

The concept of species in plants is not rigid. Entities classified as species may be in various stages of evolutionary divergence from other species in the genus or from subspecies or from other genera. Entities that have been classified as species may be reclassified as subspecies and vice versa; species may be reclassified as genera, or genera merged. In general, a species is composed of a population of freely interbreeding individuals, sharing similar morphological, physiological, ecological, cytogenetic, and genetic characteristics. Species are often reproductively isolated from one another, reinforcing species identity. Revised taxonomic classifications are based on increased knowledge about the phylogenetic and evolutionary relationships between the biological entities in question. Thus, a discussion of interspecific hybridization must concede that any two species to be hybridized may be at one of a number of stages of differentiation. Such differentiation determines reproductive or genetic isolation by several mechanisms (Clausen 1951): 1. Ecological barriers- physiological differentiation adapted to ecological habitat. 2. Morphological or physical barriers- may be due to adaptation to pollinator species. Genetic barriers do not exist, but entities maintained by constant selection via pollinator preference. Selection prevents hybrid swarms from migrating out of areas of overlapping pollinators. 3. Cytogenetic barriers- differentiation in chromosome number or repatterning, resulting in impaired products of meiosis. 4. Genetic barriers- morphological and cytological similarity, and adaptability to similar habitats, but with genetic barriers preventing hybridization or survival of hybrid entities. Most commonly, sets of related entities develop gradual genetic and morphological differentiation, with small steps of genetic, chromosomal, and morphological changes (Clausen 1951).

REPRODUCTIVE BARRIERS AND THEIR IMPLICATIONS FOR GENE EXCHANGE

Reproductive barriers, or isolating mechanisms, that limit the use of interspecific hybridization have been classified as premating and postmating (Levin 1971). Premating mechanisms include habitat differences, differences in time of flowering, and mechanical barriers such as structural differences in floral morphology that preclude cross pollination by specialized pollinators. Postmating isolating mechanisms are further divided into prezygotic and postzygotic barriers (Levin 1971). Prezygotic barriers, such as autogamy and pollen-pistil incompatibility, prevent interspecific fertilization and zygote formation. When prezygotic barriers do not limit the cross, postzygotic barriers may restrict successful gene exchange. The zygotes from crosses with postzygotic barriers may be manifested as inviable seed. Viable seed may give rise to weak F1 individuals or develop into vigorous F1 individuals. The F1 hybrids that survive to sexual maturity may display varying degrees of sterility. Postzygotic phenomena can extend into subsequent generations, where it is often referred to as hybrid breakdown (Levin 1971, Stebbins 1971). Hybrid breakdown in advanced generations is manifested as weak, abnormal, or sterile progeny.

Many terms have been used in describing phenomena that result from both prezygotic and postzygotic barriers. Genic disharmony (Stebbins 1971), interspecific sterility (Clausen 1951), interspecific cross incompatibility (Hadley & Openshaw 1980), hybrid inviability and weakness (Stebbins 1958 1971), hybrid breakdown (Stebbins 1958 1971, Levin 1971, Hadley & Openshaw 1980), and cryptic structural hybridity (Stebbins 1950) are some of the many terms that have been used to describe phenomena at various stages of dysfunction in a wide hybrid. Causes invoked for these phenomena include lack of chromosome homology (Zohary 1973), interaction between nucleus and cytoplasm, structural cytogenetic differences between parents, cryptic meiotic abnormalities, and incompatibility between embryo and endosperm (Stebbins 1950, 1958).

INCONGRUITY

Reproductive isolation between taxa, whether in sympatric species or in allopatric species can lead to divergence in the genomes (Clausen 1951, Stebbins 1971) which can result in incongruity (Hogenboom 1973, 1984). Incongruity is a term that was used initially by Hogenboom (1973) to describe pollen-pistil dysfunction sometimes observed in wide crosses. Later, the definition was extended to include intracellular as well as intercellular interactions (Hogenboom 1984). Genetic incongruity is a passive phenomenon which is displayed when sufficient evolutionary divergence has occurred between two taxa such that, when brought together as parents in a wide hybrid, some of the genes mismatch, and are unable to function efficiently, if at all. Selection does not operate to maintain incongruity, but it does operate to favor the less affected individuals that may be more vigorous and fertile. The stage of development at which dysfunction occurs may vary according to which genes have been affected during evolutionary divergence. Dysfunction may occur prezygotically, preventing fertilization, as in some species of *Papaver* (McNaughton & Harper 1960, Ojala & Rousi 1986, Ojala, et al 1990), *Carica* (Manshardt & Wenslaff 1989a 1989b), and *Pennisetum* (Mohindra & Minocha 1991). Dysfunction may also occur postzygotically, affecting the F1 hybrid generation, as in species of *Datura* (Satina, et al 1950), *Gossypium* (Phillips & Merritt 1972, Phillips & Reid 1975, Phillips 1976), *Cucurbita* (Whitaker & Bemis 1964), and *Amaranthus* (Pal & Khoshoo 1972) or influencing advanced generations, as in species of *Bromus* (Nielsen, et al 1962), *Lycopersicon* (Martin 1966), *Amaranthus* (Pal & Khoshoo 1972), and *Triticum* (Hermsen 1963, Zeven 1970).

Prezygotic dysfunctions may be expressed as cross sterility, or reduced fertility, while postzygotic dysfunctions may be expressed as embryo death, endosperm dysfunction or seed failure of unknown causes, slow seedling growth or seedling mortality, unusual susceptibility to disease, abnormal organ development, chlorophyll abnormalities, tumors (cellular proliferations), lack of flowering, sterility without apparent cause, or other dysfunctions or abnormal developmental patterns. (Stebbins 1958, Hogenboom 1984).

Thus, the term incongruity encompasses many of the reproductive isolating mechanisms and barriers, unifying a body of literature with often-confusing terminology.

AMELIORATING INCONGRUITY

Several researchers have described methods of overcoming interspecific barriers. Viehmeyer (1958) reported the creation of a panmictic multispecies gene pool in *Penstemon*. Reproductive isolation was effectively reversed by repeated intercrossing among interspecific hybrids. Rhodes (1959) combined five species of *Cucurbita* germplasm into one common gene pool by backcrossing, intercrossing and outcrossing. This gene pool became a bridging population for interspecific gene transfer. Haghighi and Ascher (1988) demonstrated a return to fertility after repeated backcrossing to alternating parents (congruity backcrossing) for eight generations, using *Phaseolus vulgaris* and *P. acutifolius*. In these instances, amelioration of incongruity was achieved by complex interspecific hybridization procedures that brought genomes of several species together or brought them together repeatedly.

INTRODUCTION TO GRAPES

The domesticated grape, *Vitis vinifera* L., is perhaps the most economically important fruit crop in the world (Einset & Pratt 1975). The fruit is consumed fresh, as well as processed in the form of raisins, juice, jelly and wine. Many of the wine-grape cultivars grown today are thought to be ancient, while the majority of table grape cultivars were developed in the 20th century (Einset & Pratt 1975). Wild members of the genus *Vitis* are found in most parts of the world, varying in their allotment of economic traits. Many show disease resistance, insect resistance, cold hardiness, or other traits which are lacking or limited in the domesticated *Vitis vinifera*. Rarely do the wild *Vitis* species show the desirable fruit characteristics that are found in *Vitis vinifera*. Cultivars with a combination of quality fruit characteristics, disease and insect resistance, and cold hardiness would enable the industry to expand into currently marginal growing regions.

Many vineyards consist of vines grafted onto resistant rootstocks to avoid damage by the insect pest, phylloxera (*Daktulosphaira vitifoliae* Fitch), and the use of resistant cultivars would eliminate the labor intensive grafting step. Virtually all commercial vineyards use fungicide sprays to reduce the effects of powdery mildew (*Uncinula necator* (Schw.) Burr), downy mildew (*Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni) and botrytis (*Botrytis cinerea* Pers.). Incorporation of resistance to some or all of these diseases would reduce the number and frequency of fungicide-spray events, affecting not only labor and materials costs, but ecological and possible health costs as well. However, the incorporation of resistance and hardiness traits into breeding lines while maintaining quality fruit characteristics has met with variable success.

TAXONOMY, CYTOGENETICS, AND PLOIDY LEVEL

Grapes are placed in the family Vitaceae, the genus *Vitis* L. *Vitis* is further subdivided into subgenera: *Vitis* Moore (*Euvitis* Planch). and *Muscadinia* Planch. *Vitis* is characterized by a haploid chromosome number of 19, while the haploid number for *Muscadinia* is 20 (Einset & Pratt 1975, Olmo 1976). It is thought that the genus is an ancient allopolyploid (Olmo, 1976). Meiotic studies in the F1 hybrid resulting from a cross between subgenus *Vitis* and *Muscadinia* indicate that 13 homeologous pairs are formed. The remaining chromosomes, 6 from *Vitis* and 7 from *Muscadinia*, are unable to pair (Patel & Olmo 1955). Thus, the ancient haploid numbers that comprise *Muscadinia* are (6+7)+7=20, while those that comprise *Vitis* are (6+7)+6=19. Diploidization has occurred in both subgenera, however, resulting in regular bivalent pairing (Olmo, 1976). *Vitis vinifera* and the other species of *Vitis* dealt with in this study belong to the subgenus *Vitis* (*Euvitis).* *Vitis vinifera* is a member of the series Viniferae Munson, while the American species used in this study are members of the series Aestivales Planch., Cinerescentes Planch., Labruscae Planch., and Ripariae Munson. *Vitis aestivalis* Michx. and its botanical variety *V. aestivalis* var. *lincecumii* (*V. lincecumii* Buckl.) are included in series Aestivales. Series Cinerescentes contains *V. cinerea* Engelm. and *V. berlandieri* Planch. Series Labruscae consists of *V. labrusca* L., while *V. riparia* Michx. and *V. rupestris* Scheele. are members of series Ripariae (Munson 1909, Moore 1991).

REPRODUCTIVE BIOLOGY: FLORAL AND SEED CHARACTERISTICS

Grape flowers are borne in clusters of compound panicles (Morley 1969). In the wild, *Vitis* species are dioecious, bearing male and female flowers on separate plants, with extremely rare hermaphroditic mutations (Munson 1909). The flowers are 5-merous, and the fruit is a berry (Morley 1969, Einset & Pratt 1975, Galet

1979). The ovary is composed of two fused carpels, forming two locules, each containing two ovules (Morley 1969, Einset & Pratt 1975). One to four seeds per fruit are common (Morley 1969).

CENTER OF ORIGIN AND EVOLUTIONARY HISTORY

The center of origin for grapes is thought to be in the Trans-Caucasian mountain region in central Eurasia, close to the centers of origin for many of our crop plants (Einset & Pratt 1975, Olmo 1976). The earliest fossil remains are of the fossil species, *Vitis sezannensis*, from the Lower Eocene Epoch, an era when large mammals were emerging as the dominant animal type (Hyams 1965). As the Northern Hemisphere became warmer, the vines retreated to the far north, and when the land masses moved apart, *Vitis* was split into two races: the American race and the Eurasian race. The races responded to local environmental pressures, and differentiation between the races developed (Hyams 1965). A series of changes evolved, some of which are documented in fossil remains, and by the end of the Pliocene Epoch, when man was known as *Homo pekinensis*, *Vitis vinifera*, the cultivated grape, emerged more or less as we know it (Hyams 1965).

HISTORY OF GRAPES AS A CROP

Grapes have had an association with mankind since the beginnings of oral and written history. In the Bible (Genesis 9:20-21) we are told that Noah planted grapevines as one of his first actions once the flood was over, and subsequently got drunk on the wine. Grapes were an integral part of Greek mythology: the goddess Hera wore a crown made of grapevines, and Dionysus, known to the Romans as Bacchus, was the god of wine. Wine became an integral part of ritual for many of the major religions of the world (Hyams 1965). Culture, mystique, as well as industry developed around this ancient fruit. The origins of many of the cultivars we still grow today were never recorded, and can be guessed to be hundreds of years old, or even older (Hyams 1965, Einset & Pratt 1975).

Most cultivars, since the beginning of recorded history, have been hermaphroditic. It is likely that early viticulturists recognized and selected hermaphroditic mutations for their production value, as vineyard space need not be allotted for a pollenizer to effect berry set on the fruiting vines (Hyams 1965, Olmo 1976). Quality fruit traits such as large berries, large, compact clusters, delicate flavors, and high sugar content were also selected by early viticulturists. Desirable genotypes were multiplied and maintained asexually, by cuttings (Hyams 1965, Olmo 1976).

ECONOMICALLY IMPORTANT SPECIES OF VITIS AND THEIR CHARACTERISTICS

Until the seventeenth century, *Vitis vinifera* was the only species of economic consequence in the world, and it remained the only species of any major significance until the nineteenth century. Thereafter, several American species assumed importance, as sources of disease and insect resistance in breeding and as rootstocks:

Vitis rupestris, V. aestivalis, V. berlandieri, V. labrusca, and *V. riparia*. (Barrett 1958a 1958b, Einset & Pratt 1975, Galet 1979). American species of grapes evolved in isolation from the European species. Subjected to pests, diseases, soil conditions and climatic conditions that *Vitis vinifera* never encountered, the American species developed resistances to downy mildew and powdery mildew, to the insect phylloxera, and in some species, to high lime soils or to extreme cold. The fruit quality also evolved differently from *V. vinifera*. Most of the American species retained small, loose clusters, with small, usually black, berries and variable, often unpleasant, flavors (Barrett 1958a 1958b, Hyams 1965, Galet 1979).

NATURAL HYBRIDS

Species of *Vitis*, subgenus *Vitis* have been thought to be completely interfertile (Einset & Pratt 1975). Natural hybridization among wild species with overlapping ranges has been thought to occur frequently (Munson 1909, Einset & Pratt 1975). However, a recent study by Comeaux, et al (1987) found very few natural hybrids among *Vitis* species in North Carolina. Vast variation within species was clinal (Comeaux, et al 1987). The early American cultivars such as 'Catawba' and 'Isabella' are thought to be natural interspecific hybrids between cultivated *Vitis vinifera* and wild *V. labrusca* (Munson 1909, Barrett 1958a 1958b, Einset & Pratt 1975, Galet 1979).

EARLY HYBRIDS IN AMERICA

The first deliberate attempts at hybridization of *V. vinifera* with American species were carried out on the American continent (Hyams 1965, Galet 1979). European grapes in America were attacked by phylloxera as well as by powdery and downy mildew (Barrett 1958a 1958b, Hyams 1965, Galet 1979). The American species did not suffer destruction from these pests as *V. vinifera* did, and efforts were made to hybridize the species in hopes of developing high quality, pest resistant progeny (Barrett 1958a 1958b, Hyams 1965, Galet 1979). Some early hybrids with *V. labrusca*, such as 'Catawba', 'Delaware', and 'Isabella', were grown widely in the United States for their dessert and winemaking qualities (Galet 1979).

FRENCH HYBRIDS

When the mildew diseases and phylloxera were inadvertently carried back to Europe, they attacked the susceptible European vineyards, threatening destruction of the grape and wine industry. Private European growers began active interspecific breeding programs using primarily *Vitis rupestris*, *V. aestivalis*, *V. berlandieri*, *V. labrusca*, and *V. riparia* for sources of resistance. The hybrids that resulted from their breeding efforts are referred to as Direct Producers or French Hybrids (Barrett 1958a 1958b, Einset & Pratt 1975, Galet 1979).

EVIDENCE OF INCONGRUITY IN INTERSPECIFIC HYBRIDS OF GRAPES

Documentation concerning the fertility of the initial interspecific crosses is rare, but there is evidence that aberrant or off-type seedlings appeared in some of the F1 hybrid progenies and their offspring (Barrett 1958a 1958b). The aberrant seedlings were culled in favor of individuals with more normal appearance. Through subsequent crosses and complex intercrosses, breeding sometimes to a *V. vinifera* parent, sometimes to an American species, and sometimes among the interspecific hybrids, the number of aberrant seedlings declined and some relatively successful cultivars emerged (Barrett 1958a 1958b).

ATTRIBUTES OF *VITIS RIPARIA*, A SOURCE OF COLD HARDY GERMPLASM

Vitis riparia, or Riverbank Grape, is the major grape native to Minnesota, its natural range extending through much of the Midwest and eastern United States (Pierquet & Stushnoff 1978, Swenson 1985). It is extremely cold hardy, surviving the severe winters of the Upper Midwest, and portions of Canada (Munson 1909, Einset & Pratt 1975, Pierquet & Stushnoff 1978, Galet 1979). Plants are dioecious, female clusters bearing reflexed stamens with functionally sterile or no pollen. Male flowers have vestigial or absent pistils (Munson 1909, Morley 1969, Remaily 1987). Berry color is blue-black with uncommon white mutation (Munson 1909, Swenson 1965). The desirable traits that *V. riparia* carries are cold hardiness, resistance to the root form of phylloxera, ease of growth (rooting) from cuttings, ease of grafting, and powdery and downy mildew resistance in some clones (Munson 1909, Pierquet & Stushnoff 1978, Galet 1979). Bloom is earlier than other species in overlapping ranges, similar to *V. rupestris* (Munson 1909), presenting a temporal barrier to hybridization with later-blooming species.

Its negative characteristics include small berry size and cluster size. Berries are highly acid and variable in levels of soluble solids and anthocyanin pigments. (Munson 1909, Pierquet & Stushnoff 1978, Moore 1986, Remaily 1987).

BREEDING HISTORY USING VITIS RIPARIA:

In the nineteenth century, two breeders, Charles Arnold of Canada and Louis Suelter of Minnesota independently demonstrated the value of using *V. riparia* when breeding for cold hardiness (Moore 1986). Suelter developed the cultivar, 'Beta', which was one of four named cultivars from a cross of a white-berried mutant *V. riparia* with 'Concord' (Munson 1909, Swenson 1985, Moore 1986), a *V. labrusca* seedling (Munson, 1909). 'Beta' and its offspring have been used extensively in subsequent breeding programs (Swenson 1985, Moore 1986). In the twentieth century, Elmer Swenson, a Wisconsin grape breeder, began working with crosses using *Vitis riparia* and the *V. riparia*-based clones derived from 'Beta' as a source of cold hardiness (Swenson 1985). Swenson's successful varieties have resulted from unrelated intercrosses of interspecific hybrids with *V. riparia* ancestry (Swenson 1985). Problems in breeding included reduced berry (and therefore seed) set, and aberrant seedlings (Swenson 1985, personal communication). Similar problems had been reported before in the production of French Hybrids (Barrett 1958a 1958b). As in the development of the French Hybrids, the types of crosses where abnormalities were seen and the frequencies of aberrant seedlings were not documented.

OBJECTIVE AND RATIONALE

The millenia of isolation of *Vitis vinifera* from the American species of *Vitis* may have led to the evolution of passive crossing barriers manifested as incongruity in the interspecific crosses. As a result of this incongruity, progeny sizes would be reduced, restricting opportunities for selection. Although useful progeny sizes can be generated by increasing the number of pollinations, progenies may display undesirable characteristics. Incongruity may interfere with recombination of traits, or prevent expression of desired traits. Further studies of these phenomena would be useful as interspecific hybridization is used so widely in *Vitis* improvement.

The purpose of this project was to investigate the apparent barriers to interspecific hybridization encountered in hybrids of *Vitis riparia* crossed to clones with varying degrees of phylogenetic similarity. Passive crossing barriers would be expected to be greater when *V. riparia* is crossed to *V. vinifera* than when crossed to *V. riparia* itself. The greater the number of intercrosses in a hybrid parent involving *V. vinifera*, previous to the use of *V. riparia* as a direct parent, the greater is the opportunity for recombining the incongruent portions of the genome. French Hybrids often have a large number of intercrosses in their pedigrees, and therefore would be expected to show amelioration of symptoms.

The use of multiple intercrosses between interspecific derivatives, as well as backcrosses to both original species parents, may reduce the incongruity effect.

Mitigation of incongruity should offer the opportunity to expand the gene pool available to grape breeders. The expanded germplasm base may provide a means for introducing the cold hardiness of *V. riparia* into cultivars with economic fruit characteristics. This work could be relevant to breeders of other crops whose use of exotic germplasm in breeding has proved disappointing due to loss of desired characters or addition of undesired characters.

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INCONGRUITY IN THE INTERSPECIFIC CROSSES OF *VITIS* L.

Reproductive Expression in the F1 Progeny

For submission to Euphytica

KEY WORDS: Grapes, interspecific barriers, interspecific hybridization, reproductive efficiency

ABSTRACT:

F1 progeny from three classes of crosses were studied for indications of incongruity in the form of reproductive dysfunction. Percent fruit set was found to be the inverse of percent aborted flowers, as no main effects were significant for percent shot berries. In interspecific crosses, barriers were manifested as reduced fruit set and seed number per berry. These two characters were used to create an index of reproductive efficiency. Nine F1s from crosses of the most divergent class, *V. riparia* x *V. vinifera* (R x V) , displayed significantly lower reproductive efficiency, percent fruit set, and seed number per berry. Four female F1s from the least divergent class, *V. riparia* x *V. riparia* (R x R), had the highest reproductive efficiency, percent fruit set and seed number per berry, when pollinated by two *V. riparia* pollen sources. Seventeen F1s from the highly-intercrossed class, *V. riparia* x French Hybrid (R x FH), displayed intermediate levels of reproductive efficiency, percent fruit set, and seed number per berry. The R x FH class involved *V. vinifera*, *V. rupestris*, *V. aestivalis*, *V. labrusca*, *V. berlandieri*, *V. cinerea*, and *V. riparia*. It is proposed that the complex intercrossing in the pedigrees of the R x FH class ameliorated incongruity. In the most divergent class, R x V, progenies of one of the *V. riparia* parents displayed fewer incongruity effects than progenies of the other *V. riparia* parents. Progeny testing of many proposed parental combinations may be used

to uncover useful congruent combinations.

ABBREVIATIONS:

R x FH	*Vitis riparia* x French Hybrid
R x R	*V. riparia* x *V. riparia*
R x V	*V. riparia* x *V. vinifera*

INTRODUCTION:

Interspecific hybridization has been used successfully in many genera to introduce valuable genes into a breeding program. However, due to the differences in genomes of the hybridizing partners, crosses are not always successful and crosses that succeed may produce offspring that do not thrive. Failures due to passive interspecific barriers to hybridization have been termed "incongruity" by Hogenboom (1973 1975 1984). Incongruity may be expressed at prezygotic or postzygotic stages in any generation of a cross. If a wide cross is sufficiently congruent to survive prezygotic barriers, the resulting generations may express a myriad of symptoms created by the lack of congruity between the constitutive genomes. Any system may be affected, as genes for all systems are vulnerable to incongruity (Hogenboom 1984). In the F1 generation, reproductive systems are commonly affected. Reproductive dysfunction may be expressed as reductions in fruit set, seed set, and germination. Of these, reduced fruit set can be further divided to distinguish between times of failure: prefertilization and postfertilization. Prefertilization barriers prevent zygote formation in a pollinated flower, precluding further fruit development signals, and resulting in floral abortion. Postfertilization barriers are inferred when a fruit begins to develop, but is then aborted. Both phenomena contribute to reduced fruit set.

Symptoms appearing in any generation beyond the original cross are considered "postzygotic" regardless of the stage at which incongruity is expressed in that generation (Levin 1971, Stebbins 1971). Morphological floral abnormalities such as reduced or malformed petals and reproductive apparatus have been reported in the interspecific F1 generation of *Papaver* spp. (McNaughton & Harper 1960, Ojala & Rousi 1986), *Amaranthus* spp. (Pal & Khoshoo 1972), and *Cucurbita* spp. (Whitaker & Bemis 1964). Reproductive dysfunction in the form of partial to complete sterility has been reported in the interspecific F1 generation of *Mimulus* spp. (Vickery 1964), *Papaver* spp. (McNaughton & Harper 1960), *Amaranthus* spp. (Pal & Khoshoo 1972), *Phaseolus* spp. (Haghighi & Ascher 1988), *Gossypium* spp. (Stephens 1946), *Iris* spp. (Yabuya 1991), *Allium* spp. (Gonzalez & Ford-Lloyd 1987), and *Carica* spp. (Manshardt & Wenslaff 1989a 1989b).

Various researchers have reported methods to circumvent or alleviate the problems of interspecific crossing barriers. Procedures which bring genomes of several species together, or involve repeated exposure of the alien genomes through alternating, or congruity, backcrossing (Haghighi & Ascher 1988) or through repeated intercrossing have been found to ameliorate incongruity. After eight generations of congruity backcrossing, interspecific crosses of *Phaseolus* had completely regained fertility (Haghighi & Ascher 1988). Repeated intercrossing in *Cucurbita* resulted in improved fertility (Rhodes 1959) and the establishment of a five-species gene pool. Viehmeyer (1958) developed a panmictic interspecific hybrid gene pool in *Penstemon* via complex intercrossing between sections of *Penstemon*. He indicated that alleviation of problems of interspecific hybridization by repeated intercrossing could, in theory, reverse evolution, resulting in the return of widely divergent germplasm to a common gene pool, which could be used as a bridge for divergent taxa in the transfer of valuable traits.

In grapes (*Vitis* spp.), reproductive barriers between species are not complete, and grape breeders frequently use interspecific hybridization (Einset & Pratt 1975). Before the turn of the twentieth century, the American grape pests, phylloxera (*Daktulosphaira vitifoliae* Fitch), powdery mildew (*Uncinula necator* (Schw.) Burr.), and downy mildew (*Plasmopara viticola* (Berk. & Curt.) Berl & de Toni) were introduced into European vineyards, where no resistances existed in *V. vinifera* L. These pests were carried to Europe on specimens brought back from the American continent. Private breeders produced interspecific hybrids using the resistant American species and the high quality *V. vinifera*, attempting to develop resistant cultivars of commercial quality. The hybrids resulting from their efforts have been termed "French Hybrids". Although it was physically possible to produce the interspecific hybrids, the F1's were usually not commercially useful. Most of the interspecific F1 seedling selections were eliminated due to "...low production, insufficient wine quality and small-sized fruit..." (Barrett 1958a 1958b). The next stages of French Hybrid development entailed backcrossing to both the quality parent, *V. vinifera*, and to donor American species, and repeated intercrossing among the better hybrids. Although it was not well documented, progeny from subsequent stages apparently displayed fewer production problems than the F1 generation (Barrett 1958a 1958b). The few pedigrees that are available indicate that the named selections of French Hybrids have backgrounds that include repeated intercrossing and backcrossing involving different clones of *V. vinifera* L., *V. rupestris* Scheele., *V. aestivalis* Michx., *V. labrusca* L., *V. berlandieri* Planch., *V. cinerea* Engelm., and *V. riparia*

Michx. (Barrett 1958a 1958b, Galet 1979).

The North American species, *V. riparia*, has been used extensively as a source of cold hardiness and disease and insect resistance in several breeding programs in cold climates, including the University of Minnesota (Luby 1991). The northern clones display exceptional cold hardiness, far beyond the hardiness shown by *V. vinifera* or the French Hybrids. Like other wild species of grape, *V. riparia* is dioecious, requiring a male clone to provide pollen for the fruit-bearing plants.

Working in Minnesota and Wisconsin, Swenson (1985) indicated that progeny from his early crosses involving *V. riparia* had production problems that were later ameliorated with further intercrossing. To explore the possibility that the reproductive dysfunction alluded to by Barrett and Swenson might be an indication of incongruity, we examined reproductive characters in F1 hybrid individuals in three classes of crosses involving *V. riparia*. Reproductive dysfunction was expected to be lowest in intraspecific crosses, and highest in interspecific crosses. Interspecific crosses involving parents of complex interspecific pedigrees were expected to show intermediate levels of or no dysfunction if repeated intercrossing had indeed ameliorated incongruity.

MATERIALS AND METHODS:

F1 species-hybrids from four female clones of *V. riparia* crossed to *V. vinifera* and to French Hybrids were produced in the late 1970's at the University of Minnesota Horticultural Research Center. Hybrids chosen for use in this study had survived approximately ten years, unprotected, in the research vineyard. Thus, natural selection occurred for fitness and winter survival. Additionally, selection for hermaphroditism was imposed for this study.

Three classes of crosses were surveyed (Table 1): 1. Narrow, or intraspecific *V. riparia* (R x R). 2. Intermediate, or intercrossed interspecific: *V. riparia* x French Hybrid (R x FH). 3. Wide, or first generation interspecific: *V. riparia* x *V. vinifera* (R x V). Four clones of *V. riparia* were chosen as common maternal parents in a series of half-sib families across the three classes. Three of the clones, #s 37, 39, and 64, were collected from the wild in Manitoba, the fourth, # 89, in Minnesota. Two male clones of *V. riparia* were used as pollen sources for the R x R class. One, designated Brandon, was from Manitoba, and the other, designated Farm 2, was from Minnesota. Two cultivars of *V. vinifera* were used: 'Limberger' and 'Carmine'. Five French Hybrid selections were used to produce the intermediate, intercrossed class: SV18-283, SV18-307, S11701, B4672, and L4511. The pedigree for S11701 is not available, but the remaining French Hybrids used in this study had highly intercrossed interspecific backgrounds involving five to seven species and five to seven generations of crossing (Galet 1988). The pedigree for L4511 entails 7 generations of crossing, resulting in the following hypothetical levels of species composition: 4% *V. labrusca*, 1.5% *V. riparia*, 21% *V. rupestris*, 7% *V. aestivalis*, 1.5% *C. cinerea*, 16% *V. berlandieri*, and 49% *V. vinifera* (Galet 1988). SV18-283 and SV18-307, full sibs, were derived from 6 generations of crossing, generating the following postulated levels of species composition: 7% *V. labrusca*, 4% *V. riparia*, 30% *V. rupestris*, 9%

V. aestivalis, 1.5% *V. cinerea*, and 48% *V. vinifera*. B4672 was produced over 5 generations with hypothetical species composition of 25% *V. berlandieri*, 6% *V. rupestris*, 5% *V. aestivalis*, 2% *V. cinerea*, and 63% *V. vinifera*.

Characters evaluated were percent aborted flowers, percent shot berries (aborted fruit), percent berry set, average number of berries per cluster, average seeds per berry, and percent germination. In addition, average seeds per berry was multiplied by percent berry set to calculate average seeds per flower, or reproductive efficiency.

At flowering, hermaphroditic F1 hybrids were covered with pollination bags (#421, Lawson Bags, Northfield, IL). Flowers were allowed to open in the bags and self pollinate. When one or two flowers per cluster opened on the dioecious *V. riparia* clones, the open flowers were removed and the clusters were covered with bags. Two days later, bags were removed for pollination. If most of the flowers on the cluster were not yet open, then a second pollination was performed the following day. Unopened flowers remaining, following the second pollination, were removed. *Vitis riparia* pollen was collected in sterile petri dishes and applied with a finger tip. Fingers were cleaned with 90% ethanol between pollinations. Bags were replaced following pollination. Pollination bags were left on the clusters throughout the growing season, ensuring the retrieval of all flowers and berries on each cluster. At least three clusters per vine were treated.

Bags were collected when grapes were ripe. Aborted flowers, shot (aborted) berries and mature berries were counted. If the ovary had begun to swell, but had not continued to maturity, then it was classified as a shot berry. Total flowers were calculated as the sum of aborted flowers, shot berries, and mature berries. Seeds per berry were counted in a random sample of 20 berries per cluster in 1988, and 10 berries per cluster in 1989. Percent berry set, percent aborted flowers, percent shot berries, and seeds per berry were determined for each vine as a mean of three clusters. Seeds were mechanically scarified during extraction with an electric blender, on pulse. Counted seeds were stratified in moist sand at 3°C for 3.5-4 months and germinated in #50 Pro-trays (TLC Polyform, Plymouth, MN) in Bacto Pro Plant Mix (Michigan Peat Co., Houston, TX). Germination data were available for only 1989 because watering problems in the greenhouse in 1988 caused tray cells to dry out at a critical point. In the 1989 season, trays were kept evenly moist on the greenhouse bench by using Vattex F capillary mats (O.S. Plastics, Norcross, GA). Daylength was extended to 14 hours using 1000-watt metal-halide high intensity discharge (HID) lamps.

Analyses for all characters were executed using PC-SAS (Cary, NC), GLM procedures with type III sums of squares. A factorial analysis of variance was performed on the data for all characters, with year, family and class of cross as main effects. Means of classes of crosses and families were compared using LSD.

RESULTS:

Despite severe drought in 1988, year effects were non-significant for all characters except percent aborted flowers (Table 2). The variance among families was not significant for any character. Percent shot berries was the only character for which class of cross was not significant (Table 2). Two-way interactions are discussed with the specific character.

PERCENT ABORTED FLOWERS: The percent aborted flowers was significantly ($p=0.0120$, Table 2) higher in 1988 (51%) than in 1989 (35%). The classes of crosses were significantly different from one another ($p=0.0002$, Table 2) with R x V the highest and R x R the lowest (Figure 1). Although family effects were not significant ($p > 0.10$), the family x class of cross interaction, was significant ($p= 0.0138$, Table 2 and Figure 2).

PERCENT SHOT BERRIES: Despite large apparent differences for percent shot berries, the error variance was sufficiently large that main effects and two-way interactions were not significant (Table 2). Only the three-way interaction, year x family x class was significant ($p=0.0350$).

BERRIES PER CLUSTER: The only main effect that was significant for number of berries per cluster was class of cross ($p=0.0220$, Table 2, Figure 3). The R x V class had significantly lower average numbers of berries per cluster (50.4) than the R x FH or the R x R (78.5 and 69.3, respectively). The p-value for the family x class interaction, was 0.0648 (Table 2, Figure 4).

AVERAGE BERRY SET: With few shot berries, means for percent berry set were essentially the inverse of percent aborted flowers. Class of cross was significant ($p=0.0020$, Table 2, Figure 1), as was the two-way interaction, family x class ($p=0.0299$, Table 2, Figure 5).

AVERAGE SEEDS PER BERRY: The only significant effect for average number of seeds per berry was class of cross (p=0.0003, Table 2). The R x R class had more seeds per berry than the two interspecific classes (Figure 6).

PERCENT GERMINATION: There were no significant differences for percent germination (Table 2).

REPRODUCTIVE EFFICIENCY: Reproductive efficiency was calculated by multiplying average number of seeds per berry by percent berry set, resulting in a value for average number of seeds per flower. The three classes were significantly different from one another with R x V being the lowest and R x R being the highest (Table 2 and Figure 7).

DISCUSSION:

We were not surprised to find that year effects were significant for aborted flowers, as severe drought characterized the entire growing season in 1988. However, although we would have expected sensitivity to drought stress in the other characters as well, year effects were not significant. Some factors other than drought were apparently responsible for the significant year effects observed in percent aborted flowers. Although percent berry set was the inverse of percent aborted flowers combined with percent shot berries, main effects were not significant for percent shot berries, thus, variation in percent berry set essentially should have reflected variation in percent aborted flowers. Nevertheless, the significant difference between the two seasons in percent aborted flowers was not reflected in percent berry set.

Although environmental factors not included in this study probably contribute to aborted flowers, the differences among the classes of crosses indicates that a genetic factor was operating. Higher levels of aborted flowers in wider crosses were assumed to be the manifestation of incongruity.

Although shot berries may be evidence of early postfertilization incongruity, other complex factors appear to be involved. The year x family x class interaction (Table 2), in the absence of other significant effects, indicates that the causes are not sufficiently accounted for in this study. The interaction may have resulted from environmental influences or yield component compensation masking the effect of class of cross.

Every flower has the potential of developing into a berry. A reproductively successful individual maximizes berry set, as berries are vectors of seeds. Therefore, percent berry set is an indication of the reproductive fitness of a grapevine, or an indicator of incongruity. Based on percent berry set the R x V class exhibits the greatest amount of incongruity, while the R x R class exhibits the least amount of incongruity.

The number of berries per cluster were determined, in addition to percent berry set, to rule out the possibility that percent berry set might be a reflection of fluctuating total flower numbers per cluster with non-fluctuating maximum sink (berry) load in the cluster. Pruning of unopened flowers in the R x R class during pollination was minimal, and was not accounted for in determination of berries per cluster. We had expected that cluster sizes might be more similar within families than among families. However, unless it was masked by the family x class interaction, which was only marginally significant (p= 0.0648, Table 2), family was not a causative effect for berries per cluster. The R x R and the R x FH classes were not significantly different from one another, while the R x V was significantly lower. Incongruity may have reduced the maximum sink load possible in the R x V class. Both R x R and R x FH classes are perhaps sufficiently congruent in their genomes that they are able to achieve a similar sink limit, while R x V, the widest cross, exhibits enough prefertilization or early postfertilization incongruity that the sink limit cannot be achieved.

Successful seed set, measured by seeds per berry, indicates successful fertilization. Although low numbers of seeds may result from poor pollination, it is unlikely that pollen availability was the cause of lower seed numbers in the two interspecific classes, as sufficient self-pollen should have been available inside the pollinating bags. Hand pollination of the *V. riparia* clones was possibly more effective in delivering pollen to the stigma than self-pollination within the bags. Assuming that pollen delivery was equivalent on the three classes, a reduced number of seeds per berry in the F1 generation is an indication of incongruity, possibly in the form of ovule sterility, pollen inviability, or postfertilization lethality. The reduced seed set in our hybrids was not as severe as that found in interspecific F1 hybrids in other genera such as *Amaranthus* (Pal & Khoshoo 1972) or *Papaver* (McNaughton & Harper 1960) which may indicate that *Vitis* species are less differentiated.

Poor germination is common for grape seeds (Einset & Pratt 1975). Grape seeds from self pollinations do not germinate as well as seeds from cross pollinations (Olmo 1942). If this were the case in our study, we might have expected the cross-pollinated R x R class to have a significantly greater level of germinable seeds. Choice of female parent has also been found to influence germinability of grape seeds, whether the cross is interspecific or intraspecific (Olmo 1942). Under these conditions, we might have expected significant family differences. However, neither class of cross, family, nor interaction effects were significant for percent germination. High error variance probably contributed to the lack of significant effects. If incongruity is operating to affect the function of the reproductive systems of the F1 hybrids in this study, then inviable zygotes are apparently screened out before the seed achieves maturity.

Reproductive efficiency was calculated to provide an overall index of incongruity up to the point of seed maturation. The ranking of classes (Figure 7) is highly supportive of the hypothesis of increasing incongruity with increasing distance of cross, and amelioration of incongruity by repeated intercrosses.

Although there was a trend for main effects to vary as predicted, family differences within class of cross were apparent. Family by class of cross interactions were significant (Table 2) for percent aborted flowers, average berries per cluster, and percent berry set. The F1 in the R x V class from family 89 displayed the fewest aborted flowers, the largest cluster size and the highest percent berry set (Figures 2, 4, & 5). The superior performance did not, however, carry into the remainder of family 89, in the R x FH or the R x R classes of crosses. In the particular combination of *Vitis riparia* 89 crossed with 'Carmine,' interspecific barriers that cause aborted flowers and influence cluster size and percent berry set apparently are attenuated. In a highly variable species, some clones may prove to be better parents, as *V. riparia* 89 did. Sampling different individual parents in interspecific combinations may reveal opportunities for circumventing incongruity.

The results in this study are consistent with findings in other genera. Rhodes (1959) with *Cucurbita*, Viehmeyer (1958) with *Penstemon*, and Haghighi and Ascher (1988) with *Phaseolus*, all found improved fertility in complex interspecific hybrid populations. Although documentation of precise pedigrees in the development of the French Hybrids is scant, it is clear that the successful cultivars were advanced intercrosses of several species. This seemingly haphazard method of breeding may have contributed to the realization of an interspecific hybrid gene pool, ameliorating incongruity. The presence of family x class interactions indicates variation for congruent combinations among specific parental pairs and demonstrates that it may be possible to avoid incongruity between taxa by testing several parental partners.

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Table 1. Half-sib families produced by interspecific and intraspecific hybridization using four *Vitis riparia* female clones and three classes of pollen parents.

	Family			
Class of Cross	37	39	64	89
V. riparia x *V. vinifera* (R x V)	Limberger	Limberger	Limberger Carmine	Carmine
V. riparia x French Hybrid (R x FH)	SV18-283 B4672	SV18-283 S11701	S11701	L4511 SV18-307
V. riparia x *V. riparia* (R x R)	Farm 2 Brandon	Farm 2 Brandon	Farm 2 Brandon	Farm 2

Table 2. P-values for the F-tests in the analyses of variance for the characters examined.

Source	df	Percent aborted flowers	Percent shot berries	Average berries/ cluster	Average berry set	Average seeds/ berry	Average germin- ation*	Repro- ductive effi- ciency
Year (Y)	1	.0120	ns	ns	ns	ns	NA	ns
Family (F)	3	ns	ns	ns	ns	ns	ns	ns
Class of cross (C)	2	.0002	ns	.0220	.0020	.0003	ns	.0001
Y x F	3	ns	ns	ns	ns	ns	NA	ns
Y x C	2	ns	ns	ns	ns	ns	NA	ns
F x C	6	.0138	ns	.0648	.0299	ns	ns	ns
Y x F x C	6	ns	.0350	ns	ns	ns	NA	ns

ns - not significant ($p > 0.10$, error df=42, except as noted)

NA - effects involving years are not applicable as germination was observed in only one year.

* - error df=19

Figure 1. Mean percent aborted flowers, percent shot berries, and percent berry set, over two seasons, presented by class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*). Mean percentages are displayed as components of percent total flowers. Sections with the same letter on them do not represent significantly different means (critical p-value = 0.05).

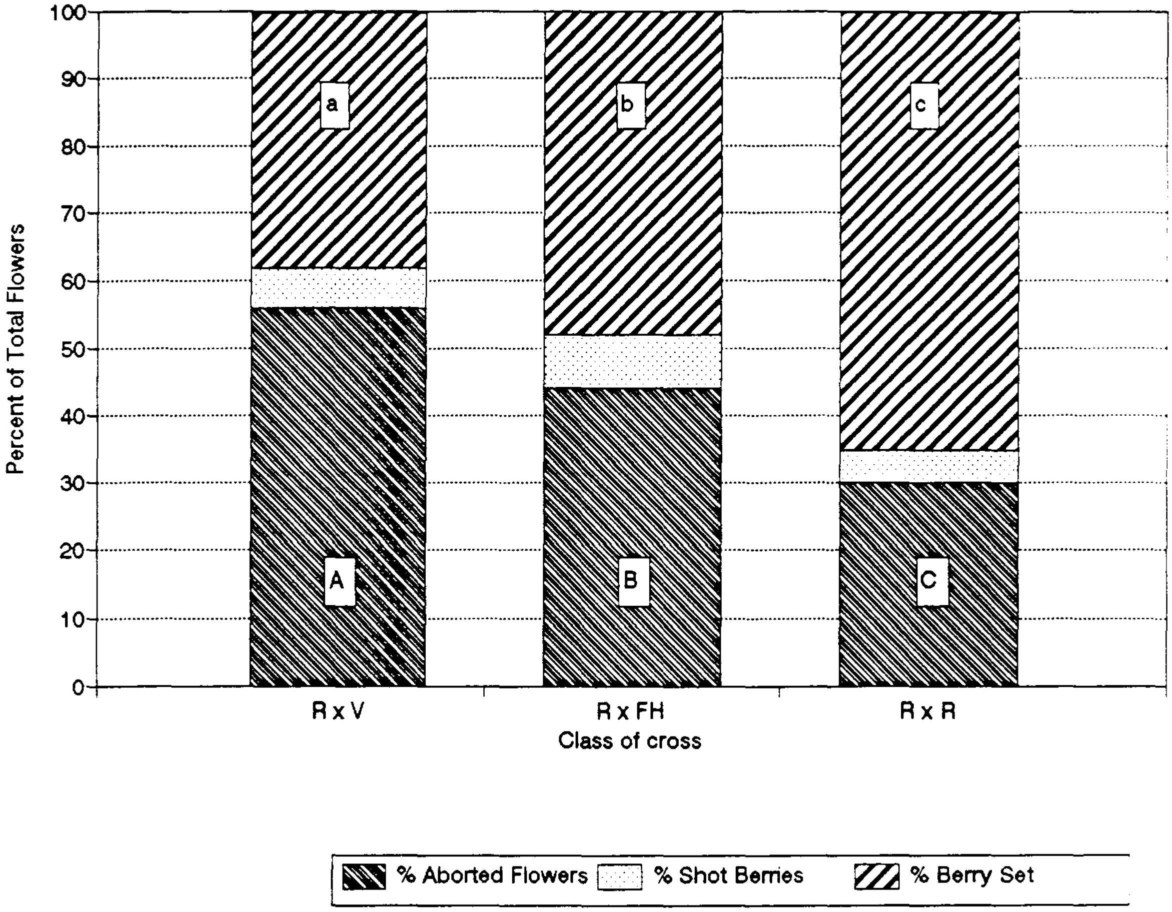
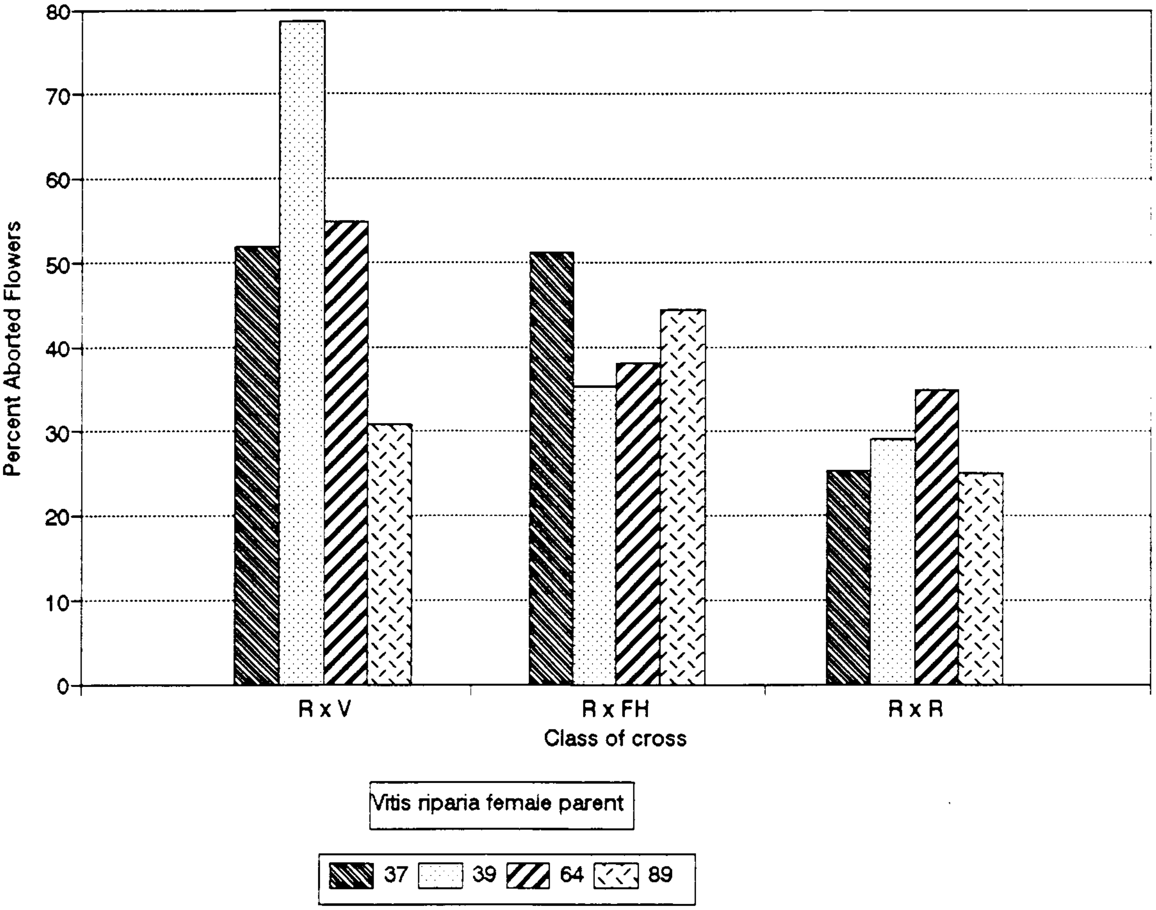

100
90
80
70
60
50
40
30
20
10
0
Percent of Total Flowers
a
b
c
A
B
C
R x V
R x FH
R x R
Class of cross
% Aborted Flowers
% Shot Berries
% Berry Set

Figure 2. Mean percent aborted flowers over two seasons, presented by family, within class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*).



Percent Aborted Flowers
0
10
20
30
40
50
60
70
80
R x V
R x FH
R x R
Class of cross
Vitis riparia female parent
37
39
64
89

Figure 3. Average number of berries per cluster over two seasons, presented by class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*). Bars with the same letter on them do not represent significantly different means (critical p-value = 0.05).

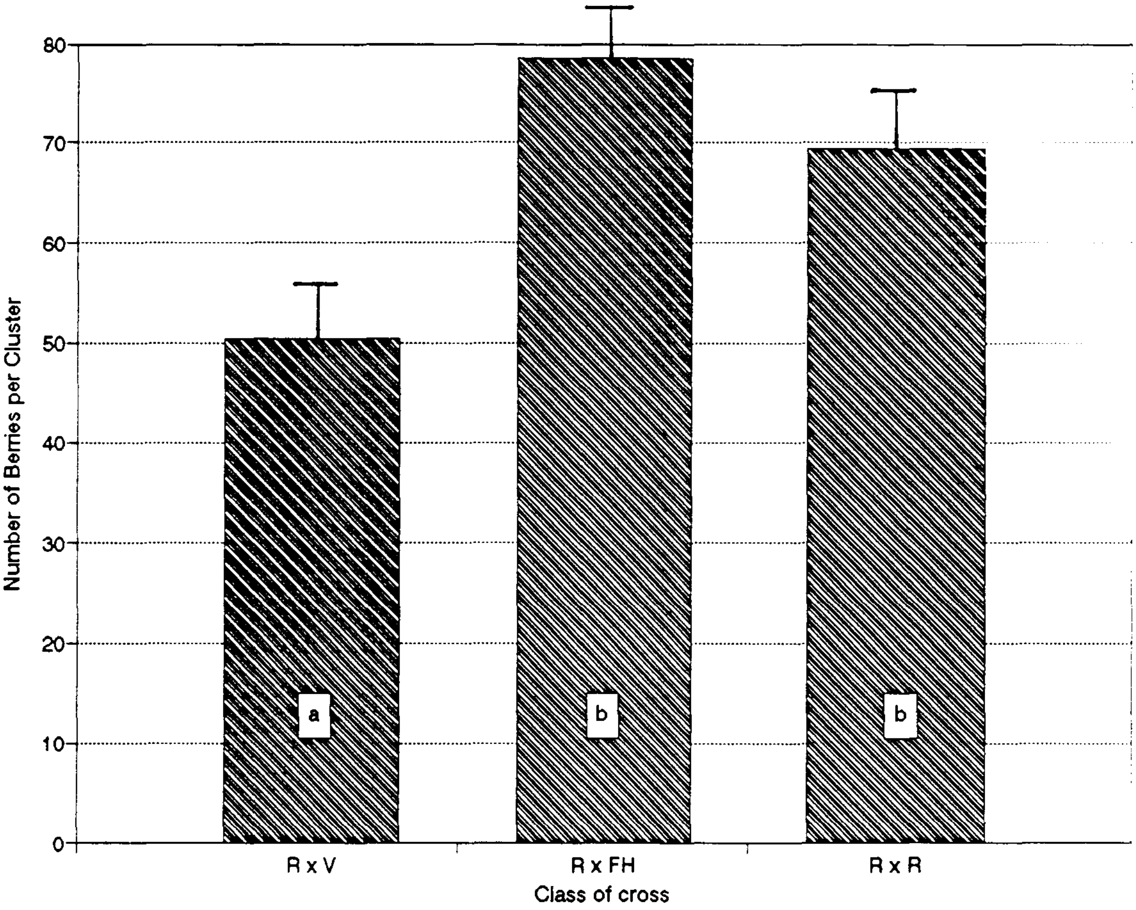
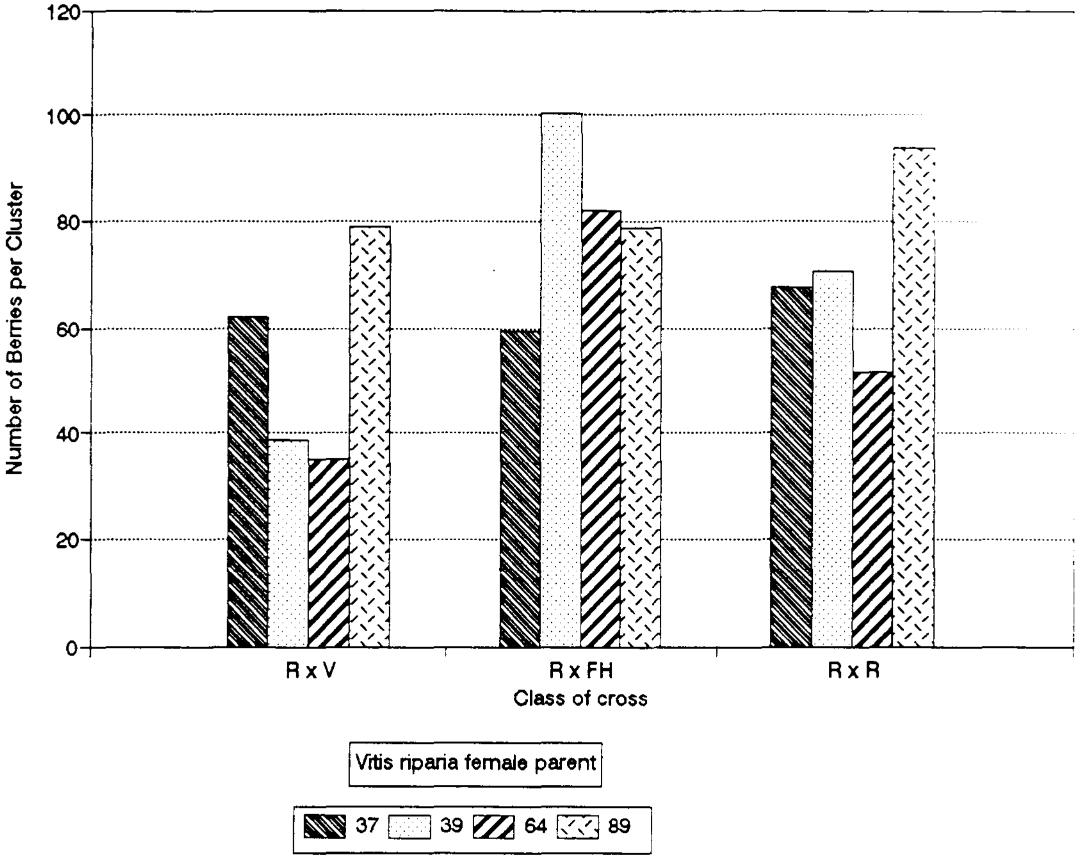

Number of Berries per Cluster
0
10
20
30
40
50
60
70
80
a
b
b
R x V
R x FH
R x R
Class of cross

Figure 4. Average number of berries per cluster over two seasons, presented by family, within class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*).



Number of Berries per Cluster
120
100
80
60
40
20
0
R x V
R x FH
R x R
Class of cross
Vitis riparia female parent
37
39
64
89

Figure 5. Mean percent berry set over two seasons, presented by family, within class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*).

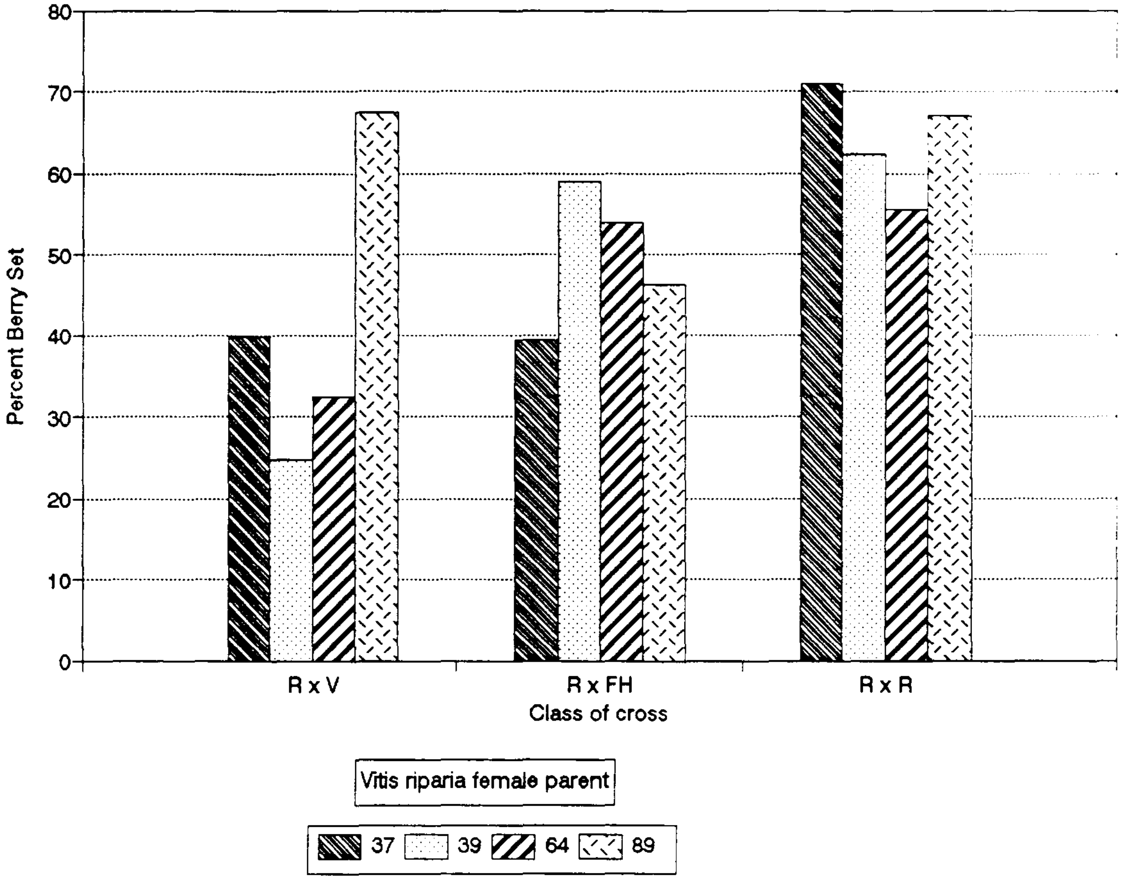

80
70
60
50
40
30
20
10
0
Percent Berry Set
R x V
R x FH
R x R
Class of cross
Vitis riparia female parent
37
39
64
89

Figure 6. Average number of seeds per berry over two seasons, presented by class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*). Bars with the same letter on them do not represent significantly different means (critical p-value = 0.05).

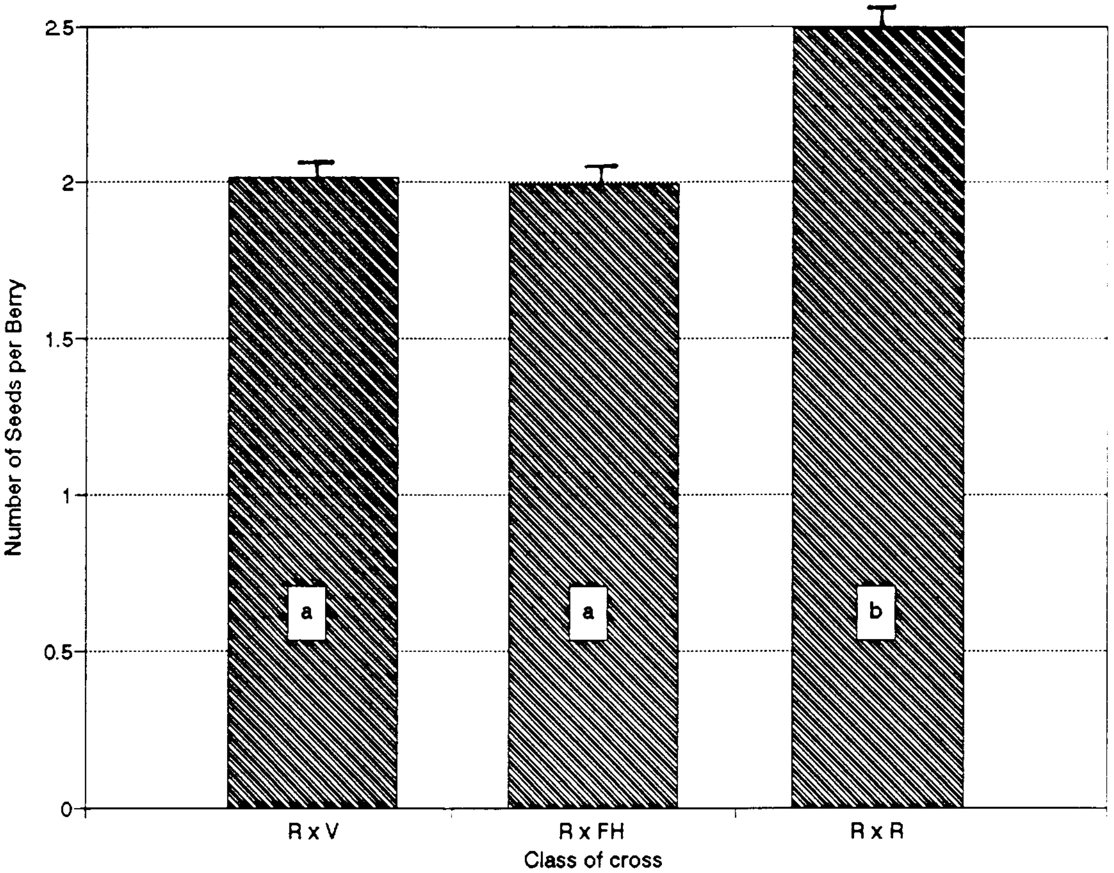
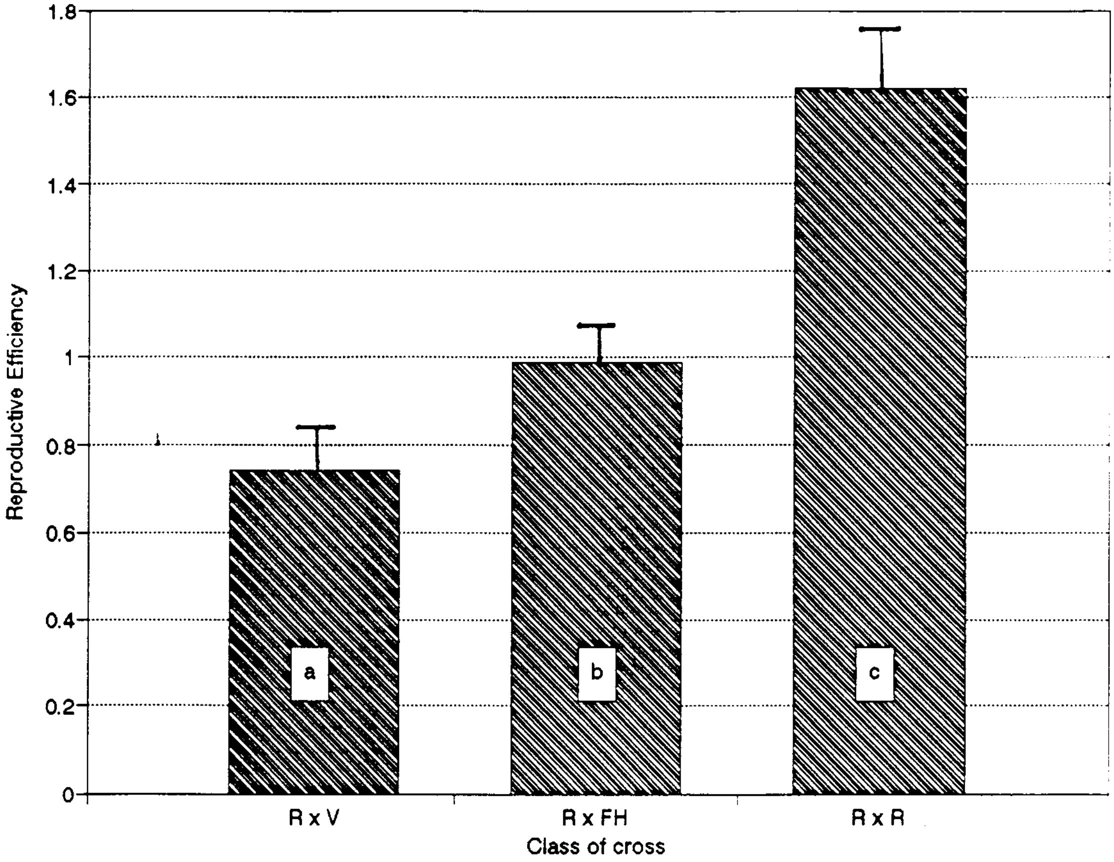

Number of Seeds per Berry
2.5
2
1.5
1
0.5
0
a
a
b
R x V
R x FH
R x R
Class of cross

Figure 7. Mean reproductive efficiency over two seasons, presented by class of cross (R x V = *V. riparia* x *V. vinifera*, R x FH = *V. riparia* x French Hybrid, R x R = *V. riparia* x *V. riparia*). Bars with the same letter on them do not represent significantly different means (critical p-value = 0.05).



1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Reproductive Efficiency
a
b
c
R x V
R x FH
R x R
Class of cross

INCONGRUITY IN THE INTERSPECIFIC CROSSES OF *VITIS* L.

Morphological Abnormalities in the F2 Progeny

For submission to Euphytica

KEY WORDS: Grapes, hybrid breakdown, interspecific barriers, interspecific hybridization.

ABSTRACT:

The F2 generation of interspecific crosses of *Vitis* were examined for symptoms of incongruity, manifested as morphological abnormalities in the seedlings. Dwarfing, achlorophyllic variegation, deformed leaves, yellow mottling, sparse roots, and enation were documented. Most comparisons between classes of crosses were significantly different. The *V. riparia* x *V. vinifera* class exhibited the greatest frequency of abnormalities, the *V. riparia* x *V. riparia* class displayed the lowest frequency of abnormalities, and the highly-intercrossed *V. riparia* x French Hybrid class demonstrated an intermediate frequency of abnormal plants. When incongruity was viewed as a syndrome, or collection of symptoms, differences between classes were consistently evident. The highly-intercrossed nature of the French Hybrid ancestry appears to have been responsible for attenuating incongruity.

ABBREVIATIONS:

R x FH	*Vitis riparia* x French Hybrid
R x R	*V. riparia* x *V. riparia*
R x V	*V. riparia* x *V. vinifera*

INTRODUCTION:

Interspecific hybridization has been used in many genera to introgress desirable genes from the wild into domesticated cultivars (Moav, et al 1968, Zeven 1970, Phillips 1976). In several crops, use of interspecific hybridization for introgression is limited by barriers to gene flow between taxa: tomato (Martin 1966), cotton (Stephens 1946, Phillips & Merritt 1972, Phillips & Reid 1975, Phillips 1976), tobacco (Moav, et al 1968), poppy (McNaughton & Harper 1960), and wheat (Zeven 1970). Interspecific barriers have been classified by several researchers. Stebbins (1958) differentiates between haplontic sterility of F1 hybrids and hybrid breakdown of the F2 and advanced generations. He theorizes that the principal difference is that, in some organisms, the barriers prevent gametes or gametophytes from functioning but, in others, where the gametes are viable and zygotes form, the barriers affect later stages of development. Levin (1971) distinguishes between premating and postmating reproductive isolating mechanisms. The postmating barriers are further classified into prezygotic (autogamy and pollen-pistil incompatibility) and postzygotic. Postzygotic mechanisms include seed inviability, hybrid inviability or weakness, hybrid floral isolation, hybrid sterility, and hybrid breakdown (Levin 1971).

Hogenboom (1973 1975 1984) proposed the term "incongruity" to indicate the dysfunction between united interspecific genomes. Incongruity is the passive byproduct of evolutionary divergence. When the genomes in an intimate partner relationship lack information concerning a relevant character in one another, dysfunction results, and the expression may range from deterioration of pollen-pistil interactions

(Hogenboom 1973) to postzygotic manifestations (Hogenboom 1984). Incongruity may include such hybrid dysfunctions as cross failure, F1 inviability or weakness, F1 reproductive breakdown, and F2 and advanced generation hybrid breakdown. Thus "incongruity" unifies an otherwise confusing terminology.

The gene system controlling any trait may be disrupted by genetic incongruity. Some dramatic morphological displays have been documented in the F2 and later generations of wide crosses. In tomato, frosty spot eruptions were seen in backcrosses and in the F2 and F3 following repeated backcrossing (Martin 1966). In *Zantedeschia*, virescence was documented in the F1 and F2 generations of the interspecific cross (New & Paris 1968). Chromosome instability has been seen in various generations of *Nicotiana* interspecific crosses (Moav, et al 1968). Lack of vigor and reduced fertility were seen in the F2 of *Mimulus* crosses (Vickery 1964). The F2 generation of interspecific crosses in bromegrass displayed a series of abnormalities involving plant type, chlorophyll disturbances, summer vigor, necrosis, mosaic, and reduced fertility (Nielsen, et al 1962). Clausen (1951) found that despite high vigor in the F1 generation, the F2 generation of *Hemizonia* and *Zauschneria* interspecific crosses both displayed severe dwarfing, inviability and sterility. Incongruity may have a negative affect on fitness of interspecific progeny and may impede introgression of valuable traits in breeding programs.

Incongruity may sometimes be circumvented by surveying the desired species for prospective parental combinations that show less malfunction in their progeny. Progeny from crosses involving similar genomes would be expected to show fewer symptoms of incongruity than those composed of divergent genomes. Also, interspecific breeding procedures that bring genomes of several species together, or that bring them together repeatedly, have been shown to ameliorate incongruity (Viehmeyer 1958, Rhodes 1959, Haghighi & Ascher 1988). Viehmeyer (1958) postulated that evolution could, in a sense, be reversed by these breeding procedures, and widely divergent germplasms returned to a common gene pool. Such a gene pool could be used to bridge divergent species, as a source of valuable traits to be transferred to adapted germplasm, or developed into new materials not currently defined by taxonomy.

In *Vitis* L., interspecific hybridization was used at the turn of the century in the production of the so-called French Hybrids. The primary species used in the production of the French Hybrids were the American species, *V. cinerea* Engelm., *V. rupestris* Scheele, *V. aestivalis* Michx., *V. berlandieri* Planch., *V. labrusca* L., and *V. riparia* Michx., and the European *V. vinifera* L. (Barrett 1958a 1958b, Galet 1988). The French Hybrids were developed by private breeders, primarily in France, in an attempt to introgress genes for resistance to powdery mildew (*Uncinula necator* (Schw.) Burr.), downy mildew (*Plasmopara viticola* (Berk. & Curt.) Berl & de Toni), and the insect phylloxera (*Daktulosphaira vitifoliae* Fitch). These pests were introduced into European vineyards via infected stock brought from North America. The American species evolved in the presence of these pests and many developed disease and insect resistance, as well as cold hardiness and tolerance to drought or calcareous soils. The traits that breeders were seeking to preserve from the domesticated *V. vinifera* were cluster size, berry size, various fruit quality traits, and hermaphroditism.

The initial crosses used in French Hybrid production were between American species and *V. vinifera*. In the later stages of French Hybrid development, genomes were brought together repeatedly through backcrossing to useful species and intercrossing of valuable interspecific offspring. Some of the valuable French Hybrids have pedigrees that include 5 to 7 species, and may have been subjected to up to 7 generations of intercrossing (Galet 1988). Barrett (1958b) stated that there were often "aberrant or variable" seedlings in the intercrossed progenies. He indicated that these aberrant seedlings were discarded in favor of more normal-appearing individuals. As intercrossing progressed, quality improved and aberrations diminished.

Vitis riparia is the predominant grape species native to Minnesota. It covers a large range, from Texas to southern Canada and from the east coast to the Rocky Mountains. Some of its salient characteristics are disease resistance, phylloxera resistance, and extreme cold hardiness in the northern clones. *Vitis riparia* does, however, have several disadvantages. It has small clusters, tiny berries with high acid, characteristic off-flavors, and it is dioecious (Munson 1909, Pierquet & Stushnoff 1978, Galet 1979).

Vitis riparia has been indispensable as a source of cold hardiness and disease resistance in several breeding programs, including the program at the University of Minnesota (Luby 1991). However, as a source of quality traits, complex interspecific hybrids have been more successful than *V. vinifera* (Swenson 1985). Swenson's breeding program in Wisconsin and Minnesota used a variety of interspecific hybrids as quality parents, including French Hybrids, while using *Vitis riparia*-based germplasm for sources of cold hardiness (Swenson 1985, Luby 1991). Analogous to Barrett's (1958a 1958b) account, Swenson also found occasional "off-types" in some of his early progenies. The frequency of these abnormal seedlings apparently diminished following several generations of intercrossing (E.P. Swenson, personal communication). No attempt was made to document these aberrants, or their frequencies in different types of crosses, and they were culled from the breeding program.

The appearance of aberrant or off-types in *Vitis* crosses suggested that incongruity might be interfering with normal seedling development. If this were the case, then progeny of crosses where the genomes had not previously encountered one another would be expected to contain the greatest percentage of aberrant seedlings. Progenies of the highly-intercrossed French Hybrids would be expected to show attenuated levels of incongruity, if any, and if incongruity affected mating pairs of the same species, it would be expected to be minimal.

A previous study of F1 progenies from three classes of *Vitis* crosses involving *V. riparia* indicated that incongruity symptoms were present in the most divergent class, were somewhat attenuated in the intercrossed class, and were the lowest in the intraspecific class (Filler 1992). The F1 individuals of the interspecific classes were not severely affected by incongruity. They were reasonably vigorous, and sufficiently fertile to produce an F2 generation. Serious interspecific barriers, however, may not be manifested until the F2 generation, after there has been an opportunity for recombination (Clausen 1951). Incongruity exhibited in the F2 progeny may be just as effective in blocking gene flow between species. Abnormal F2 plants, affected by incongruity, may be too weak to reach sexual maturity, blocking opportunities for recombination and introgression of desirable traits. Even vigorous individuals may be culled from a breeding program if other aberrations are manifested.

The objective of this study was to determine whether incongruity was affecting the F2 progeny of wide crosses involving *Vitis riparia*, and to evaluate whether the complex interspecific breeding procedures used in the development of the French Hybrids ameliorated incongruity in the F2 generation. The null hypothesis was one of no differences: that all classes would display the same level of incongruity or lack thereof. The alternative was that the F2 progeny of the divergent R X V class (*V. riparia* x *V. vinifera*) would most frequently show symptoms of incongruity, the progeny of the R x R class (*V. riparia* x *V. riparia*) would show the lowest frequency of symptoms, and that the F2 progeny of the highly-intercrossed R X FH class (*V. riparia* x French Hybrid) would show intermediate frequencies of incongruity symptoms, indicating that intercrossing had ameliorated incongruity in that class.

If attenuation of incongruity can be achieved by intercrossing multiple species, then breeding progress in grapes would be expected to be enhanced by the purposeful use of complex interspecific hybrids as quality parents, rather than pure *V. vinifera*, in order to bridge the divergent genomes.

MATERIALS AND METHODS:

The plant material used for this study were F2 progenies that fell into three classes of crosses:

1. R X V: *V. riparia* X *V. vinifera* (wide interspecific; New World X Old World).
2. R X FH: *V. riparia* X French Hybrid (complex interspecific; New World X advanced interspecific containing New and Old World).
3. R X R: *V. riparia* X *V. riparia* (New World intraspecific; control).

F2 families were derived from a random subset of the F1 individuals examined in the previous study (Filler 1992). The three classes of F1 hybrids were produced using four female clones of *V. riparia*, resulting in four half-sib families (Table 1). The history and species ancestry of the parents are presented in detail in the previous study (Filler 1992).

In order to generate sufficient numbers of progeny and still maintain controlled pollination, and to preserve the genomic complement of the primary crosses without confounding with an additional genome, the interspecific F1 hybrids were selfed to produce F2 progenies. This is not a common practice in grape breeding programs, as inbreeding depression is a problem in grapes (Einset & Pratt 1975). In this case, though, we felt that results confounded with inbreeding depression would be preferable to results confounded by the addition of another genome. Comparisons of the two inbred progenies (R X V and R X FH) were attempted to resolve the confounding with inbreeding depression.

Intraspecific *Vitis riparia* crosses were produced using the same *V. riparia* females employed in construction of the interspecific hybrids (Table 1). Intraspecific hybrids were produced because *V. riparia*, like other wild grapes, is dioecious and cannot be selfed. These intraspecific crosses were intended as analogs to selfings in the absence of hermaphroditism. Crosses between sibs were not possible, as the *V. riparia* clones used were collected from the wild in the 1970s and sib relationships were unknown.

Self pollination of hermaphrodites and cross pollination of dioecious *Vitis riparia* clones was described by Filler (1992). Pollinating bags remained on the clusters until they were collected when berries were ripe.

Seeds were scarified during extraction, using an electric blender, on pulse, to prevent excessive chipping of the seed coats. Seeds were dried, and counted numbers of each seed lot were placed in moist sand in plastic bags and stratified at 3°C for 3.5-4 months. When removed from stratification, seeds were planted in #50 Pro-trays (TLC Polyform, Inc., Plymouth, MN) in Bacto Pro Plant Mix (Michigan Peat Co., Houston, TX) and kept moist using Vattex F capillary mats (O.S. Plastics, Norcross, GA) on the greenhouse benches. Metal-halide high intensity discharge (HID) supplemental light was used to extend daylength to 14 hours. After emergence, seedling flats were fertilized with Peters 20-10-20 (400 ppm N) once a week, and supplemented with 0.5% S.T.E.M. (Soluble Trace Element Mix, Peters Fertilizer Products, Fogelsville, Pa.) once a month. Evaluation began at seven weeks from planting and extended over a period of several weeks. Measurements and photographs were taken in the greenhouse.

The frequencies of aberrant seedlings were compared between all pairs of classes using 2x2 contingency tables with a χ^2 one-degree-of-freedom test for significance.

RESULTS:

The symptoms seen in the F2 progeny included dwarfing, achlorophyllic variegation, deformities of leaf shape, yellow mottling, poor roots, enation of the leaf surface, disturbances in cotyledon morphology, and various kinds of necrosis.

DWARFING: Any individual with average internode length less than two standard deviations shorter than the intraspecific mean for its *V. riparia* family was defined as a dwarf (Figure 1). *Vitis riparia* clones appeared to differ in average internode length, so separate normal and dwarfing categories were established for each female *V. riparia* half-sib family.

The R X V group contained a high frequency of dwarfs, varying considerably among families, from 21.1% to 85.7% (Table 2). Most of the R X R group displayed a low frequency of dwarfs, but in the R X R family derived from Riparia 39, there were 21.9% dwarfs, approximately 7 times the frequency of dwarfs in the next highest family in the R X R category. No dwarfing was observed in two of the families in the R X FH group, while the other two contained 17.6% and 20.8% dwarfs.

ACHLOROPHYLLIC VARIEGATION: Variegation ranged from faint streaking to large achlorophyllic regions (Figure 2), with the greatest frequency and severity in one family in the R X V class (Table 2). In the R X FH class, variegation was limited to one family. Significant differences were observed only in comparisons involving these two families (Table 3).

DEFORMED LEAVES: Several different leaf-shape deformities (Figure 3) were common in both of the interspecific classes. Some characteristic types of deformities were seen regularly in conjunction with the more extreme cases of variegation (Figure 2). Most of the deformities appeared in the R X V and R X FH classes, and few were detected in the R X R class (Table 2). Family 89 displayed few deformities in any class, and all comparisons for this family were non-significant (Table 3).

YELLOW MOTTLE: Yellow mottling (Figure 4) appeared in blotches on leaves in all three classes (Table 2). Except for family 89, significant differences were seen when the R X V and R X R classes were compared (Table 3). In comparisons of R X FH to R X R, 39 was the only family not exhibiting significant differences. The frequency of mottling was lower in the R X FH progeny of Riparia 89 than in the R x R progeny.

SPARSE ROOTS: Root systems in normal plants were full, long, and heavily branched. Root systems classed as sparse were decidedly scant, short, and had restricted branching (Figure 5). Some profoundly dwarfed plants had such a meager root system, that the condition of the roots was likely at least partially responsible for the severity of the dwarfing. The frequency of sparse roots in R X V, family 39, and R X FH, family 37, was 0 and not significantly different from the R X R, and none of the comparisons were significant in family 89 for sparse roots. The frequencies in the other families in the interspecific classes were significantly different from the intraspecific class (Tables 2 and 3).

ENATION: Enations (outgrowths) were observed on the upper surface of some leaves (Figure 6). The outgrowths made the surface appear bubbled, while the underside of the leaf was unaffected. While this character was observed in all classes of all families, the largest proportions were manifested in the R X V and the R X FH classes in three of the four families (Table 2). However, family 64 in the R X V class displayed a significantly lower proportion of affected plants (Table 3). Outgrowths on the lower surface of grape leaves are a symptom of the rare grape enation disease, caused by an unknown agent (Pearson & Goheen 1988). As the outgrowths were observed on the upper surface of the leaves, it seems unlikely that grape enation disease was responsible for this phenomenon.

OTHER OBSERVATIONS: In addition to the six characters described above, various types of necroses and disturbances of cotyledon morphology were observed. In the progenies of one of the half-sib families (Riparia 64), necrosis ranged from flecks barely visible to the naked eye, to splotches, to an overall necrosis of the leaves that eventually killed the plant. Tricotyledons were observed in three families. In two families in the R X V class, frequencies were 7/43 and 1/11, respectively. In one R X FH family, the frequency was 1/12. Fused cotyledons were also seen.

ANALYSIS OF HALF-SIB FAMILIES: The R X V and R X R families were significantly different from one another for most characters studied (17 of 24 comparisons, Table 3). When the R X FH families were compared to the respective R X R families, fewer significant differences (12 of 24, Table 3) were apparent. Slightly fewer (10 of 24 comparisons) were exhibited between the R X V and R X FH classes, as would be expected with the large *V. vinifera* component present in the French Hybrids. Both of the latter comparisons, however, concern the R X FH class, and less incongruity, indicated by fewer differences, is what would be expected given the highly intercrossed nature of the French Hybrids.

If incongruity is impeding function in the widest crosses, and is ameliorated by intercrossing, as predicted, then the frequency differences should be predominantly in one direction, rather than random. Of the significant differences in the family comparisons (Table 3), there is a preponderance (37 of 40) of those in the predicted direction ("+") over those that are significant, but with the direction of differences reversed ("-").

Sample sizes for some of the families were small, and may have affected the precision of the analysis (Table 2). The small sample sizes reflected the combined effect of low berry set, few seeds per berry, and low germination percentages (Filler 1992) and may be another indication of incongruity.

POOLED ANALYSIS: When the data were pooled across families, so that class sizes were larger (Table 4), then the percentage of individuals with a given abnormality was highest in the R X V class, followed by R X FH, then R X R, for most of the characters. In the majority (14 of 18) of comparisons, the differences between classes were significant. This order of classes would be expected if less incongruity was associated with crosses between more similar, or more highly intercrossed parents.

DISCUSSION:

Reisch and Watson (1984) proposed a single recessive gene to account for leaf variegation in F1 and S1 progenies derived from both interspecific hybrids and cultivars of *Vitis vinifera*. Although our sample sizes are small, our results would best be explained by the presence of duplicate genes. All family-class combinations except the R x V progeny of family 64 fit a 15:1 ratio as would be expected with duplicate genes with both loci in the heterozygous condition. The R x V progeny from family 64 did not fit a 15:1 ratio ($\chi^2 > 7.8$), but fit a 3:1 ratio ($\chi^2 < 1.4$), and may be the result of the same duplicate genes, one locus in the heterozygous condition, and the other in the homozygous recessive condition. The gene reported by Reisch and Watson may be the same as one of those observed in our study, but as in the R x V progeny of family 64, the additional gene we postulate would have to be in the homozygous recessive condition.

High frequencies of dwarfing were observed in some families (Table 2), and the frequencies do not fit single gene hypotheses. Dwarfing has been associated with aneuploids in wheat and oats (Burnham 1962, Kush 1973), and may suggest aneuploidy in this case. No cytology was done to determine if dwarfing was associated with aneuploidy in this study. However, grapes are thought to be ancient allopolyploids (Olmo 1976), and may be tolerant of a low level of aneuploidy.

Multiple abnormalities were commonplace among the observed populations. Leaves with the most severe cases of variegation tended to be accompanied by a characteristic shape deformity (Figures 2 and 7). Less severely variegated leaves rarely exhibited this particular deformity. As might be expected, absence of roots was associated with acute dwarfing. However, less profoundly affected root systems were not consistently identified with dwarfing, and severe dwarfing was seen even when root systems were abundant and lush. No other relationships between specific traits were observed.

The presence of multiple aberrations led us to contemplate incongruity as a collection of symptoms, or a syndrome (Figure 7). This is consistent with Hogenboom's (1984) observations that incongruity may be seen as a complex combination of expressions. "...as evolutionary divergence proceeds, more complex combinations of different expressions of incongruity will occur." To evaluate the validity of considering incongruity as a syndrome, each class of cross was tested for independence assortment of traits (Table 5). The observed frequencies of each character were used to calculate the expected number of abnormalities. Results of tests for all three cross classes were significant, indicating multiple abnormalities were observed at a greater frequency than would be expected given the frequencies of the individual abnormalities (Table 5).

Differences in multiple abnormalities were also found between the cross classes (Table 6). Half of the R X R class had at least one symptom per plant, but the frequencies in the R X FH and the R X V classes were even higher. The percentage of individuals showing two or more, or three or more abnormalities was progressively lower in the R x R class, as well as in the two interspecific classes. The R x V class always had a higher proportion of individuals with multiple abnormalities compared to the R x FH class.

A detailed look at multiple abnormalities within families indicated differences among full-sib families. For example, compared to the R x R crosses involving Riparia 64, the F2 progeny of 64/Lm2 did not have a higher proportion of individuals with multiple abnormalities, but progeny from a full-sib, 64/Lm1, did contain more. Such inconsistencies were not noted in the other R X V families. Differing responses in full-sib families might be due to the random gametic contributions of Riparia 64 and 'Limberger', the parents of the F1's in these two families. Congruent combinations may result from random gametic unions, just as incongruent genetic combinations may result, especially in a genus like *Vitis*, in which reproductive barriers are not complete.

In this study, incongruity was confounded with inbreeding depression in comparisons of R x R with selfed families in the R x V and R x FH crosses. Comparisons of the R x FH with the respective R x V selfed populations partially resolve the confounding, as both populations were inbred by one generation. A majority of the R X V vs R X FH comparisons in Table 3 were not significant, indicating that inbreeding depression may be responsible for the differences between these two classes and the intraspecific R X R class. However, in the pooled analysis (Table 4), most of the differences between the two selfed interspecific populations are significant, adding to the evidence for incongruity. When multiple abnormalities were considered, all comparisons between R X V and R X FH were significant (Table 6), and incongruity, rather than inbreeding depression, can be inferred.

Although some of the differences between classes of crosses may be due to inbreeding depression, the data support the hypothesis of increasing incongruity with increasing parent divergence. When incongruent combinations occur in wide hybrids, genes may be present in a hemizygous condition, due to divergent evolution of the parental genomes. As no dominant allele is present to mask a recessive allele in the hemizygous condition, it would be expected to be expressed identically to the same recessive allele present in the homozygous state. Therefore, recessive deleterious alleles expressed as a result of inbreeding, may also be expressed as a result of incongruity. In the inbred progeny of hybrids of divergent parents, differentiating between inbreeding depression and incongruity may be impossible.

The fact that frequencies did not differ between classes for some characters may indicate that the genes affecting those characters are not incongruent in the parental material of that cross. In *Vitis*, reproductive barriers and barriers to recombination are not complete, and congruent combinations do exist, as is evidenced by the prevalent use of interspecific hybridization in successful grape breeding programs (Einset & Pratt 1975, Swenson 1985, Luby 1991). In a perennial genus like *Vitis*, fixation of mutations in a population may take a very long time, despite isolation, as parental combinations may be preserved for hundreds of years, impeding the effects of drift.

Minimal symptoms were noted in the progeny of the F1, 64/Lm2, compared to the progeny of its full sib, 64/Lm1, which manifested severe symptoms of incongruity. In a highly heterozygous crop, such as grapes, random assortment of chromosomes will lead to different gene complements available among gametes, making it possible to develop full-sibs displaying different levels of incongruity.

CONCLUSIONS:

In this study, the interspecific hybrid complex of *V. riparia* and *V. vinifera* genomes appeared to be the stimulus for symptoms of incongruity to emerge. This is particularly notable when incongruity is regarded as a syndrome. Complex interspecific intercrossing, as in the production of the French Hybrids, a process which brought several genomes together repeatedly, appears to have ameliorated the effects of incongruity with *V. riparia*.

As breeding progresses, progenies showing minimal negative effects are most likely to be chosen by the breeder as subsequent parents, and the most incongruent combinations are apt to be dropped from the crossing schemes. Repeated intercrossing of several species, followed by selection for normal individuals, may reduce the number of incongruent combinations, and the portions of genomes remaining in the complex hybrids may be available for recombination. However, if aneuploidy or severe structural chromosomal problems are not the cause of aberrations, then aberrants may represent recombinant individuals. In that case, aberrant progeny that can be maintained to reproductive maturity may provide a link to rapid recovery of desireable traits from disparate parents.

In breeding programs where exotic germplasm is employed and symptoms of incongruity are displayed, breeders may need to find methods of circumventing or ameliorating incongruity. When initial trials of hybridization of divergent germplasm are unsuccessful, further attempts to effect hybridization are often abandoned. Our data indicate that repeated trials with different individuals representing the divergent taxa may be effective. Where reproductive barriers are incomplete, the use of advanced interspecific hybrids may help to overcome symptoms of incongruity, opening up opportunities for recombination between taxa, and providing a bridge for more effective gene transfer.

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Table 1. Male clones used to produce half-sib families of three classes of interspecific and intraspecific hybrids using four *V. riparia* female clones.

	V. riparia female parent			
Class of Cross	37	39	64	89
V. riparia X *V. vinifera* (R X V)	Limberger (Lm)	Limberger (Lm)	Limberger (Lm) Carmine (Cm)	Carmine (Cm)
V. riparia X French Hybrid (R X FH)	SV18-283 (V2)	SV18-283 (V2)	S11701 (S1)	L4511 (L4)
V. riparia X *V. riparia* (R X R)	Farm 2 (Fm) Brandon (Br)	Farm 2 (Fm) Brandon (Br)	Farm 2 (Fm) Brandon (Br)	Farm 2 (Fm)

Table 2. Percentage of each half-sib family affected with each abnormal character, within the three classes of crosses [*V. riparia* X *V. vinifera* (R X V), *V. riparia* X French Hybrid (R X FH) and intraspecific hybrids of *V. riparia* (R X R)].

			Character					
Cross class	Female parent	n	Dwarf	Varie-gation	Deformed leaves	Mottle	Sparse roots	Enation
R X V	37	11	36.4	9.0	27.3	45.5	27.3	27.3
	39	8	75.0	0	37.5	75.0	0	37.5
	64	109	21.1	29.4	29.4	37.6	37.6	15.6
	89	13	84.6	0	7.7	61.5	23.1	46.2
R X FH	37	12	0	8.3	25.0	66.7	0	58.3
	39	17	17.6	0	29.4	41.2	35.3	70.6
	64	24	0	0	29.2	54.2	20.8	37.5
	89	24	20.8	0	4.2	4.2	20.8	4.2
R X R	37	50	2.0	0	2.0	0	2.0	4.0
	39	32	21.9	9.4	6.3	46.9	3.1	9.4
	64	79	1.3	4.0	2.5	25.3	1.3	39.2
	89	32	3.1	9.4	12.5	46.9	6.3	3.1

Table 3. Summary of the p-values for the chi-square analyses comparing the three classes of crosses [*V. riparia* X *V. vinifera* (R X V), *V. riparia* X French Hybrid (R X FH) and intraspecific hybrids of *V. riparia* (R X R)] for six characters, classed by half-sib family.

		Character					
Cross class	Female parent	Dwarf	Variegation	Deformed leaves	Mottle	Sparse roots	Enation
R X V	37	++++	++	+++	++++	+++	++
vs	39	++	ns	++	ns	ns	++
R X R	64	++++	++++	++++	+	++++	---
	89	++++	ns	ns	ns	ns	++++
R X FH	37	ns	++	+++	++++	ns	++++
vs	39	ns	ns	+++	ns	+++	++++
R X R	64	ns	ns	++++	+++	++++	ns
	89	++	ns	ns	---	ns	ns
R X V	37	++	ns	ns	ns	+	ns
vs	39	+++	ns	ns	ns	++	ns
R X FH	64	++	+++	ns	ns	ns	---
	89	++++	ns	ns	++++	ns	+++

ns not significant; + $P<.10$; ++ $P<.05$; +++ $P<.01$; ++++ $P<.001$

--- $P<.01$, but with direction of differences reversed

Table 4. Pooled frequencies for symptoms observed in interspecific crosses of *V. riparia* X *V. vinifera* (R X V) and *V. riparia* X French Hybrid (R X FH) and in intraspecific crosses within *V. riparia* (R X R). Chi-square values and p-values are presented comparing the classes of crosses to one another.

		Character					
Cross class	n	Dwarfs	Variegation	Deformed leaves	Mottle	Sparse roots	Enation
R X V	141	31.2	23.4	27.7	42.6	33.3	20.6
R X FH	77	10.4	1.3	20.8	37.7	20.8	37.7
R X R	193	5.2	4.7	4.7	25.9	2.6	19.2
R X V							
vs	χ^2	40.71	26.03	20.78	10.22	58.58	0.10
R X R	P	0.000	0.000	0.000	0.001	0.000	0.752ns
R X FH							
vs	χ^2	2.40	1.75	17.01	3.67	25.39	10.19
R X R	P	0.121ns	0.186ns	0.000	0.055	0.000	0.001
R X V							
vs	χ^2	11.88	18.49	1.25	0.49	3.82	7.53
R X FH	P	0.001	0.000	0.264ns	0.484ns	0.051	0.006

Table 5. Chi-square comparisons of observed versus expected number of abnormalities per individual for each class of cross. Expected values are derived based on independent assortment of the six characters, using the experimentally observed frequencies for each character.

Class of Cross	n	0	1	2	3	≥4	df	χ^2	P
				Abnormalities per individual					
R x V									
obs	141	14	41	36	29	21			
exp		15.51	42.3	46.53	26.79	9.87	4	15.30	0.004
R x FH									
obs	77	21	20	20	16[a]	--			
exp		16.17	30.8	21.56	8.47[a]	--	3[a]	12.04	0.000
R x R									
obs	193	91	51	38	13[a]	--			
exp		96.5	75.27	19.3	1.93[a]	--	3[a]	89.75	0.000

[a] as expected values were <1 for ≥4 group, this group was bulked with the group below, resulting in 3df, and a more valid test.

Table 6. Percent individuals in each class displaying one or more (≥ 1), two or more (≥ 2), and three or more (≥ 3) abnormalities, followed by the chi-square values and p-values comparing the three classes of crosses.

Cross class		Abnormalities per individual		
		≥ 1	≥ 2	≥ 3
R X V	%	90.0	61.0	35.5
R X FH	%	72.7	46.8	20.8
R X R	%	52.8	26.4	6.7
R X V				
vs	χ^2	52.37	40.24	43.93
R X R	P	0.000	0.000	0.000
R X FH				
vs	χ^2	8.96	10.41	11.32
R X R	P	0.003	0.001	0.001
R X V				
vs	χ^2	11.12	4.10	5.09
R X FH	P	0.001	0.043	0.024

Figure 1. Dwarf and normal F2 individuals of the same age.

Figure 2. Leaf showing achlorophyllic variegation and leaf-shape deformity characteristic of the severely variegated leaves.

Figure 3. One of several examples of deformed leaves.

Figure 4. Leaf displaying yellow mottling.

Figure 5. Roots of F2 plants. Left plant shows normal roots, middle and right plants show increasing degrees of sparse roots.

Figure 6. Enations (outgrowths) on upper surface of leaf.

Figure 7. Individual exhibiting dwarfing, achlorophyllic variegation, and leaf-shape deformities.

GENERAL DISCUSSION

The prevalent use of interspecific hybridization in successful grape breeding programs (Einset & Pratt 1975, Swenson 1985, Luby 1991) indicates that interspecific barriers are not complete in *Vitis*. However, the symptoms observed in both the F1 and F2 generations of interspecific crosses involving *Vitis riparia* are consistent with the attributes of incongruity, a phenomenon resulting from passive evolutionary divergence between taxa (Hogenboom 1984). Although parental combinations of genes could be preserved for hundreds or even thousands of years in long-lived perennials, delaying the effects of drift, *V. riparia* and *V. vinifera* have been reproductively isolated from one another for perhaps 40 million years (Hyams 1965), which could be an adequate interval for passive divergence to occur. The multiple-species, repeatedly-intercrossed complexes of the French Hybrids appear to have ameliorated incongruity in hybrids involving *V. riparia*, as symptoms are attenuated in both the F1 and the F2 generations of the intercrossed class. Results correspond with allusions made to the occurrence of abnormalities in *Vitis* hybrids from previous interspecific breeding programs (Barrett 1958a 1958b, E.P. Swenson personal communication).

Over both generations, *Vitis riparia* families were variable in their display of incongruity symptoms. Differences in progeny performance were also seen in instances where multiple pollen partners were used for one *V. riparia* parent in a class of cross. Even in *V. riparia* x *V. riparia* crosses, a low level of abnormalities was common. Therefore, in comparing interspecific progeny, those individuals with 0, or only 1 abnormality could be considered normal. As the breeder is most likely to be interested in efficient methods to effect gene transfer in divergent crosses, avoiding incongruity may be the most expedient course of action. In a highly heterozygous and variable genus such as *Vitis*, it may be possible to circumvent incongruity by surveying the desired species for more congruent parental combinations.

I propose the use of a congruity index, whereby the potential of a self- or cross-mating could be evaluated. High ratings in the congruity index would be expected to produce a higher percentage of useful progeny for screening. For *Vitis*, the congruity index would be defined as reproductive efficiency of an individual and its pollen partner, multiplied by the frequency of normal individuals in its progeny. A hermaphroditic F1 individual would be indexed by multiplying its reproductive efficiency, when selfed, by the frequency of normal plants in the F2 progeny (Table 1). In taxa where self pollination is impossible due to dioecy or self-incompatibility, an index of the cross would be calculated from the reproductive efficiency of the mating partners, multiplied by the frequency of normal individuals in the F1 progeny (Table 2). If further information is available, such as variation for fertility, reproductive efficiency, or germination of the progeny, it could be included in calculation of the index.

Development of the congruity index could be started during progeny evaluation from initial trial crosses. The additional generation time spent in procuring the F2 data is well spent, as any good F2 individual can be entered directly into further amelioration schemes or into the breeding program, and the additional meiotic cycle offers another opportunity for recombination. Sampling several F1 individuals from a cross would be advisable, as variation for index values among F1 plants may be great. For example, the separate congruity index values for the two F1 individuals of the cross Riparia 64 x Limberger were 0.12 and 0.87 (Table 1).

In any breeding program, aberrant progeny are commonly culled. As many symptoms of incongruity appear following a meiotic cycle, as in the F2 generation of the *Vitis* hybrids studied, the aberrant individuals may be those resulting from recombination. Therefore, breeders may wish to consider maintaining some aberrant individuals as putative recombinants that may be valuable in hastening gene transfer.

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Table 1. Congruity indices for 10 F1 hybrids from interspecific crosses involving *Vitis riparia*.

Vitis riparia female parent	Male parent	Pollen parent class[a]	F1 reproductive efficiency[b]	Frequency normal progeny[c]	Congruity index[d]
37	Limberger	V	1.39	0.18	0.25
39	Limberger	V	0.53	0.25	0.13
64 [e]	Limberger	V	0.47	0.26	0.12
64 [e]	Limberger	V	1.2	0.72	0.87
64	Carmine	V	0.59	0.5	0.3
89	Carmine	V	1.2	0.23	0.28
37	SV18-283	FH	1.4	0.33	0.47
39	SV18-283	FH	1.26	0.24	0.3
64	S11701	FH	0.92	0.46	0.42
89	L4511	FH	0.47	0.92	0.43

a V = *Vitis vinifera*, FH = French Hybrid

b Number of seeds per berry x percent berry set

c 0-1 morphological abnormalities per seedling

d Reproductive efficiency x frequency of normal progeny

e Full sibs

Table 2. Congruity indices for 7 intraspecific *Vitis riparia* crosses.

Female parent	Male parent	Cross reproductive efficiency[a]	Frequency normal progeny[b]	Congruity index[c]
37	Brandon	1.38	1.0	1.38
39	Brandon	1.04	0.63	0.65
64	Brandon	0.82	0.59	0.49
37	Farm 2	1.22	0.91	1.12
39	Farm 2	2.29	0.67	1.53
64	Farm 2	1.16	0.63	0.74
89	Farm 2	2.09	0.75	1.57

[a] Number of seeds per berry x percent berry set

[b] 0-1 morphological abnormalities per seedling

[c] Reproductive efficiency x frequency of normal progeny

APPENDIX A: Identification codes.

Code	Meaning
37	Riparia 37 (Manitoba female)
39	Riparia 39 (Manitoba female)
64	Riparia 64 (Manitoba female)
89	Riparia 89 (Minnesota female)
Fm	Farm 2 Riparia (Minnesota fenceline male)
Br	Brandon (Manitoba male) 3rd from the north
Cm	'Carmine'
Lm	'Limberger'
V2	SV18-283
V3	SV18-307
L4	L4511
B4	B4672
S1	S11701

Examples: 64/Lm-2 = Riparia 64 x 'Limberger', F1 plant # 2.

64xBr = Riparia 64 x Brandon male

APPENDIX B: Percent aborted flowers, percent shot berries, average number of berries per cluster, percent berry set, average number of seeds per berry, and reproductive efficiency for 1988 and 1989, and percent germination for 1989, only. Multiple clusters were averaged to obtain one value per plant.

ID code	*V. riparia* family	Cross class	Year	% abort. flowers	% shot berries	Avg. berries	% berry set
37/Lm-1	37	R x V	1988	57.97	13.25	42	26.34
37/Lm-2	37	R x V	1988	63.36	4.16	61.33	30.85
37/Lm-3	37	R x V	1988	80.33	0	18	14.75
37/V2-1	37	R x FH	1988	65.01	5.73	57	28.53
37/V2-2	37	R x FH	1988	55.41	2.14	48.33	41.02
37/V2-3	37	R x FH	1988	73.22	1.99	43	22.62
37/V2-4	37	R x FH	1988	53.27	3.31	73	43.13
37/V2-5	37	R x FH	1988	79	2.01	57.75	17.69
37/B4-1	37	R x FH	1988	88.12	1.98	10	9.9
37xBr	37	R x R	1988	31.62	2.42	71.67	62.68
37xFm	37	R x R	1988	0	0	85.67	96.52
39/Lm-1	39	R x V	1988	94.44	4.25	4	1.31
39/Lm-2	39	R x V	1988	72.12	0.61	57.25	45.93
39/V2-1	39	R x FH	1988	55.02	1.95	139.83	42.98
39/V2-3	39	R x FH	1988	42.99	13.32	113.67	42.9
39/S1-1	39	R x FH	1988	57.92	2.9	103.67	38.79
39/S1-2	39	R x FH	1988	39.38	1.88	93	58.13
39/S1-3	39	R x FH	1988	3.23	0.54	89	95.7
39xBr	39	R x R	1988	56.49	0	59.5	43.16
39xFm	39	R x R	1988	26.01	0	130.67	73.62
64/Lm-1	64	R x V	1988	69.85	12.67	31	16

ID code	*V. riparia* family	Cross class	Year	% abort. flowers	% shot berries	Avg. berries	% berry set
64/Lm-2	64	R x V	1988	72.48	3.56	23.67	21.45
64/Cm-1	64	R x V	1988	71.7	0.75	38	26.66
64/S1-2	64	R x FH	1988	37.36	9.26	84	52.65
64xBr	64	R x R	1988	29.59	1.22	54.5	69.2
64xFm	64	R x R	1988	31.6	2.21	41.25	64.27
89/Cm-1	89	R x V	1988	36.29	2.47	99	61.25
89/V3-1	89	R x FH	1988	39.09	17.25	80.33	38.23
89/V3-2	89	R x FH	1988	54.31	13.5	97.5	32.19
89/L4-1	89	R x FH	1988	44.23	1.52	67	54.25
89/L4-2	89	R x FH	1988	41.79	14.54	96	42.6
89/L4-3	89	R x FH	1988	53.15	7.8	106.33	39.05
89xBr	89	R x R	1988	19.78	9.75	121	70.19
89xFm	89	R x R	1988	28.28	7.34	82.5	59.96
37/Lm-1	37	R x V	1989	55.23	1.42	77	38.75
37/Lm-2	37	R x V	1989	32.41	9.73	107.33	57.39
37/Lm-3	37	R x V	1989	21.7	1.18	66.33	70.66
37/V2-1	37	R x FH	1989	59.17	19.75	22.33	21.08
37/V2-2	37	R x FH	1989	14.53	16.1	62.33	68.35
37/V2-3	37	R x FH	1989	36.42	6.94	97.67	56.48
37/V2-4	37	R x FH	1989	33.63	1.75	99	64.62
37/V2-5	37	R x FH	1989	37.26	8.06	120.33	52.99
37/B4-1	37	R x FH	1989	20.14	49.55	23	45.47
37xBr	37	R x R	1989	33.52	0.58	58	64.78
37xFm	37	R x R	1989	36.09	0	54	59.57
39/Lm-2	39	R x V	1989	68.92	3.9	54.33	26.86
39/V2-1	39	R x FH	1989	27.13	2.18	112.33	69.76

ID code	*V. riparia* family	Cross class	Year	% abort. flowers	% shot berries	Avg. berries	% berry set
39/V2-3	39	R x FH	1989	48.23	5.4	98	46.25
39/S1-1	39	R x FH	1989	31.4	16.19	95	51.5
39/S1-2	39	R x FH	1989	33.92	1.56	57.33	57.73
39/S1-3	39	R x FH	1989	13.51	0.41	101.33	86.08
39xBr	39	R x R	1989	17.74	21.11	32	53.92
39xFm	39	R x R	1989	15.85	4.59	60	78.83
64/Lm-1	64	R x V	1989	42.31	26.09	48.67	29.63
64/Lm-2	64	R x V	1989	20	10	20	66.67
64/Cm-1	64	R x V	1989	52.04	4.6	49	33.89
64/S1-2	64	R x FH	1989	38.45	5.66	80	54.95
64xBr	64	R x R	1989	43.94	12.98	39.33	38.4
64xFm	64	R x R	1989	34.07	8.77	71.5	49.44
89/Cm-1	89	R x V	1989	25.21	0.94	59	73.49
89/V3-1	89	R x FH	1989	31.84	1.98	95.67	64.96
89/V3-2	89	R x FH	1989	24.08	1.71	55	72.05
89/L4-1	89	R x FH	1989	48.45	1.44	82.33	41.95
89/L4-2	89	R x FH	1989	47.42	8.26	77.33	43.81
89/L4-3	89	R x FH	1989	59.81	4.38	30.67	32.79
89xFm	89	R x R	1989	26.89	0.96	78	70.72

ID code	*V. riparia* family	Cross class	Year	Seeds/ berry	Reprod. effic.	% germ.
37/Lm-1	37	R x V	1988	2.33	.	0.61
37/Lm-2	37	R x V	1988	2.47	.	0.76
37/Lm-3	37	R x V	1988	2.4	.	0.35
37/V2-1	37	R x FH	1988	1.57	.	0.45
37/V2-2	37	R x FH	1988	2.03	.	0.83
37/V2-3	37	R x FH	1988	1.5	.	0.34
37/V2-4	37	R x FH	1988	2.49	.	1.07
37/V2-5	37	R x FH	1988	2.4	.	0.42
37/B4-1	37	R x FH	1988	1	.	0.1
37xBr	37	R x R	1988	2.65	.	1.66
37xFm	37	R x R	1988	2.65	.	2.56
39/Lm-1	39	R x V	1988	2.2	.	0.03
39/Lm-2	39	R x V	1988	1.98	.	0.91
39/V2-1	39	R x FH	1988	2.25	.	0.97
39/V2-3	39	R x FH	1988	1.9	.	0.82
39/S1-1	39	R x FH	1988	2.18	.	0.85
39/S1-2	39	R x FH	1988	2.15	.	1.25
39/S1-3	39	R x FH	1988	2.68	.	2.56
39xBr	39	R x R	1988	2.04	.	0.88
39xFm	39	R x R	1988	2.8	.	2.06
64/Lm-1	64	R x V	1988	1.53	.	0.24
64/Lm-2	64	R x V	1988	2.12	.	0.45
64/Cm-1	64	R x V	1988	1.94	.	0.52
64/S1-2	64	R x FH	1988	1.87	.	0.98
64xBr	64	R x R	1988	2.23	.	1.54
64xFm	64	R x R	1988	2.43	.	1.56

ID code	*V. riparia* family	Cross class	Year	Seeds/ berry	Reprod. effic.	% germ.
89/Cm-1	89	R x V	1988	1.65	.	1.01
89/V3-1	89	R x FH	1988	1.53	.	0.58
89/V3-2	89	R x FH	1988	2.65	.	0.85
89/L4-1	89	R x FH	1988	2.08	.	1.13
89/L4-2	89	R x FH	1988	2.35	.	1
89/L4-3	89	R x FH	1988	1.6	.	0.62
89xBr	89	R x R	1988	3.25	.	2.28
89xFm	89	R x R	1988	2.93	.	1.76
37/Lm-1	37	R x V	1989	2.2	33.33	0.85
37/Lm-2	37	R x V	1989	2.43	28	1.39
37/Lm-3	37	R x V	1989	2.2	57.33	1.55
37/V2-1	37	R x FH	1989	1.17	62.23	0.25
37/V2-2	37	R x FH	1989	2.05	42.67	1.4
37/V2-3	37	R x FH	1989	1.43	50.67	0.81
37/V2-4	37	R x FH	1989	2.35	86.75	1.52
37/V2-5	37	R x FH	1989	2.4	76	1.27
37/B4-1	37	R x FH	1989	2.05	13.5	0.93
37xBr	37	R x R	1989	2.13	82.66	1.38
37xFm	37	R x R	1989	2.05	46.7	1.22
39/Lm-2	39	R x V	1989	1.97	30.67	1.53
39/V2-1	39	R x FH	1989	1.8	68	1.26
39/V2-3	39	R x FH	1989	2.23	73.67	1.03
39/S1-1	39	R x FH	1989	1.8	66.17	0.93
39/S1-2	39	R x FH	1989	1.53	53.33	0.88
39/S1-3	39	R x FH	1989	2.13	60	1.83
39xBr	39	R x R	1989	1.93	10.77	1.04

ID code	*V. riparia* family	Cross class	Year	Seeds/ berry	Reprod. effic.	% germ.
39xFm	39	R x R	1989	2.9	86.67	2.29
64/Lm-1	64	R x V	1989	1.6	57.33	0.47
64/Lm-2	64	R x V	1989	1.8	64.3	1.2
64/Cm-1	64	R x V	1989	1.73	65.17	0.59
64/S1-2	64	R x FH	1989	1.67	33.33	0.92
64xBr	64	R x R	1989	2.13	37.77	0.82
64xFm	64	R x R	1989	2.35	73	1.16
89/Cm-1	89	R x V	1989	1.63	60	1.2
89/V3-1	89	R x FH	1989	1.9	.	1.23
89/V3-2	89	R x FH	1989	3.05	44.3	2.2
89/L4-1	89	R x FH	1989	2.27	58.67	0.95
89/L4-2	89	R x FH	1989	2.33	44	1.02
89/L4-3	89	R x FH	1989	1.43	75.73	0.47
89xFm	89	R x R	1989	2.95	84.1	2.09

APPENDIX C: Number of F2 individuals displaying each character, arranged by class of cross and F1 family.

F1 ID	n	Dwarf	Varie-gated leaves	De-formed leaves	Mottled leaves	Sparse roots	Ena-tion
<u>R x V</u>							
37/Lm-2	11	4	1	3	5	3	3
39/Lm-2	8	6	0	3	6	0	3
64/Lm-1	43	17	14	10	18	15	3
64/Lm-2	18	0	4	4	9	6	1
64/Cm-1	48	6	14	18	14	20	13
89/Cm-1	13	11	0	1	8	3	6
<u>R x FH</u>							
37/V2-2	12	0	1	3	8	0	7
39/V2-1	17	3	0	5	7	6	12
64/S1-2	24	0	0	7	13	5	9
89/L4-3	24	5	0	1	1	5	1
<u>R x R</u>							
37 x Fm	34	1	0	0	0	1	2
37 x Br	16	0	0	1	0	0	0
39 x Fm	24	1	3	2	11	1	3
39 x Br	8	6	0	0	4	0	0
64 x Fm	52	0	2	2	17	0	19
64 x Br	27	1	1	0	3	1	12
89 x Fm	32	1	3	4	15	2	1

APPENDIX D: Number of F2 plants with multiple abnormalities, arranged by class of cross and F1 family.

F1 ID	n	0	1	2	3	≥4
R x V						
37/Lm-2	11	1	1	4	5	0
39/Lm-2	8	0	2	2	3	1
64/Lm-1	43	3	8	15	12	5
64/Lm-2	18	3	10	4	0	1
64/Cm-1	48	6	18	8	5	11
89/Cm-1	13	1	2	3	4	3
R x FH						
37/V2-2	12	2	2	5	3	0
39/V2-1	17	0	4	9	2	2
64/S1-2	24	6	5	6	5	2
89/L4-3	24	13	9	0	1	1
R x R						
37 x Fm	34	27	5	1	1	0
37 x Br	16	14	2	0	0	0
39 x Fm	24	10	6	5	1	2
39 x Br	8	1	4	1	2	0
64 x Fm	52	18	15	14	5	0
64 x Br	27	11	5	10	1	0
89 x Fm	32	10	14	7	1	0