HYBRIDS AND HERBIVORY: GENETIC PATTERNS

OF TOLERANCE IN HYBRIDS

THESIS

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HYBRIDS AND HERBIVORY: GENETIC PATTERNS

OF TOLERANCE IN HYBRIDS

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INTRODUCTION

When genetically divergent populations meet and reproduce in nature, such matings may result in the production of fertile hybrid offspring. In the past, hybridization has been perceived as a mostly maladaptive occurrence (Mayr 1942), with hybrid individuals demonstrated as being unfit relative to the parental species (Harrison 1986). However, it is now known that hybrid fitness is often highly variable, with some hybrid classes actually revealing fitness measures equal to or exceeding those of parental taxa (Endler 1973, Moore 1977, Arnold 1997). Such variable hybrid fitness can result in a wide range of ecological and evolutionary consequences when divergent taxa become sympatric and hybridize. If hybrids are generally unfit relative to the parental species, then prezygotic isolation may be favored by natural selection, resulting in the further isolation (and divergence) of the parental lineages (i.e. reinforcement: Schluter 2001, Coyne and Orr 2004). On the other hand, if hybrids experience increased fitness relative to the parental species, then hybrids may facilitate introgression across species boundaries (Rieseberg and Wendel 1993, Arnold 1997, Kim and Rieseberg 1999). For plants, a major environmental factor that impacts fitness is the presence of herbivores, with herbivore attack generally acting to reduce plant fitness (McNaughton 1983, Marquis 1984, Strauss et al. 1996, Strauss 1997, Maron 1998, Agrawal 1997,

1999). However, plants have evolved a diverse array of defense mechanisms to reduce both the chance and effects of herbivory (Strauss and Agrawal 1999). Defense mechanisms are traditionally classified into two broad categories: resistance (i.e. traits that reduce the incidence and / or level of herbivore damage) and tolerance (i.e. the degree to which plant fitness is reduced by herbivore damage relative to the fitness of the plant had it been undamaged) (Strauss and Agrawal 1999). As with many other important measures of fitness, when interspecific hybridization occurs between plant species, the fitness resulting from resistance / tolerance mechanisms in hybrid progeny may vary considerably. Hybrid fitness may be intermediate to the parental species, similar to one parent, or significantly higher or lower than the mean fitness of both parents. The degree to which hybrids differ in resistance / tolerance traits relative to their parents will likely depend on the underlying genetic basis of defense. While the genetic basis of plant resistance has been studied extensively, and a number of well-known mechanisms are related to resistance to herbivores [such as: a well-developed cuticle (Gaines 1985), tri-chromes (Levin 1973), biochemical compositions that are harmful to the herbivore (Fox 1981)], these traits may have arisen from selective pressures other than those associated with herbivory (Lindroth et al. 2001). For example: a well developed cuticle may have evolved as a result of drought tolerance / prevention of water loss (Jones et al. 1981); evolution of tri-chromes may have been the result of selective pressure for 'shading' in high-sun environments (Gupta 1979, Lam 1980); and a change in biochemical composition may have been selected for due to an increase in nutrient content, which may in turn be harmful to some herbivores (Loveless 1961, 1962; Medina et al. 1990). While effective resistance mechanisms result in reduced herbivory, the ability of a plant

to tolerate herbivory (maintain pre-herbivory fitness levels in the face of tissue loss) may be a more efficient method of defense than tolerance due to the potentially less costly nature of such preventative trade-offs (McNaughton 1983). Because of the direct effects of tolerance on plant fitness, and because tolerance mechanisms are likely not directly related to resistance mechanisms, it is prudent in this case to study tolerance independently of resistance.

Tolerance initiates at the onset of herbivory, and in fact seems to be a direct product of herbivory itself. Tolerant plants, by definition, have higher fitness compared to plants that do not have tolerance mechanisms to compensate for growth lost during herbivory. Insect herbivory has been shown to initiate a tolerance response in many plant species, (Paige 1992, Trumble et al. 1993, Zimmerman et al. 1996). However, insect herbivory is difficult to experimentally mimic because artificial mechanical damage does not mimic the chemical interactions between insect and plants that may be important in initiating tolerance responses to insect herbivory (Lehtilä 2003). Macroherbivory (i.e. the removal of large amounts of plant material) by ungulates, rabbits, and rodents has also been shown to initiate tolerance responses in a number of plant systems (Paige 1992, 1999; Agrawal 2000). Furthermore, a number of studies have successfully identified a diverse array of tolerance responses in plants by mimicking macroherbivory through the mechanical removal of plant tissue in nature, as well as in greenhouse experiments: *Panicum:* Wallace 1981; *Ipomopsis:* Paige and Whitham 1987; *Betula:* Hjalten et al. 1993; *Salix:* Bach 1994; *Sorghastrum, Artemisia, Aster:* Hickman and Hartnett 2002*; Helianthus:* Pilson and Decker 2002; *Geum, Prenanthes ,Carex and Luzula:* Moser and Schutz 2006; and *Pinus:* Hodar et al. 2008.

Many plant species have evolved multiple forms of tolerance mechanisms (Strauss and Agrawal 1999, Tiffin 2001). However, the underlying genetic basis of individual mechanisms is not widely understood. It is known that plants express heritable, genetic variation for many tolerance traits (Gartside and McNeilly 1974, Fokar et al. 1998, Strauss and Agrawal 1999), and the mechanisms of tolerance vary widely across taxonomic groups (Tiffin 2001). Hybridization results in increased genetic diversity (especially in late-generation hybrid classes) and can create new allelic combinations that can directly influence plant tolerance, especially if parental lineages have evolved different tolerance mechanisms. Selection may then act on these novel allelic combinations, potentially favoring some combinations not previously observed in parental genetic backgrounds; thereby potentially contributing to adaptive evolution, either through the creation of novel or extreme tolerance traits (hybrid vigor: Grant 1975, Rieseberg and Carney 1998), or through the introgression of alleles (which contribute to the expression of tolerance) across species boundaries (Stutz and Thomas 1964, Reiseberg and Wendel 1993, Jiang et al. 2000). Therefore, determining the basic genetic architecture of plant tolerance may help to predict the evolutionary outcomes of hybridization.

By examining the tolerance of known experimental hybrid classes, and comparing them to the tolerance of their progenitors, the underlying genetic architecture may be teased apart. Fritz et al. (1994) suggested four general alternative testable models of genetic inheritance for resistance traits: additive, dominance, hybrid susceptibility, and hybrid resistance based on the patterns (and genetic basis) of resistance exhibited among known hybrid genotypes (i.e. F1, F2, or BC) and parental taxa. A purely additive genetic model

of hybrid tolerance would result in hybrid tolerance measures being intermediate between those of the parental taxa. If the tolerance of hybrids differs significantly from one parent, but not the other, this pattern suggests that one or more genes influencing tolerance act in a dominant fashion (Fritz et al. 1994). Hybrids may also be more susceptible to herbivory (or less able to tolerate herbivory) than either parent [Hybrid Susceptibility hypothesis] (Whitham 1989; Keim et al. 1989; Boecklen and Spellenberg 1990), which may be due to the breakup of coadapted gene complexes (Dobzhansky 1937; Muller 1939). Alternatively, hybrids may be more resistant to herbivory than either parent [Hybrid Resistance hypothesis] (Fritz et al. 1994), which may be due to overdominance or positive epistasis (Whitlock et al. 1995, Carr and Dudash 2003). Thus, by examining patterns of herbivore tolerance among known hybrid classes and parents, we can distinguish among these various hypotheses, and begin to understand the underlying genetic mechanisms responsible for the observed patterns, even if we are unaware of the particular ecological / phenotypic mechanisms that underlie the differences.

Here, I utilize a model system, Louisiana *Iris*, which has been shown to be particularly amenable to examining the evolutionary consequences of natural hybridization, as well as examining the genetic architecture of reproductive isolation and adaptive introgression (Arnold 2000). The Louisina *Iris* species complex (which consists four interfertile species: *Iris brevicaulis, I. fulva, I. hexagona, I. nelsonii*) has been shown to experience significant herbivory from white-tailed deer (*Odocoileus virginianus*), nutria (*Myocastor coypus*) (Tobler et al. 2006), and swamp rabbits (*Sylvilagus aquaticus*) (Chapman 1984), in south-central Louisiana, potentially reducing the fitness of individual plants

experiencing such herbivory. Thus, selection for tolerance mechanisms that mediate the effects of macroherbivory might be expected in these plants.

The motivation for this research was therefore to determine whether or not the Louisiana Iris species Iris brevicaulis and Iris fulva, and their F1 and reciprocal backcross hybrids show differential tolerance to macroherbivory. I performed a common garden experiment using cloned *I. brevicaulis*, *I. fulva*, F1 and reciprocal backcross genotypes under varying levels of simulated herbivory to specifically determine: 1. Are there significant differences among cross types in asexual fitness measures? 2. If differences are found, what is the mode of genetic inheritance associated with tolerance (whether additivity, dominance, or epistasis is expressed)? And finally: 3. Does the genetic architecture of tolerance support assumptions of hybrids acting as a bridge or a barrier to gene flow between species?

METHODS

a. Study System

Louisiana *Iris* are long-lived sexually and asexually reproducing perennial plants which are abundant in the cypress swamps and hardwood forests of Louisiana and the Mississippi River valley and range from Florida to Texas. A number of reproductive isolating barriers have been documented in this complex (Martin et al. 2005, 2006, 2007, 2008; Taylor et al. 2009) yet hybrid zones persist in nature (Cruzan and Arnold 1993). Because reproductive isolation is incomplete, this system is amenable to crossing among all members of the species in the complex; thus, the genetic patterns of tolerance to herbivores may be studied.

b. Simulated Macroherbivory

One wild-collected individual from both of two commonly hybridizing species of Louisiana iris (*Iris brevicaulis* and *I. fulva*) were used to produce an F_1 generation (*I. brevicaulis*: maternal parent, *I. fulva*: pollen parent). Reciprocal, interspecific backcross populations (backcross to *Iris brevicaulis* and backcross to *I. fulva*, hereafter referred to as BCIB and BCIF, respectively) were then produced by mating F1 clones (pollen parents) to several clones of wild-collected *I. brevicaulis* and *I. fulva* parents (Bouck et al. 2005). These reciprocal backcross populations are the same mapping populations that have been used to map a variety of reproductive isolating barriers between *I. fulva* and *I. brevicaulis* (Bouck et al. 2005, 2007; Martin et al. 2005, 2006, 2007, 2008; Taylor et al. 2009). The experimental plants utilized for the present study included 88 *I. fulva*, 184 *I. brevicaulis*, 61 F1 hybrids of *I. fulva* and *I. brevicaulis*, 512 BCIB and 380 BCIF hybrid individuals (individual plants).

Plants were first clipped of all leaves and roots on 3 November 2009. Bare rhizomes were immediately weighed and potted in 8in. azalea pots and allowed to grow for four months (until 22 February 2010), at which time all plants were randomly assigned one of three tolerance treatments: in which 0%, 50% or 100% of the above-ground tissue was removed. On 22 February 2010, plants were clipped of leaves wherein: one clone of each grouping was left intact (0% of above ground biomass removed) for use as a control and the other two clones of each grouping were either clipped of 50% or 100% of their total height. Paired triplicate clones of the aforementioned cross types were placed in a randomized design at the Texas State University Greenhouse grounds. Both experimental

and control plants were then allowed to grow throughout the flowering season (February-May 2010).

c. Response variables

I utilized a number of measures of asexual fitness as the response variables in this study: plant height, leaf weight, root weight and rhizome weight. These variables were recorded in the following manner: plant height was assessed (following approximately three months of growth) on 1 June 2010, by measuring the two tallest leaves of each plant. An average of the two measurements was used to estimate individual plant height (an estimate of plant growth in the face of herbivory). Leaves and roots of each plant were clipped immediately after the measurement of height, placed into separately labeled paper bags, and dried at 80 degrees Celsius for a minimum of 10 days. Dry weight of leaves and roots were then measured separately on 10 June 2010. Rhizome weight was measured on 1 June 2010, immediately following leaf and root removal, for each individual plant.

d. Statistical analyses

All dead or missing plants prior to executing treatments were removed before beginning any statistical analyses. A fully crossed three-way multiple analysis of covariance (MANCOVA) was used to assess differences between treatment (0%, 50%, or 100% simulated herbivory) and cross type (*I. brevicaulis*, BCIB, F1, BCIF, and *I. fulva*). Four response variables (plant height, leaf weight, root weight and rhizome weight), one covariate (initial rhizome weight), and all possible two- and three-way interactions

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between these variables were assessed. The interaction of most interest was the cross type by treatment interaction, because a significant interaction between these two variables would indicate that cross types are responding differently to treatments. Following MANCOVA, separate analyses of covariance (ANCOVAs) were performed for each of the four response variables, using the same predictors and covariate as in the MANCOVA. If a significant treatment by cross type interaction was observed, two different measures of tolerance were then obtained for each cross type: mean tolerance at 50% simulated herbivory, and mean tolerance at 100% simulated herbivory. These measures were obtained by dividing the mean of the cross type at 50% treatment by the mean of the same cross type at 0% treatment and the mean of the cross type at 100% treatment divided by the mean of the same cross type at 0% treatment, respectively. Estimating tolerance in this manner allows a straight-forward interpretation of results. If the ratio of damaged / undamaged is one, the plant is wholly tolerant; a ratio greater than one suggests over-compensation; and as the ratio approaches zero, the tolerance declines. A measure of variance was calculated for this ratio using the equation:

$$\mathbf{Var(X/Y) = (X/Y)^2 \{[Var(X)/X^2] + [Var(Y)/Y^2] - 2[Cov(X,Y)/XY]\}}$$

Standard error was then calculated for each cross type ratio at each treatment level (50% and 100% damage) by taking the square root of the variance from the equation calculated above (Manly et al. 1993).

To determine whether F1 hybrid means differed from a null model of pure additivity, planned linear contrasts were then performed on each significant response variable. The least square means tolerance data were used in these analyses to determine whether

differences between the means of parents and F1s significantly differed from the mid-parent values. Two other planned linear contrasts were then performed to determine whether each back cross was different from the mid-parent value of the F1 and recurrent parent means. This line cross analysis was performed to test whether backcross lines differed significantly from an additive-dominance model. Deviation from the model indicates epistasis. Therefore, three independent contrasts were run for each response variable (when a significant interaction was observed) to determine whether the mean value of F1 tolerance was different than the mid-parent (ie. between *I. brevicaulis* and *I. fulva*) mean value of tolerance, as well as if the mean tolerance value of the back cross classes differed from the mid-parent value of the F1 and recurrent parent mean tolerance.

Survivorship and flowering data were collected to determine the extreme fitness effects, if any, to simulated herbivory. In other words, if plants are wholly intolerant, they would not survive, and if herbivory directly affected sexual fitness, flowers (and therefore potentially more offspring) may be produced. Plants that did not survive after treatments were recorded, and consequently removed from the above statistical analyses. A logistic regression was performed to determine whether there was a significant effect of cross type, treatment, or an interaction between the two, on survivorship after treatment. Those plants which flowered were also recorded, and a logistic regression was performed to assess whether there were any significant differences among cross type, treatment, and the interaction between these two predictor variables, with respect to the nominal data: presence or absence of flower per plant.

RESULTS

The MANCOVA revealed significant main effects of cross type, treatment, and initial weight, as well as all two-way and the three-way interaction, except the treatment by initial weight interaction (Table 1). The cross type by treatment interaction, which describes the difference in fitness effects of cross types due to herbivory treatment, was significant (p-value = 0.0049). Therefore, further statistical examination of the data by was performed using separate ANCOVAs to determine the true nature of the differences.

Because *Iris brevicaulis* plants are generally shorter, broad-leaf plants found in "dry" habitats in open sun, and *I. fulva* plants are taller, narrow-leaf plants found in shaded habitats inundated with up to approximately 30cm of water year-round, differences in response variables (plant height, leaf weight, root weight, and rhizome weight) may be expected to differ among cross types. ANCOVAs revealed that all main effects (cross type, treatment, and initial weight) were significant for all response variables (plant height, leaf weight, root weight, rhizome weight) (Table 2). Separate ANCOVAs also revealed significant cross type by treatment interactions in two of the four response variables: plant height (p-value = 0.001) and root weight (p-value = 0.0036) (Figure 1) again suggesting difference in tolerance among cross types with respect to herbivory treatments.

Linear contrasts performed on the means of the two response variables that revealed significant interactions (plant height and root weight) at both 50% herbivory level, and 100% herbivory level revealed differential tolerance responses within and among response variables and treatment levels. Line-cross analysis did not reveal significant

departures from the assumption of additivity for plant height at 50% damage level, in that no significant differences were found between F1 tolerance levels and the mid-parent tolerance level between *I. brevicaulis* and *I. fulva* ($F_{1,97} = 0.3864$, p-value = 0.5357). Further, no significant deviation from the additive pattern was found between each back cross [BCIB ($F_{1,97} = 0.4083$, p-value = 0.5236) and BCIF ($F_{1,97} = 2.5162$, p-value = 0.1147)] for tolerance to 50% damage revealed by the response variable: plant height (Figure 2a). For root weight, F1 hybrid tolerance was found to be significantly lower than the expectation of additivity (suggestive of underdominance) [F1 ($F_{1,162} = 4.7309$, p-value = 0.032)]. Back crosses were found to be significantly lower than the additive dominance model, suggesting epistasis for root weight tolerance after 50% damage [BCIB ($F_{1,162} = 16.1395$, p-value < 0.0001), and BCIF ($F_{1,162} = 4.6954$, p-value = 0.0317)] (Figure 2b).

All three contrasts examining tolerance after 100% damage were significantly different from the expectation of additivity / additive dominance, for both fitness response variables: plant height [F1 ($F_{1,97} = 9.7572$, p-value = 0.0024), BCIB ($F_{1,97} = 11.019$, p-value = 0.001), BCIF ($F_{1,97} = 31.8489$, p-value < 0.0001)], and root weight [F1 ($F_{1,162} = 113.396$, p-value < 0.0001), BCIB ($F_{1,162} = 80.9884$, p-value < 0.0001), and BCIF ($F_{1,162} = 172.591$, p-value < 0.0001)].

Significant differences were found among cross types with respect to survivorship ($\chi^2 = 22.993$, p-value < 0.0001), with the lowest survivorship found in *I. fulva* (74% survival), and the highest found in BCIF (92% survival). No significant effect of herbivory treatment was observed with respect to survivorship ($\chi^2 = 0.0164$, p-value = 0.8981).

Flowering was not affected by cross type ($\chi^2 = 10.390$, p-value = 0.2387), or by treatment ($\chi^2 = 1.096e-5$, p-value = 1.0000).

DISCUSSION

Louisiana *Iris* have been shown to possess multiple pre- and post-zygotic isolating barriers which are incomplete (Cruzan and Arnold 1994, Emms et al. 1996, Martin et al. 2007, Martin et al. 2008). Hybridization in this complex does occur in nature, and many aspects concerning the maintenance of these natural hybrid zones have been documented. In this study, I attempted to determine whether the effects of herbivory could be acting as another mechanism by which hybrid zones persist in nature, thereby allowing interspecific gene flow in this system. Significant differences were found for fitness response measurements (plant height and root weight) of tolerance to simulated macroherbivore damage. Furthermore, the mode of inheritance by which tolerance traits are passed from parental species to hybrids can be attributed to epistasis, wherein hybrid fitness significantly deviates from a null model of additivity / additive dominance. These findings support the current literature that hybrids in the Louisiana *Iris* species complex may be acting as a bridge to gene flow between pure species progenitors.

Concerning the four models of genetic inheritance (additive, dominance, hybrid susceptibility, and hybrid resistance/tolerance) proposed by Fritz et al. (1994), the two response variables that were found to be affected by simulated macroherbivory (plant height and root weight) express similar modes of inheritance at the 100% damage treatment level. At 100% damage, the combination of divergent alleles culminates in an over-expression of tolerance in the F1 generation (with respect to the parental species)

which may be best explained by the hybrid resistance/tolerance model. However, allelic breakdown is expressed in the back cross generation for both plant height and root weight (Figures 3a and 3b), presumably due to epistasis.

Epistasis may be a major contributing factor to genetic diversity among species (Doebley et al. 1995). Any pattern deviating from the null model of additivity or additive dominance can be attributed to epistasis, which may be thought of as the phenotypic result of interacting non-allelic loci (Kelly 2005). Epistasis may be the most powerful mechanism underlying selection, leading to the evolution of favorable gene combinations (Wright 1930). In this study, it may be assumed that the divergent species (*I. brevicaulis* and *I. fulva*) have evolved different genetic mechanisms for the phenotypic expression of tolerance, and when these different alleles are recombined, overdominance is expressed in the F1 generation, which quickly breaks down within a single generation in back cross hybrids (Figures 3a and 3b). This is presumably due to heterosis, the condition in which heterozygous individuals express overdominance, which disperses in any generation thereafter, due to the breakup of coadapted gene complexes. Heterosis was indicated for two measures of asexual fitness (plant height and root weight). F1 fitness was significantly greater than the midparent, and back crosses were significantly lower than the expectation of additive dominance, which is consistent with a model of negative epistasis reducing the tolerance response of backcross hybrids. However, while backcrosses generally have significantly lower tolerance than that expected under an additive-dominance model, such responses are generally not significantly different from the parents, suggesting that this break down of fitness may not be a strong barrier to gene flow. In other words, the lower fitness of back crosses observed in this study may not be

acting to limit gene flow between species, because the lower mean fitness of back crosses is generally not lower than the mean fitness of the either of the pure species parents.

The overdominance in the F1 generation in this study mirrors the F1 hybrid fitness expressed by this same cross in sexual fitness traits (proportion of flowering plants, proportion of plants that set fruit, and number of fruits per flower) as well as asexual reproduction (asexual growth points) recorded in a previous study (Taylor et al. 2009). While high F1 fitness has been documented in multiple systems (reviewed in Arnold and Hodges 1993), the breakdown of fitness due to heterosis is common in back cross generations (Coyne and Orr 2004). This is not to say that reduced fitness in back cross generations will necessarily decrease the potential for gene flow across species boundaries. Because genotypic diversity is much higher in later generation hybrids, the range of fitness will be much greater in back cross generations. Although the mean fitness is lower than parents in the back cross generation in this study, the most fit back cross hybrid may be more fit than either parent, facilitating introgression of adaptive alleles across species boundaries. It may also be noted that any level of fitness in hybrids greater than zero allows the potential for gene flow between species (Arnold 1997).

Regarding survivorship differences among cross types: Louisiana *Irises* are found in a range of environments in the cypress swamps and hardwood forests of central and south Louisiana. Differing tolerance to environmental variants such as sunlight, shade, flooding and drought have been documented in this complex (Taylor et al. in review). The two species used in this study have been found to show differential tolerance to flooding, with *I. fulva* showing significantly higher survivorship when inundated by flood water for an

extended period of time (more than 8 weeks) (Martin 2006). For this experiment, plants were left outside following treatments, in central Texas for two months to allow re-growth. Perhaps the substantial difference in humidity and climate found between the natural habitat of these plants and the conditions under which they were grown for this experiment may explain the high mortality rate in *I. fulva* (which is most tolerant to excessive water conditions).

In this study, the tolerance of pure-species and hybrid Louisiana Iris was examined, but only in a single environment. Tiffin (2000) explains that more tolerant plants will tolerate herbivory in a range of environments (or levels of herbivory). Measuring fitness in different environments, or in relation to differing degrees of stress / damage is important in understanding the evolution and expression of tolerance in plants. Although a genotype may be more tolerant at one stress level, the overall tolerance may be misinterpreted by only measuring the effect of one level of stress (Simms 2000). This is inherent in the definition of tolerance: the degree to which plant fitness is affected by damage relative to the fitness in an undamaged state. In this study, the degree to which plants tolerated herbivory at the 50% treatment level did significantly differ than that measured at the 100% treatment level for the plant height response variable. This may be explained by the nature of the damage that is driving selection on this trait. In nature, if macroherbivores are generally removing 100% of above-ground biomass in this complex (which is what has been observed in the field by Martin, Dobson, Shaw and Taylor-personal observations), tolerance will be more pronounced in the extremely damaged state.

Tolerance and resistance are inversely related in that plants allocate resources to both strategies (Núñez-Farfán et al. 2007). Because resources are finite, increase in the resources allotted to one defense mechanism will result in less available resources for any other defense strategy. Therefore, some study the joint evolution of resistance and tolerance mechanisms. Due to the increased number of variables involved in such a study, it may be far more difficult to determine the effects of either strategy. By limiting the confounding effects of resistance mechanisms in this study, we are able to focus on the fitness traits affected by plant tolerance. Because each of these defense mechanisms is heritable, and dependent on environmental factors, it was necessary to pair clones to limit the influence of environmental variability on the results.

Speciation is dependent on the culmination of reproductive isolating barriers, which evolve as a result of divergent natural selection on traits affecting fitness. In contrast, adaptive introgression can be thought of as a means by which divergent lineages may share traits which increase fitness. Naturally hybridizing plants allow for inspection of these two theories. In this case, I have shown that tolerance is a trait in which F1 hybrids express higher fitness than either parent. Because evidence of heterosis was observed in this study, and because backcross hybrids are fit relative to their parents the potential for gene flow is likely. Tolerance is yet another mechanism which shows that hybrids may act as a bridge to gene flow in the Louisiana *Iris* complex. This study has provided insight into the evolution and expression of herbivore defenses in plant systems, and the evolution of extrinsic, postzygotic mechanisms that may enhance or reduce the chance for interspecific gene flow in nature.

TABLES

Table 1. Results of multiple analysis of covariance (MANCOVA)

| Source of variation	Wilks' lambda	df_n,df_d	P-value
Cross type	0.8191961	16,3523.1	**<0.0001**
Treatment	0.9009644	8,2306	**<0.0001**
Initial weight	0.4591035	4,1153	**<0.0001**
Cross type*treatment	0.9524532	32,4253.6	**0.0049**
Cross type*initial weight	0.9294219	16,3523.1	**<0.0001**
Treatment*initial weight	0.9872933	8,2306	0.0639
Treatment*initial weight*cross type	0.9494704	32,4253.6	**0.0019**

Table 2. Results of analysis of covariance (ANCOVA) for each response variable.

| Response variable & Source of variation	df	SS	F Ratio	P-value
Plant height:				
Cross type	4	2318.8256	7.3011	**<0.0001**
Treatment	2	8556.0563	53.8792	**<0.0001**
Initial weight	1	5892.5089	74.2127	**<0.0001**
Cross type*treatment	8	2088.4918	3.2879	**0.0010**
Cross type*initial weight	4	3626.5835	11.4187	**<0.0001**
Treatment*initial weight	2	555.5114	3.4982	**0.0306**
Treatment*cross type*initial weight	8	502.1798	0.7906	0.6110
Leaf weight:				
Cross type	4	4.564142	2.6016	**0.0347**
Treatment	2	83.895026	95.6409	**<0.0001**
Initial weight	1	42.355544	96.5712	**<0.0001**
Cross type*treatment	8	3.242510	0.9241	0.4953
Cross type*initial weight	4	8.092596	4.6128	**0.0011**
Treatment*initial weight	2	1.996386	2.2759	0.1032
Treatment*cross type*initial weight	8	4.720407	1.3453	0.2168
Root weight:				
Cross type	4	12.323305	37.7750	**<0.0001**
Treatment	2	1.639197	10.0494	**<0.0001**
Initial weight	1	17.923024	219.7597	**<0.0001**
Cross type*treatment	8	1.874548	2.8731	**0.0036**
Cross type*initial weight	4	3.846305	11.7902	**<0.0001**
Treatment*initial weight	2	0.460031	2.8203	0.0600
Treatment*cross type*initial weight	8	1.675873	2.5686	0.0089
Rhizome weight:				
Cross type	4	855.324	4.6063	**0.0011**
Treatment	2	953.641	10.2716	**<0.0001**
Initial weight	1	22275.087	479.8450	**<0.0001**
Cross type*treatment	8	306.696	0.8258	0.5798
Cross type*initial weight	4	322.128	1.7348	0.1399
Treatment*initial weight	2	11.412	0.1229	0.8843
Treatment*cross type*initial weight	8	1784.909	4.8063	**<0.0001**
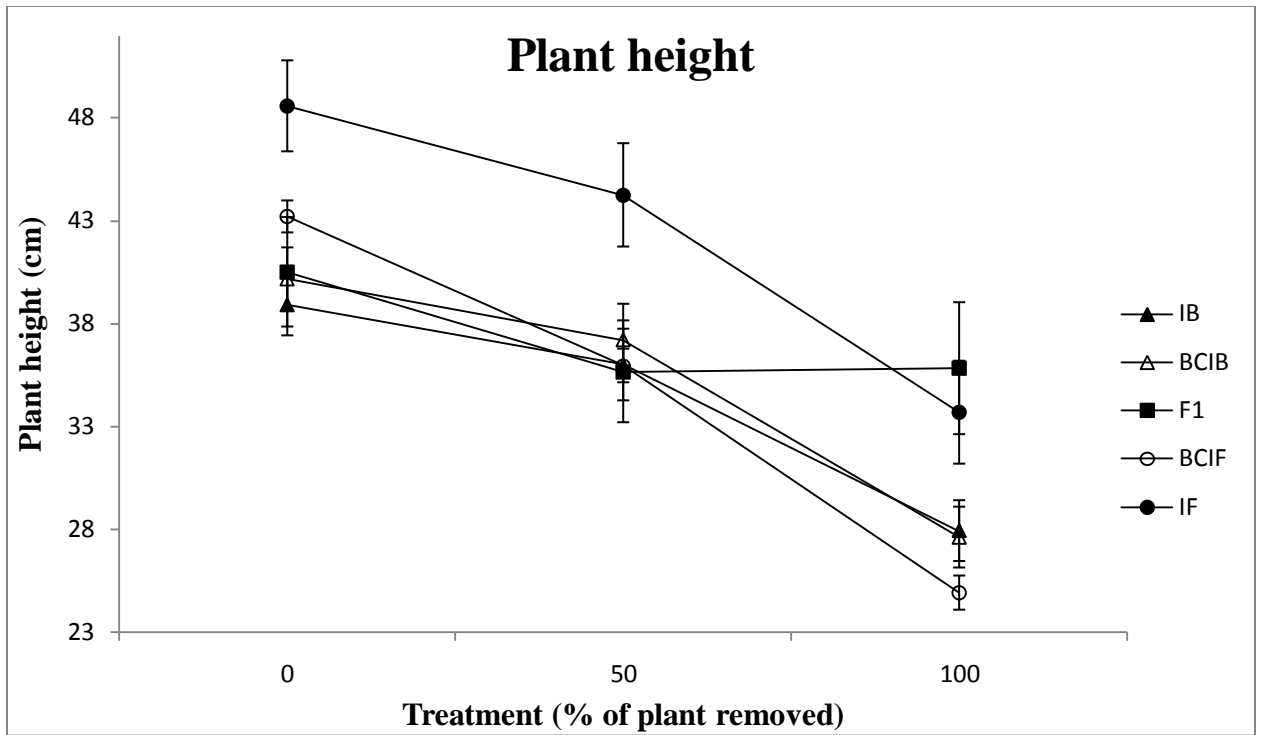
Figure 1a: Least square means (± SE) of plant height (cm) for each cross type (*I. brevicaulis*, BCIB, F1, BCIF, and *I. fulva*) plotted at each treatment or damage level (0%, 50% and 100%).
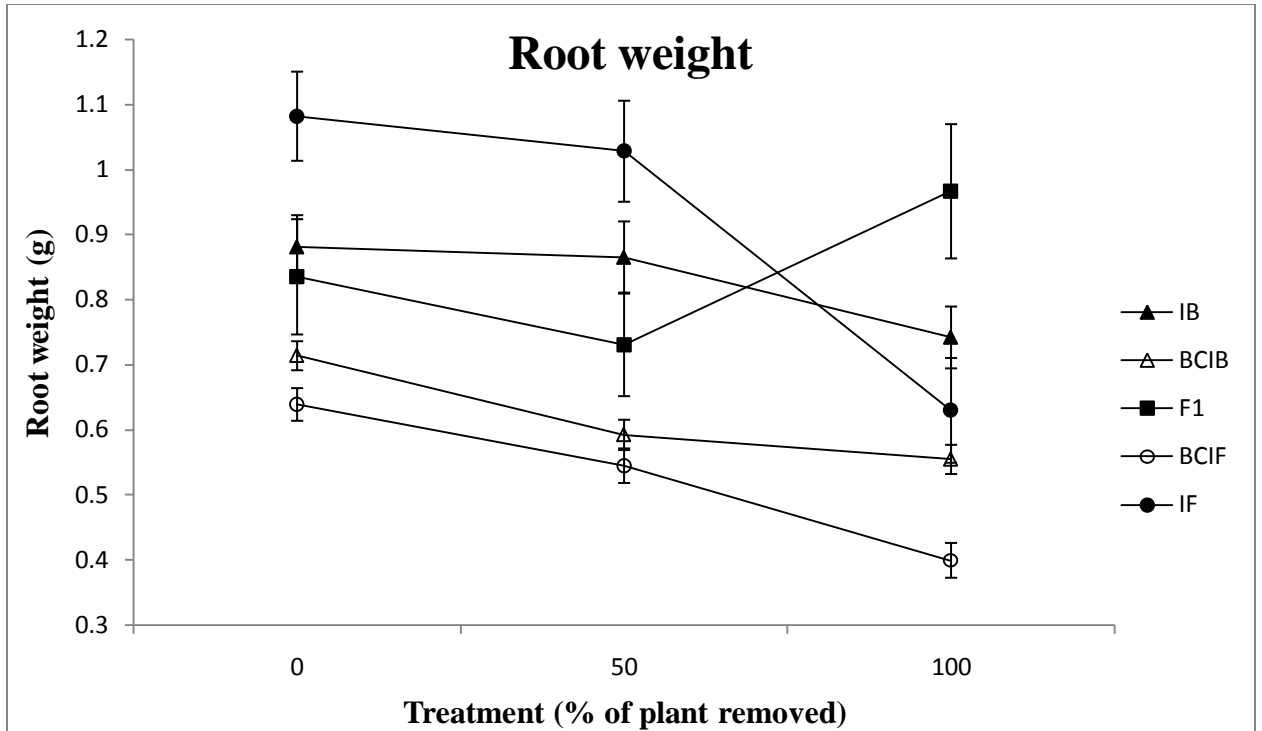
Figure 1b: Least square means (± SE) of root weight (g) for each cross type (*I. brevicaulis*, BCIB, F1, BCIF, and *I. fulva*) plotted at each treatment or damage level (0%, 50% and 100%).
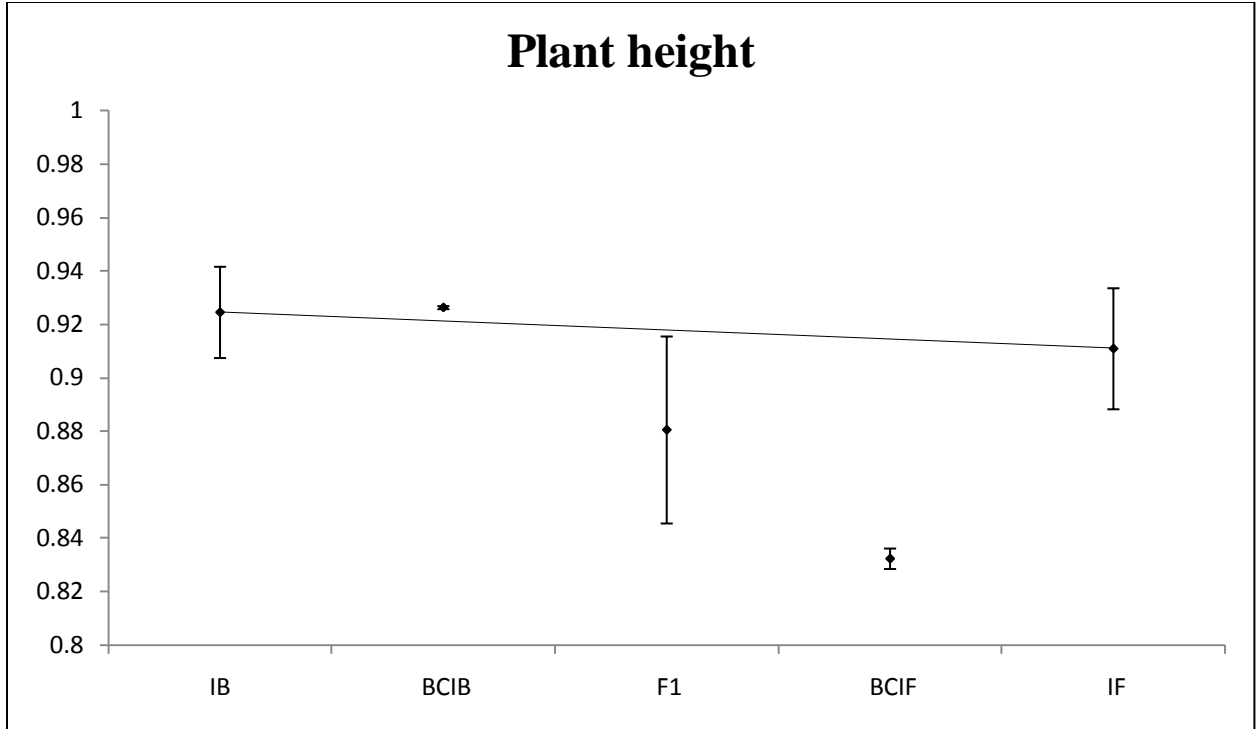
Figure 2a: Tolerance of plants with respect to plant height fitness variable (±SE) after 50% leaf removal. Tolerance is measured by LSM of cross type at 50% damage divided by LSM of same cross type at 0% damage (damage/undamage). Lines between parents denote expectations for additivity. Asterisks represent tolerance levels which are significantly different from the expectation of additivity.

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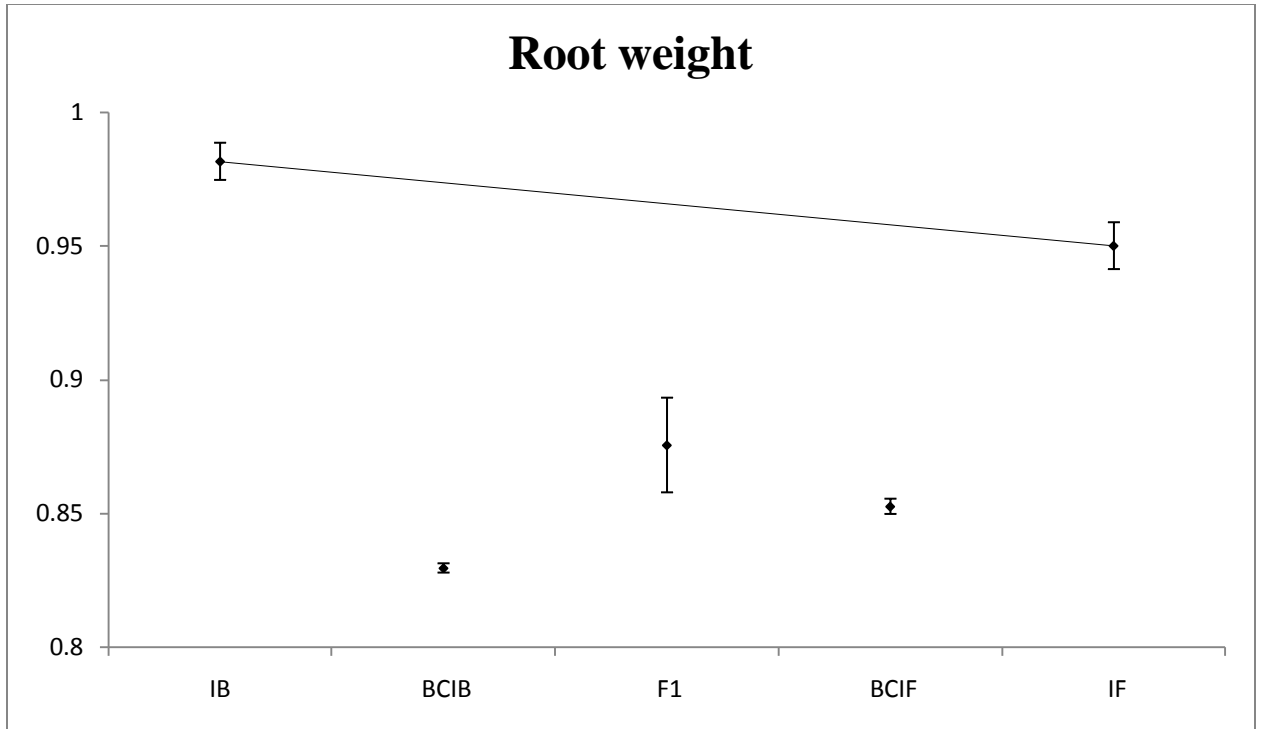
Figure 2b: Tolerance of plants with respect to root weight fitness variable (±SE) after 50% leaf removal. Tolerance is measured by LSM of cross type at 50% damage divided by LSM of same cross type at 0% damage (damage/undamage). Lines between parents denote expectations for additivity. Asterisks represent tolerance levels which are significantly different from the expectation of additivity.
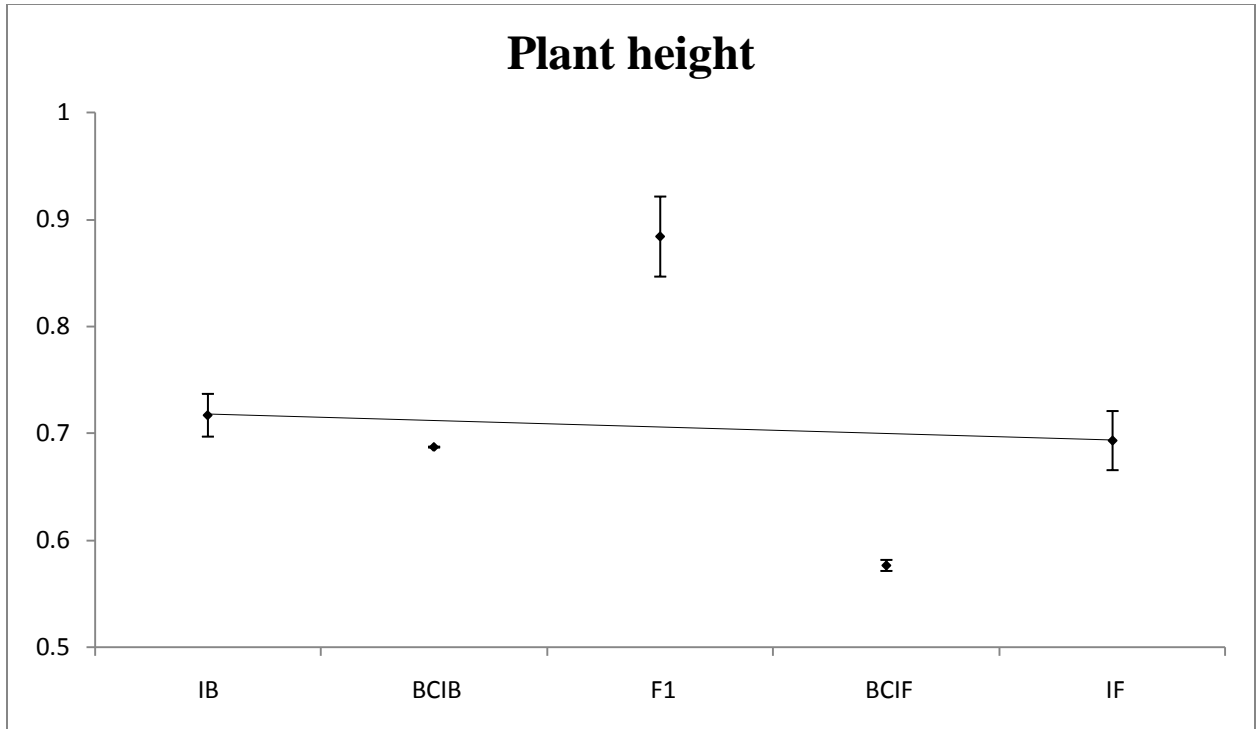
Figure 3a: Tolerance of plants with respect to plant height response variable (±SE) after 100% leaf removal. Tolerance is measured by LSM of cross type at 100% damage divided by LSM of same cross type at 0% damage (damage/undamage). Lines between parents denote expectations for additivity. Asterisks represent tolerance levels which are significantly different from the expectation of additivity
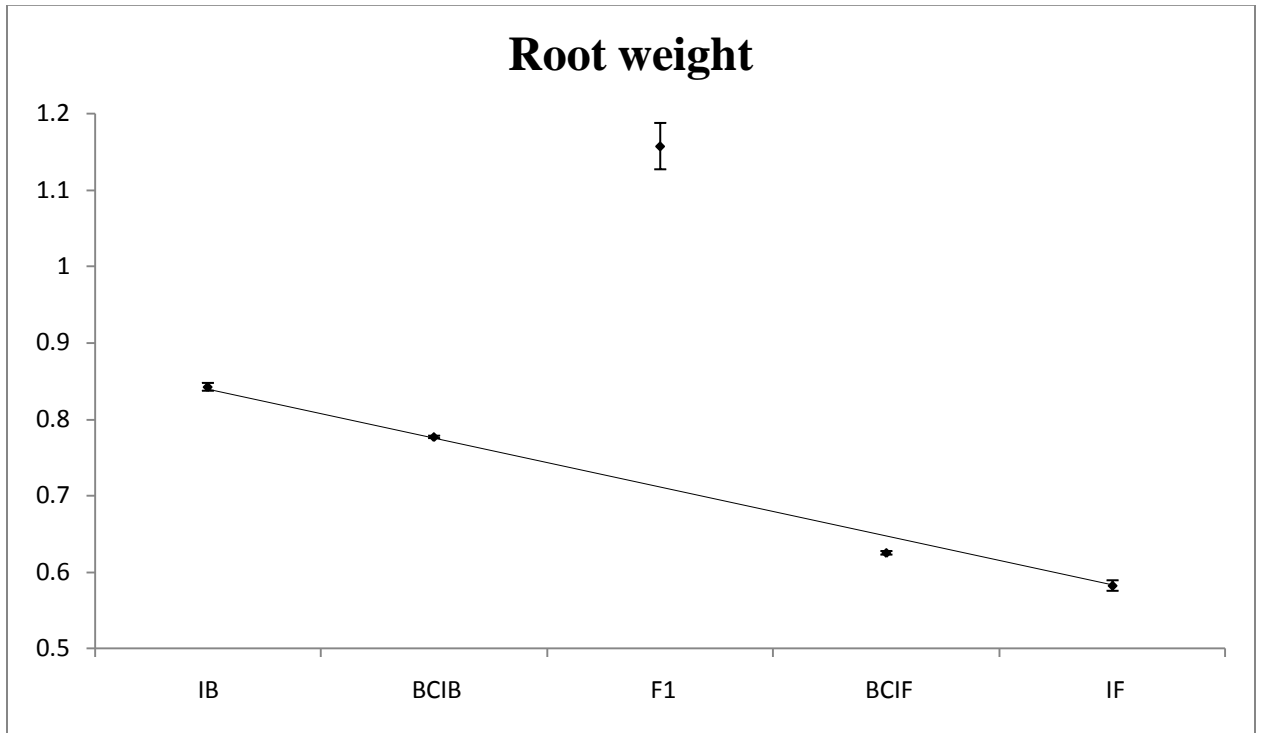
Figure 3b: Tolerance of plants with respect to root weight response variable (±SE) after 100% leaf removal. Tolerance is measured by LSM of cross type at 100% damage divided by LSM of same cross type at 0% damage (damage/undamage). Lines between parents denote expectations for additivity. Asterisks represent tolerance levels which are significantly different from the expectation of additivity.

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VITA

Mary Caroline Dobson was born in Baton Rouge, Louisiana, and graduated from Tara High School in 2000. In 2002, she moved to Cape Cod, Massachusetts, and graduated from Cape Cod Community College with an Associates Degree in Liberal Arts in 2005. Dreading another Cape Cod winter, Mary moved to Austin, Texas, and after two more years of working in the food industry, made a solemn promise to herself to never wait another table again. She then enrolled at Texas State University-San Marcos, and began undergraduate research with Noland H. Martin, her Genetics professor who boffed at her mention of med-school, and eventually strong-armed her into agreeing to finish a master's degree. Mary received her B.S. degree in 2008, in Biology, with a minor in Psychology. She has attended and presented at regional, national, and international biology conferences. Mary hopes to teach undergraduate Biology and Psychology courses at a junior college after graduation, so that she too can inspire students as she was inpired by her first college biology professor, Dr. Lynda Farley LaRocca.

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