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Patterns of herbivory and fungal infection in experimental Louisiana *Iris* hybrids

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ABSTRACT

Background: The consequences of hybridization vary depending on the fitness of hybrids and pure species plants. Herbivory and fungal infection may affect fitness and often differ in their effect on hybrid versus pure-species genotypes.

Questions: Do herbivory and fungal infection rates differ between pure species and hybrids? What is the underlying genetic basis of resistance to herbivory and fungal infection?

Study system: Two ecologically similar species of Louisiana *Iris*: *I. brevicaulis* and *I. fulva*, and their F_1 and first-generation backcross hybrids.

Methods: Mammalian herbivory (macroherbivory), insect herbivory (microherbivory), and fungal infection were assayed in an experimental plot of 930 irises, including pure species and three hybrid classes planted in native Louisiana *Iris* habitat in the Atchafalaya swamp of southern Louisiana.

Results: Macroherbivory rates were low in the field plot and did not differ significantly between crosstypes. Microherbivory and fungal infection rates differed significantly between crosstypes. Microhervibory did not differ significantly between the pure species plants and was lower than expected in one backcross class. The fungus *Puccinia iridis* infected one F_1 hybrid class at significantly greater rates than all other experimental crosstypes. Backcross hybrids towards *I. brevicaulis* were also infected by *P. iridis* at rates intermediate between the highly infected F_1 class and its recurrent parent, suggesting a pattern of underdominance for resistance traits in the F_1.

Keywords: fungal infection, herbivores, hybrid fitness, hybridization, Louisiana *Iris*.

INTRODUCTION

Natural hybridization is a commonly observed phenomenon that can result in a number of evolutionary consequences, including the introgression of adaptive traits (Arnold, 1992; Rieseberg, 1997; Martin *et al.*, 2006; Whitney *et al.*, 2006), the extinction of species due to genetic swamping (Whitham

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et al., 1999; Carney *et al.*, 2000; Haygood *et al.*, 2003; Hegde *et al.*, 2006; Prentis *et al.*, 2007), and the formation of new hybrid taxa (Stutz and Thomas, 1964; Grant, 1981; Rieseberg, 1997, 2006; Gross *et al.*, 2003; Hegarty and Hiscock, 2005). The evolutionary outcome of natural hybridization will be affected by the fitness of interspecific hybrids, and hybrid fitness has been shown to be quite variable depending on the taxa and hybrid genotypes being examined (Arnold and Hodges, 1995; Arnold, 2006). In addition, hybrid fitness is often environment-dependent such that the fitness of particular genotypes will be different when exposed to different ecological variables (Hatfield and Schluter, 1999; Egan and Funk, 2009; also reviewed by Arnold, 2006).

Plant herbivores and fungal parasites can have potentially important impacts on an individual's fitness (Marquis, 1992). Hybrid and pure-species plants often reveal differential responses to such selective agents, with hybrids revealing intermediate, equal, increased or decreased resistance compared with the pure species individuals (Boecklen and Spellenberg, 1990; Fritz *et al.*, 1994, 1996; Fritz, 1999), depending on the underlying genetic basis of resistance (Fritz *et al.*, 1994). In some systems, resistance appears to be due to the additive action of multiple genes (e.g. Boecklen and Spellenberg, 1990), where hybrid resistance is intermediate to that of the pure-species parents (Fritz *et al.*, 1994). Dominance or over-dominance at loci involved in resistance can result in hybrids with similar or greater resistance than the pure-species individuals (e.g. Whitlock *et al.*, 1995; Orians and Floyd, 1997; Carr and Dudash, 2003). Alternatively, the break-up of co-adapted gene complexes can result in the production of hybrids with less resistance to herbivores than the pure species (e.g. Keim *et al.*, 1989; Boecklen and Spellenberg, 1990; Floate *et al.*, 1993; Fritz *et al.*, 1994, 1996). Hybrids that are less resistant to herbivory or infection than the pure species may have low fitness, suggesting that susceptibility to herbivory or fungal infection may result in post-zygotic isolation between species. However, hybrids that are resistant to herbivory or infection may have high fitness and can potentially evolve on an independent evolutionary trajectory or facilitate adaptive introgression between the hybridizing species (Whitney *et al.*, 2006, 2010).

The current study was designed to examine differential resistance to herbivore (both mammalian and insect herbivores) and fungal attack among *Iris brevicaulis*, *Iris fulva*, their F_1 and reciprocal backcross hybrids planted into the natural habitat of Louisiana irises. The Louisiana *Iris* group consists of four species: *I. brevicaulis*, *I. fulva*, *I. hexagona*, and *I. nelsonii. Iris fulva* and *I. brevicaulis*, the two species utilized in the current study, are the most ecologically similar (Viosca 1935), and can be found in close sympatry in southern Louisiana (Cruzan and Arnold, 1993; Johnston *et al.*, 2001). Although F_1 hybrid formation is extremely rare due to a number of strong pre-zygotic barriers (Arnold, 2006), those F_1 hybrids that are produced between *I. fulva* and *I. brevicaulis* are fertile, viable, and reveal extremely high levels of clonal and sexual fitness relative to that of their progenitor species (e.g. Emms and Arnold, 1997; Burke *et al.*, 1998; Taylor *et al.*, 2009). Owing to this relatively high fitness of F_1 hybrids (and despite their relative rarity), natural hybrid zones consisting of late-generation hybrids are produced, and are indeed common in southern Louisiana where the two species co-occur. This has even resulted in the introgression of both genetic and morphological traits across species boundaries (e.g. Arnold *et al.*, 1990; Cruzan and Arnold, 1994).

A variety of mammalian herbivores (hereafter referred to as 'macroherbivores') consume leaves of Louisiana irises in their native habitats. Such macroherbivores remove large amounts of plant biomass likely resulting in reduced fitness to those individual plants experiencing such herbivory. These macroherbivores include whitetail deer (*Odocoileus virginianus*), nutria (*Myocastor coypus*), and swamp rabbits (*Sylvilagus aquaticus*) (Tobler *et al.*, 2006). A variety of insect herbivores (hereafter referred to as 'microherbivores') also consume

Louisiana *Iris* leaf tissue, including Lepidoptera, Coleoptera, Hemiptera, Homoptera, and Orthoptera (N.H. Martin, personal observation). These insects remove smaller amounts of leaf tissue relative to the 'macroherbivores'. Finally, the 'orange smut fungus', *Puccinia iridis*, is commonly observed to attack Louisiana *Iris* leaves in nature, causing early leaf senescence (Mains, 1934; Sobers, 1980). Each of these herbivores and fungal pathogens causes distinctly different morphological damage to Louisiana *Iris* leaf tissue and can be readily distinguished and quantified in the field and presumably may have an effect on the fitness of *Iris* plants.

By examining patterns of hybrid and parental resistance among this well-studied species pair, we hoped to identify the underlying genetic basis of resistance to different herbivore and fungal pathogens. Specifically, we tested whether hybrid resistance acted in an additive fashion or if the pattern of resistance differed significantly from additive expectations, thus suggesting a more complex genetic basis underlying resistance to herbivory and fungal infection.

MATERIALS AND METHODS

Experimental set-up

The current experiment was conducted in the spring of 2008 in an experimental plot of 930 *I. fulva*, *I. brevicaulis*, F_1, and reciprocal backcross Louisiana irises planted into the Indian Bayou swamp near Krotz Springs, Louisiana. This experimental plot is the same plot used to investigate phenology (Martin *et al.*, 2007), pollinator preference (Martin *et al.*, 2008), and hybrid fitness (Taylor *et al.*, 2009), but not the same plot that was used to investigate survival in inundated conditions (Martin *et al.*, 2006) because of the high mortality rate in those plots. Natural populations of *I. fulva* and *I. brevicaulis*, as well as the aforementioned herbivores and orange smut fungus, also exist in this region.

Plant material

The backcross individuals planted into the field plots were initially formed to serve as linkage-mapping populations and subsequently to identify quantitative trait loci (QTLs) that underlie a number of pre-zygotic and post-zygotic barriers between *I. fulva* and *I. brevicaulis* (Bouck *et al.*, 2005, 2007; Martin *et al.*, 2005, 2006, 2007, 2008; Taylor *et al.*, 2009). The backcross populations were created from crossing wild-collected pure-species individuals. The *I. fulva* genotype (*If*174) that was used in the crossing design was collected from a bayou in Terrebonne Parish, Louisiana, while the *I. brevicaulis* genotype (*Ib*25) was collected from an oak hardwood forest in St. Martin Parish, Louisiana. Clones from both individuals were used to generate initial F_1 parents and served as the recurrent parents for the reciprocal backcross hybrids. Multiple clones of a single F_1 genotype ($F_1$2) were backcrossed to clones of *If*174 to produce the BC*If* population, while clones of another F_1 genotype ($F_1$3) were backcrossed to *Ib*25 to produce the BC*Ib* population. Backcross seeds were germinated at the University of Georgia greenhouse in 1999 and were maintained until planting in the field.

In October 2005, up to five clones (i.e. ramets) of each backcross individual (BC*If*: 370 total rhizomes from 172 genotypes; BC*Ib*: 423 total rhizomes from 243 genotypes), the parental species (*I. brevicaulis*: 62 rhizomes of seven different wild-collected individuals, including *Ib*25; *I. fulva*: 43 rhizomes of five different wild-collected individuals, including *If* 174), and 47 rhizomes of the two F_1 hybrids used in the crossing experiments were planted in a randomized design at 0.5-m intervals into the experimental plot. By the spring of 2008, 370 BC*If*, 423 BC*Ib*, 54 F_1, 43 *I. fulva*, and 40 *I. brevicaulis* had survived and were utilized in the current assay of herbivory and fungal infection.

Data collection and analysis

For each of the 930 plants, the presence/absence of herbivory, the type of herbivory (i.e. macroherbivores or microherbivores), and the presence/absence of smut fungus were recorded on three dates in late winter to early spring 2008: 2 February, 3 March, and 29 March. We ultimately analysed the data from the last date where the most individuals had been subjected to herbivory and fungal infection in this study.

A logistic regression model was developed to include crosstype [parental *I. brevicaulis*, parental *I. fulva*, F_1, backcross *I. brevicaulis* (BC*Ib*), and backcross *I. fulva* (BC*If*)] as a predictor variable for the presence or absence of microherbivory, macroherbivory, and fungal infection. If a significant result was found ($P < 0.5$), *post-hoc* single paired logistic regressions were performed to determine pair-wise differences in resistance. Traditional line-cross analyses were not performed on these data because the data are categorical. Bonferroni corrections were used to adjust alpha values, and all statistical tests were performed in JMP v.8.0.

RESULTS

Macroherbivory

The presence of macroherbivory was low in this experiment. No macroherbivore damage was scored on any of the *I. fulva* plants, and only 2.5% of the *I. brevicaulis* pure-species plants had evidence of macroherbivory. The BC*Ib* plants had similarly low rates (1.90%), while BC*If* and F_1 plants had somewhat higher rates (4.3% and 7.4% respectively). However, none of the differences among the various crosstypes were significant ($\chi^2 = 10.26$, d.f. = 5, $P = 0.07$), so no graphical representation is provided.

Microherbivory

Insect-induced microherbivory was relatively rare among the plants, ranging from 5.7% to 20.9% depending on the crosstype being examined (Fig. 1), and overall there were significant differences in microherbivory rates across the various crosstypes ($\chi^2 = 29.90$, d.f. = 5, $P < 0.0001$). The two parental classes showed considerably different rates of microherbivory, with 7.5% of the *I. brevicaulis* plants and 20.9% of the *I. fulva* plants revealing evidence of insect herbivory. However, owing to the low sample sizes of the parental classes, this was not a significant difference ($\chi^2 = 3.16$, d.f. = 1, $P = 0.076$). The two F_1 hybrids each experienced the same 14.8% herbivory rates, so they were combined for the analysis comparing F_1's against the other crosstypes. F_1 hybrid herbivory rates were intermediate to those of the parents, while the BC*If* hybrids were intermediate between F_1 and *I. fulva* parents (BC*If* = 16.8%). The BC*Ib* hybrids revealed the lowest incidence of insect microherbivory (5.7%). This latter value was significantly lower than that of the BC*If* hybrids ($\chi^2 = 25.25$,

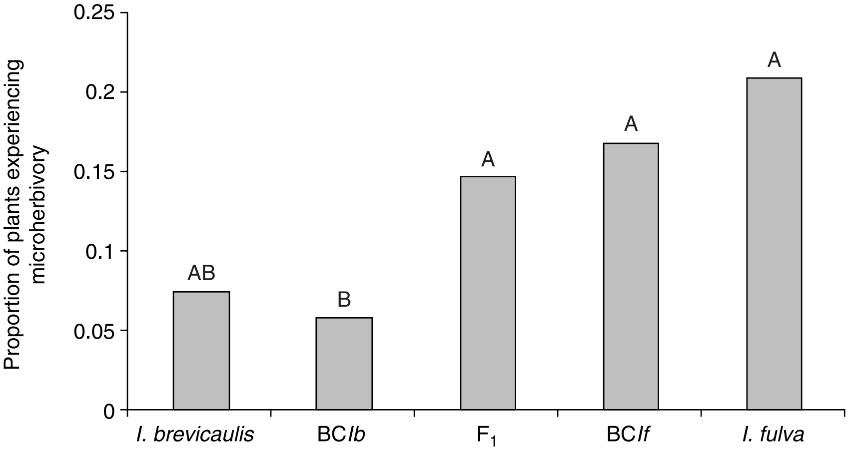

Fig. 1. Proportion of plants per crosstype affected by microherbivory. Significant differences ($\alpha = 0.05$) among crosstypes are denoted with the letters A and B.

d.f. = 1, $P < 0.0001$) and *I. fulva* genotypes ($\chi^2 = 9.8$, d.f. = 1, $P = 0.006$). Overall, patterns of resistance to microherbivory appear to follow a general pattern of additivity, with *I. fulva* being the most susceptible and *I. brevicaulis* being much less susceptible to microherbivory, and F_1 and BC*If* hybrids not deviating substantially from additive expectations (Fig. 1). BC*Ib* hybrids appear to be more resistant to microherbivory than expected under a purely additive model (and are significantly more resistant than the reciprocal backcross hybrid and *I. fulva*; Fig. 1), but again, sample sizes for the F_1 and parental classes were likely too small to provide definitive conclusions as to the underlying genetic basis of herbivore resistance.

Fungal infection

The presence of the fungus *P. iridis* varied significantly among the crosstypes ($\chi^2 = 76.54$, d.f. = 5, $P < 0.0001$), ranging from a high 63.0% infection rate in one of the F_1 hybrid classes (F_13) to a low of 2.5% in *I. brevicaulis* (Fig. 2). Pure *I. fulva* plants showed signs of fungal infection in 11.6% of the plants, but again, likely owing to reduced sample sizes of both parental classes, the differences between *I. fulva* and *I. brevicaulis* were not significant ($\chi^2 = 2.82$, d.f. = 1, $P = 0.09$). BC*If* and F_12 hybrids revealed 3% and 16% infection rates, respectively, and these hybrid classes did not differ significantly from either of the pure-species parents. However, F_13 hybrids had a dramatically high infection rate of 63%. Clones of this F_1 hybrid were used to generate the BC*Ib* hybrid population, which showed intermediate infection rates (32.4%) between its F_1 and *I. brevicaulis* parents (Fig. 2). This pattern is suggestive of under-dominance for resistance in the F_1.

DISCUSSION

In this experiment, we assayed five genotypic classes of Louisiana *Iris* (*I. fulva*, *I. brevicaulis*, F_1, and reciprocal backcross hybrids) for the presence/absence of macroherbivory, insect microherbivory, and the leaf smut fungus *P. iridis* in experimental plots within the natural

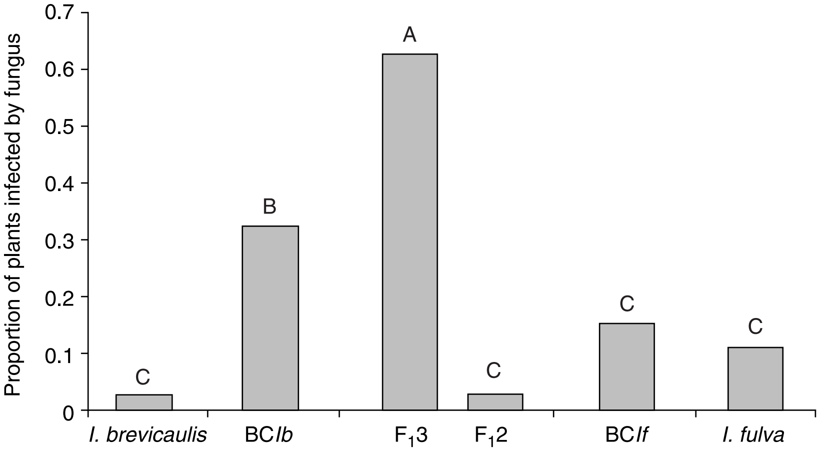

Fig. 2. Proportion of plants per crosstype affected by the smut fungus *P. iridis*. Significant differences ($\alpha = 0.05$) among crosstypes are denoted with the letters A, B, and C.

range of *I. fulva* and *I. brevicaulis* in southern Louisiana. Our study detected significant differences between Louisiana *Iris* classes in terms of damage from insect herbivores and the smut fungus (Figs. 1 and 2). The differential susceptibility to insect microherbivores and fungal infection could ultimately influence the outcome of natural hybridization between *I. fulva* and *I. brevicaulis* in nature, as both insect herbivores and fungal pathogens may have often dramatic effects on plant reproduction (e.g. Fritz, 1999; Whitham *et al.*, 1999; McIntyre and Whitham, 2003; Dechaine *et al.*, 2009).

While browsing by macroherbivores such as deer has been shown to reduce fitness in a related Louisiana *Iris* species, *I. hexagona* (Geddes and Mopper, 2006; Tobler *et al.*, 2006), macroherbivores did not appear to differentiate among the Louisiana *Iris* crosstypes in the current study. It should be noted, however, that rates of macroherbivory were low in the present study, and in years where macroherbivores focus on Louisiana irises as a food source, perhaps the various crosstypes would show significant differences.

Previous studies involving *I. brevicaulis* and *I. fulva* have revealed that F_1 hybrids often reveal considerably higher fitness than either of the parental progenitors (Wesselingh and Arnold, 2000; Johnston *et al.*, 2004; Taylor *et al.*, 2009). In fact, for a number of sexual and asexual fitness measures, F_1 hybrids (the same plants utilized for the current study) have been shown to exhibit marked heterosis, ultimately resulting in this hybrid class producing significantly more flowering stalks, flowers, and asexual growth points than either *I. brevicaulis* or *I. fulva* (Taylor *et al.*, 2009). However, Taylor *et al.* (2009) conducted their study during 2006 and 2007, during which *P. iridis* rust was not observed (N. Martin, personal observation). The current study was conducted in 2008, when a substantial proportion of the plants (24%) were infected with *P. iridis*. Furthermore, one of the F_1 hybrids, F_13, was shown to be quite susceptible to fungal infection, with 67% of the plants being infected. As *P. iridis* fungus causes early leaf senescence and a dramatic reduction in above-ground biomass (Mains, 1934; Sober, 1980), this would ultimately result in larger reductions in fitness measures of this F_1 hybrid genotype compared with previous studies (Taylor *et al.*, 2009). With respect to insect microherbivory, F_1 hybrids revealed intermediate susceptibility between the pure-species parents, indicating that microherbivores might not be able to exert as dramatic a fitness consequence on F_1 hybrids as did the smut fungus.

A common method for investigating genetic architecture is through line cross analysis, which can reveal the general mode of gene action that influences the trait(s) of interest (Mather and Jinks, 1982; Lynch and Walsh, 1998; Fritz *et al.*, 2003). Due to the qualitative nature of our data, we were not able to conduct line cross analyses directly. However, we were able to compare our results with expectations given different modes of gene action. For example, if genes that are responsible for a plant's susceptibility to herbivores or fungal pathogens act in a purely additive fashion, then F_1 hybrids should reveal intermediate susceptibility between the pure-species parents. However, deviations from additive expectations suggest that a more complex genetic architecture may underlie resistance to herbivory and fungal infection.

With respect to microherbivory by insects, the iris F_1 hybrids did not deviate from additive expectations (Fig. 1), and in fact, F_1, *I. brevicaulis*, and *I. fulva* classes did not differ significantly from one another. Considering the low sample sizes, we urge caution in interpreting these data. However, the backcross hybrids did not conform to a pattern expected given additive gene action. For example, the incidence of microherbivory was lower than expected for backcrosses to *I. brevicaulis* (BC*Ib*). This finding suggests that introgressed *I. fulva* alleles are interacting in a mostly *I. brevicaulis* genetic background to cause increased resistance to microherbivory for genotypes belonging to this hybrid class. We would predict that in natural hybrid zones, such genotypes would act as a conduit for further introgression of resistance alleles between the donor (i.e. *I. fulva*) and recipient species (i.e. *I. brevicaulis*).

There were clear departures from additive expectations with respect to susceptibility to infection by the smut fungus *P. iridis*. *Iris brevicaulis* and *I. fulva* did not differ significantly from one another with respect to fungal susceptibility, and neither did one of the F_1 hybrid genotypes or the BC*If* hybrid class. However, $F_1$3, which was used as the maternal parent for the BC*Ib* hybrids, revealed an infection rate of 67%, indicating under-dominance at loci involved in resistance to fungal infection. BC*Ib* hybrids matched well an additive-dominance model for fungal susceptibility, in that they were intermediate between the rarely infected *I. brevicaulis* parent and the highly infected F_1 parent. These data highlight the importance of non-additive gene action with respect to traits that are potentially important in affecting the fitness of hybrids. Such non-additive gene action has been observed for other fitness measures in these Louisiana irises (Burke *et al.*, 1998; Taylor *et al.*, 2009). Although hybrid breakdown is usually not seen until later generation hybrids in this system (Cruzan and Arnold, 1994; Burke *et al.*, 1998; Taylor *et al.*, 2009), the break-up of co-adapted gene complexes in the first-generation hybrids led to marked differences in F_1 susceptibility to infection by a fungal pathogen. Such genetic incompatibilities in the first generation may be an additional causal factor leading to the rarity of Louisiana *Iris* F_1 hybrids in nature (Arnold, 2006).

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REFERENCES

Arnold, M.L. 1992. Natural hybridization as an evolutionary process. *Annu. Rev. Ecol. Syst.*, **23**: 237–261.

Arnold, M.L. 2006. *Evolution Through Genetic Exchange*. Oxford: Oxford University Press.

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

Arnold, M.L., Hamrick, J.L. and Bennett, B.D. 1990. Allozyme variation in Louisiana irises: a test for introgression and hybrid speciation. *Heredity*, **65**: 297–306.

Boecklen, W.J. and Spellenberg, R. 1990. Structure of herbivore communities in two oak (*Quercus* spp.) hybrid zones. *Oecologia*, **85**: 92–100.

Bouck, A.C., Peeler, R., Arnold, M.L. and Wessler, S.R. 2005. Genetic mapping of species boundaries in Louisiana irises using *IRRE* retrotransposon display markers. *Genetics*, **171**: 1289–1303.

Bouck, A.C., Wessler, S.R. and Arnold, M.L. 2007. QTL analysis of floral traits in Louisiana *Iris* hybrids. *Evolution*, **61**: 2308–2319.

Burke, J.M., Voss, T.J. and Arnold, M.L. 1998. Genetic interactions and natural selection in Louisiana *Iris* hybrids. *Evolution*, **52**: 1304–1310.

Carney, S.E., Gardner, K.A. and Rieseberg, L.H. 2000. Evolutionary changes over the fifty-year history of a hybrid population of sunflowers (*Helianthus*). *Evolution*, **54**: 462–474.

Carr, D.E. and Dudash, M.R, 2003. Recent approaches into the genetic basis of inbreeding depression in plants. *Phil. Trans. R. Soc. Lond. B*, **358**: 1071–1084.

Cruzan, M.B. and Arnold, M.L. 1993. Ecological and genetic associations in an iris hybrid zone. *Evolution*, **47**: 1432–1445.

Cruzan, M.B. and Arnold, M.L. 1994. Assortative mating and natural selection in an iris hybrid zone. *Evolution*, **48**: 1946–1958.

Dechaine, J.M., Burger, J.C., Chapman, M.A., Seiler, G.J., Brunick, R., Knapp, S.J. *et al.* 2009. Fitness effects and genetic architecture of plant–herbivore interactions in sunflower crop–wild hybrids. *New Phytol.*, **184**: 828–841.

Egan, S.P. and Funk, D.J. 2009. Ecologically dependent postmating isolation between sympatric host forms of *Neochlamisus bebbianae* leaf beetles. *Proc. Natl. Acad. Sci. USA*, **106**: 19426–19431.

Emms, S.K. and Arnold, M.L. 1997. The effect of habitat on parental and hybrid fitness: transplant experiments with Louisiana irises. *Evolution*, **5**: 1112–1119.

Floate, K.D., Kearsley, M.J.C. and Whitham, T.G. 1993. Elevated herbivory in plant hybrid zones – *Chrysomela confluens, Populus* and phenological sinks. *Ecology*, **74**: 2056–2065.

Fritz, R.S. 1999. Resistance of hybrid plants to herbivores: genes, environment, or both? *Ecology*, **80**: 382–391.

Fritz, R.S., Nichols-Orians, C.M. and Brunsfeld, S.J. 1994. Interspecific hybridization of plants and resistance to herbivores – hypotheses, genetics, and variable responses in a diverse herbivore community. *Oecologia*, **97**: 106–117.

Fritz, R.S., Roche, B.M., Brunsfeld, S.J. and Orians, C.M. 1996. Interspecific and temporal variation in herbivore responses to hybrid willows. *Oecologia*, **108**: 121–129.

Fritz, R.S., Hochwender, C.G., Brunsfeld, S.J. and Roche, B.M. 2003. Genetic architecture of susceptibility to herbivores in hybrid willows. *J. Evol. Biol.*, **16**: 1115–1126.

Geddes, N. and Mopper, S. 2006. Effects of environmental salinity on vertebrate florivory and wetland communities. *Natural Areas J.*, **26**: 31–37.

Grant, P.R. 1981. Speciation and the adaptive radiation of Darwin's finches. *Am. Sci.*, **69**: 653–663.

Gross, B.L., Schwarzbach, A.E. and Rieseberg, L.H. 2003. Origin(s) of the diploid hybrid species *Helianthus deserticola* (Asteraceae). *Am. J. Bot.*, **90**: 1708–1719.

Hatfield, T. and Schluter, D. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. *Evolution*, **53**: 866–873.

Haygood, R., Ives, A.R. and Andow, D.A. 2003. Consequences of recurrent gene flow from crops to wild relatives. *Proc. R. Soc. Lond. B*, **270**: 1879–1886.

Hegarty, M.J. and Hiscock, S.J. 2005. Hybrid speciation in plants: new insights from molecular studies. *New Phytol.*, **165**: 411–423.

Hegde, S.G., Nason, J.D., Clegg, J.M. and Ellstrand, N.C. 2006. The evolution of California's wild radish has resulted in the extinction of its progenitors. *Evolution*, **60**: 1187–1197.

Johnston, J.A., Wesselingh, R.A., Bouck, A.C., Donovan, L.A. and Arnold, M.L. 2001. Intimately linked or hardly speaking? The relationship between genotype and environmental gradients in a Louisiana *Iris* hybrid population. *Mol. Ecol.*, **10**: 673–681.

Johnston, J.A., Donovan, L.A. and Arnold, M.L. 2004. Novel phenotypes among early generation hybrids of two Louisiana *Iris* species: flooding experiments. *J. Ecol.*, **92**: 967–976.

Keim, P., Paige, K.N., Whitham, T.G. and Lark, K.G. 1989. The genetic structure of a *Populus* hybrid swarm and the correlation of insect susceptibility to specific genetic classes. *Genetics*, **122**: S30.

Lynch, M. and Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Associates.

Mains, E.B. 1934. Host specialization in the rust of Iris, *Puccinia iridis. Am. J. Bot.*, **21**: 23–33.

Marquis, R.J. 1992. A bite is a bite is a bite – constraints on response to folivory in *Piper arieianum* (Piperaceae). *Ecology*, **73**: 143–152.

Martin, N.H., Bouck, A.C. and Arnold, M.L. 2005. Loci affecting long-term hybrid survivorship in Louisiana irises: implications for reproductive isolation and introgression. *Evolution*, **59**: 2116–2124.

Martin, N.H., Bouck, A.C. and Arnold, M.L. 2006. Detecting adaptive trait introgression between *Iris fulva* and *I. brevicaulis* in highly selective field conditions. *Genetics*, **172**: 2481–2489.

Martin, N.H., Bouck, A.C. and Arnold, M.L. 2007. The genetic architecture of reproductive isolation in Louisiana irises: flowering phenology. *Genetics*, **175**: 1803–1812.

Martin, N.H., Sapir, Y. and Arnold, M.L. 2008. The genetic architecture of reproductive isolation in Louisiana irises: pollination syndromes and pollinator preferences. *Evolution*, **62**: 740–752.

Mather, K. and Jinks, J.L. 1982. *Biometrical Genetics*. New York: Chapman & Hall.

McIntyre, P.J. and Whitham, T.G. 2003. Plant genotype affects long-term herbivore population dynamics and extinction: conservation implications. *Ecology*, **84**: 311–322.

Orians, C.M. and Floyd, T. 1997. The susceptibility of parental and hybrid willows to plant enemies under contrasting soil nutrient conditions. *Oecologia*, **109**: 407–413.

Prentis, P.J., White, E.M., Radford, I.J., Lowe, A.J. and Clarke, A.R. 2007. Can hybridization cause local extinction: a case for demographic swamping of the Australian native *Senecio pinnatifolius* by the invasive *Senecio madagascariensis? New Phytol.*, **176**: 902–912.

Rieseberg, L.H. 1997. Hybrid origins of plant species. *Annu. Rev. Ecol. Syst.*, **28**: 359–389.

Rieseberg, L.H. 2006. Hybrid speciation in wild sunflowers. *Ann. Missouri Bot. Gard.*, **93**: 34–48.

Sobers, E.K. 1980. Hosts of *Puccinia iridis* in Florida. *Plant Pathol. Circ.*, #**35**.

Stutz, H.C. and Thomas, L.K. 1964. Hybridization and introgression in *Cowania* and *Purshia. Evolution*, **18**: 183–195.

Taylor, S.J., Arnold, M.L. and Martin, N.H. 2009. The genetic architecture of reproductive isolation in Louisiana irises: hybrid fitness in nature. *Evolution*, **63**: 2581–2594.

Tobler, M., Van Zandt, P., Hassenstein, K. and Mopper, S. 2006. Negative effects of salinity stress on a leafmining insect and its host plant. *Ecol. Entomol.*, **31**: 1–7.

Viosca, P., Jr. 1935. The irises of southeastern Louisiana: a taxonomic and ecological interpretation. *Bull. Am. Iris Soc.*, **57**: 3–56.

Wesselingh, R.A. and Arnold, M.L. 2000. Pollinator behaviour and the evolution of Louisiana *Iris* hybrid zones. *J. Evol. Biol.*, **13**: 171–180.

Whitham, T.G., Martinsen, G.D., Floate, K.D., Dungey, H.S., Potts, B.M. and Keim, P. 1999. Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. *Ecology*, **80**: 416–428.

Whitlock, M.C., Phillips, P.C., Moore, F.B.-G. and Tonsor, S.J. 1995. Multiple fitness peaks and epistasis. *Annu. Rev. Ecol. Syst.*, **26**: 601–629.

Whitney, K.D., Randell, R.A. and Rieseberg, L.H. 2006. Adaptive introgression of herbivore resistance traits in the weedy sunflower *Helianthus annuus*. *Am. Nat.*, **167**: 794–807.

Whitney, K.D., Randell, R.A. and Rieseberg, L.H. 2010. Adaptive introgression of abiotic tolerance traits in the sunflower *Helianthus annuus*. *New Phytol.*, **187**: 230–239.