The impacts of hybridization on the endangered red mulberry (*Morus rubra* L.) in Canada

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Abstract

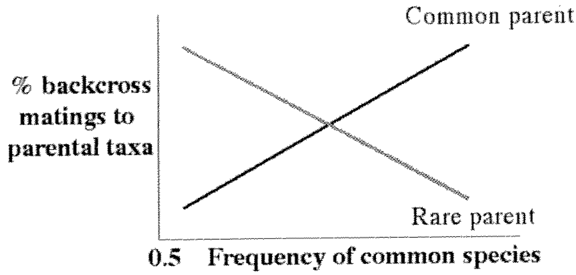
*We have developed a research program that uses molecular, morphometric and experimental approaches to measure the genetic and ecological impacts of hybridization on the endangered red mulberry (*Morus rubra *L.) by the introduced, white mulberry (*Morus alba *L.) in Canada. Although the genetic impacts of hybridization are discussed, this paper focuses on the ecological impacts of hybridization through its influence on the production of red mulberry offspring and their establishment. Results from a culling experiment show that although red mulberry offspring have similar fitness measures among culled vs. non-culled plots, the production of hybrid offspring is higher in non-culled plots. Results from transplant experiments show that red, white and hybrid mulberry are not ecologically differentiated and red mulberry is at a competitive disadvantage. This research provides insight into the evolutionary processes that occur when a rare plant hybridizes with a more abundant species, and represents an opportunity to implement management strategies based on experimental approaches that contribute to the Red Mulberry National Recovery Plan.*

Introduction

When two plant populations hybridize the impact of hybridization can be genetic and/or ecological (Arnold, 1997). Genetic impacts affect the frequency and types of novel gene combinations that are formed between hybridizing populations and have been the focus of much research in the hybridization literature (Rieseberg and Wendel, 1993; Arnold, 1997; Rieseberg and Carney, 1998; Rieseberg, 2000). The ecological impacts concern the viability of hybrids and their parents (Levin *et al.*, 1996, Wolf *et al.*, 2000), and may be equally important in determining the outcome of the hybridization process (Levin et al., 1996; Rhymer and Simberloff, 1996). For example, hybridization can create competition for the limited ovules of the hybridizing taxa as well as influence establishment through increased competition for suitable sites. Together, ecological and genetic impacts will determine the fate of hybrids and their parents.

When hybridization occurs between a small (or rare) plant population and a more abundant congener, the genetic and ecological impacts of hybridization on the rare parental population may be particularly acute (Huxel, 1999; Wolf *et al.*, 2000). As the proportion of individuals of the rare taxon decreases relative to the common congener, the percentage of backcross matings between hybrids and the common parent will increase while those to the rare parent will decrease (Figure 1).

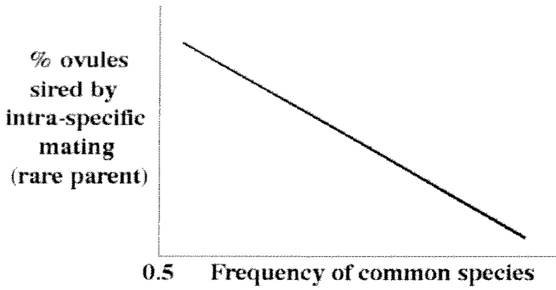
Figure 1. Genetic assimilation of the rare parent involves the incorporation of genes from the rare taxon into the genome of the common. This process is dependent on the formation of hybrids and the frequency of the common parent.



Such asymmetric gene flow between rare and common taxa, can lead to the incorporation of genes from the rare taxon into the genome of the common (Rhymer and Simberloff, 1996; Huxel, 1999). This genetic impact of hybridization on the rare taxon has often been referred to as genetic assimilation although several other terms in the literature have been applied to this process (Rhymer and Simberloff, 1996). These include: genetic deterioration, genetic takeover, genetic swamping, genetic aggression, and genetic pollution (Rhymer and Simberloff, 1996).

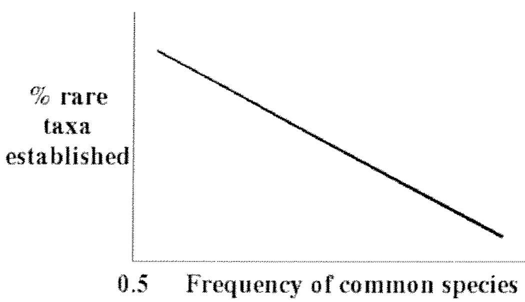
Although the genetic impacts of hybridization can have a detrimental effect on the rare parent, ecological impacts can also play an important role in their persistence (Levin *et al.*, 1996; Wolf *et al.*, 2000). For example, the rare parent may experience a mating disadvantage with the more abundant taxon. This can occur when the percentage of ovules sired by intra-specific matings among rare parents decreases as the frequency of the common species increases above that of the rare parent (Figure 2).

Figure 2. Rare parental taxa can experience mating disadvantage when the frequency of the common species causes a reduction in the % of ovules sired by intra-specific mating among rare parents.



Similarly the rare taxon may experience establishment disadvantage where the percentage of individuals of the rare parent that establish at a site also decreases as the frequency of the common species increases above that of the rare parent (Figure 3).

Figure 3. Rare parental taxa can experience establishment disadvantage when the frequency of the common species causes a reduction in the % of rare taxa that are able to get established at a site.



Hybridization and red mulberry

One rare plant species that is thought to be experiencing the genetic and ecological impacts of hybridization with a more abundant congener is red mulberry (*Morus rubra* L.) (Ambrose *et al.*, 1999). Red mulberry is a wind pollinated under-story tree species, listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). In Canada red mulberry is restricted to the Carolinian zone of southern Ontario and is only found in six core populations that contain five or more trees. These include the Hamilton Royal Botanical Gardens, Ball's Falls Conservation Area (Niagara Peninsula Conservation Authority), Niagara Glen (Niagara Parks Commission), Rondeau Provincial Park, Point Pelee National Park and Fish Point Provincial Nature Reserve (Pelee Island). Apart from habitat fragmentation due to increasing urban and agricultural land use, hybridization with the introduced and more abundant white mulberry (*Morus alba* L.) has been identified as a significant threat to remaining populations (Ambrose *et al.*, 1999). The hypothesis that hybridization is occurring between these two species is based on two lines of evidence. The first of these is the high occurrence of white mulberry at four of the remaining core populations in southern Ontario where the percentage of white mulberry within a 25 m radius of red mulberry can be as high as 92.6% (Husband and Burgess, 2000). Secondly, individuals with intermediate leaf morphology have also been found at the same populations although their parentage has yet to be confirmed with molecular markers.

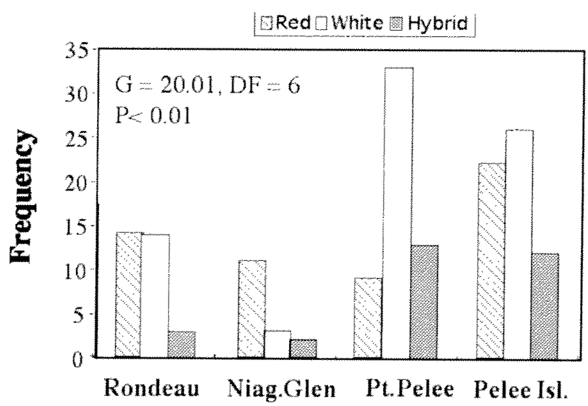
Given the suspected threat of hybridization to red mulberry in southern Ontario, both the management and scientific community have expressed an interest in this particular conservation problem (Burgess and Husband, 2001). Land managers have expressed the need for immediate restoration of the species, whereas the scientific community has developed an interest in hybridization per se as a mechanism of endangerment to small populations of red mulberry. More specifically, the primary focus of management is to reduce the impact of hybridization (which involves the identification and removal of hybrid and white mulberry). Alternatively the scientific community is interested in investigating the biology and ecology of small populations of red mulberry and achieve a mechanistic understanding of extinction by hybridization in these populations (i.e., the role of genetic assimilation, mating disadvantage and establishment disadvantage in red mulberry).

We have established a research program that attempts to meet both the needs of management and those of science (Burgess and Husband, 2001). In this paper we will present the objectives outlined in the red mulberry research program as well as preliminary results that address these objectives. The research program has two basic objectives: 1) to determine the genetic impacts of hybridization by determining the genetic structure of the population and patterns of morphological variation; and, 2) to measure the ecological impacts of hybridization i.e., the role of mating disadvantage and establishment disadvantage. As the results presented here represent preliminary work of the primary author's Ph.D. thesis, please keep in mind that a more thorough analysis of the data presented in this paper will be published in the primary literature in the future. This aside, we want to demonstrate work done to date and how we are attempting to meets the needs of both land managers and science.

Genetic impacts of hybridization

Results from our on-going genetic studies using species-specific Randomly Amplified Polymorphic DNA (RAPD) have confirmed the occurrence of hybridization at four key locations where red and white mulberry co-exist (Husband and Burgess, 1999; Husband and Burgess, 2001). As seen in figure 4, a high frequency of hybrid mulberry were detected in populations that also have the highest occurrence of white mulberry, namely Pelee National Park and Fish Point Provincial Nature Reserve.

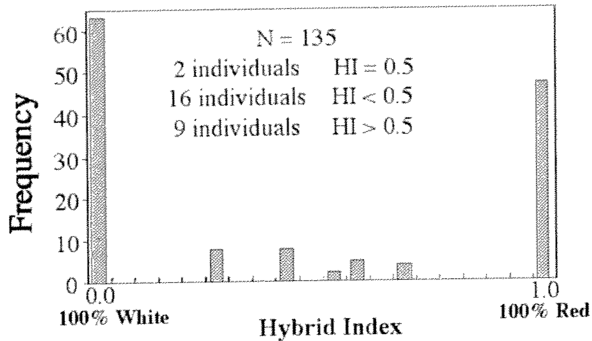
Figure 4. DNA analysis using species specific Randomly Amplified Polymorphic DNA (RAPD) showed that the variation in hybrid frequencies differs among populations in southern Ontario. Sites with the highest occurrence of white mulberry also had the highest hybrid frequency.



When individuals were ranked along an assigned value using a hybrid index (0=white, 1=red), few hybrids were found to be of first generation parentage (F1); but rather, the most hybrids were of later generation backcrosses and were more closely related to white mulberry (Figure 5).

This research gives insight into the direction of hybridization and the potential for genetic assimilation (processes of scientific interest). The results from our genetic studies also have conservation relevance; namely, the confirmation of hybridization as a potential threat to remaining populations and the existence of red mulberry in southern Ontario.

Figure 5. Hybrid index scores based on species specific RAPD markers. Of the 27 hybrids detected only two were F1 while the majority had DNA profiles that were closer to white mulberry.



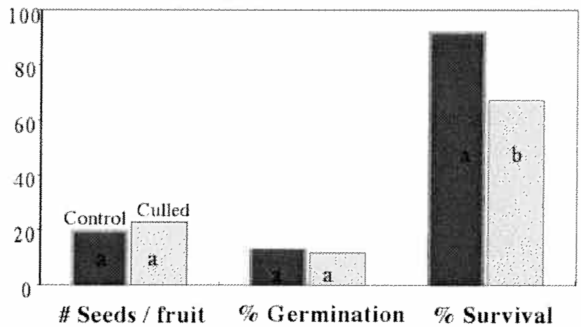
Ecological impacts of hybridization

The second objective of the red mulberry research program (and the focus of this paper) goes beyond measuring the magnitude of hybridization in remaining populations of red mulberry to investigating the impacts of hybridization on the abundance of red mulberry. Specifically we have established experiments that measure the impact of mating disadvantage and establishment disadvantage in determining the fate of red mulberry parental taxa (Husband and Burgess, 2000; Husband *et al.*, 2001, Burgess and Husband, 2002). To us, these experiments represent an adaptive management approach to addressing the conservation needs of this species, where management is conducted in an experimental framework. Once again this represents a work in progress and data from these experiments will be published elsewhere in the primary literature.

Mating disadvantage

At locations where red mulberry is less abundant than white mulberry we hypothesize that red mulberry may be experiencing mating disadvantage. Due to this numerical asymmetry we suspect that some of the red mulberry ovules which ordinarily would be sired by red mulberry pollen are being fertilized by white mulberry. To determine if red mulberry is experiencing mating disadvantage, we have established a culling experiment in two of the six remaining populations. In this experiment we identified 12 female red mulberry trees around which all white mulberry were either removed within a 25m radius (culled plots) or left standing (control plots). Seeds were then collected from each of these trees, germinated in growth chambers and grown in a greenhouse environment. Leaves were collected for DNA analysis using RAPD to determine the percentage of hybrid progeny produced in control vs. culled plots. Results showed that although there was no significant differences between treatments for the # of seeds/fruit and % germination, % survival was higher in control plots (Figure 6).

Figure 6. Results from a white mulberry removal experiment showed that although culling has no effect on seed production or quality, seedlings from control plots had higher survival rates.



Although not statistically different, the percentage of hybrids produced also tended to be higher in control plots. From a conservation perspective, this experiment not only allows researchers to determine if white mulberry removal is necessary for red mulberry restoration but also gives us an estimate for the scale of removal that may be required. From a scientific perspective these results suggest that mating disadvantage is occurring in this species.

Establishment disadvantage

At locations where red, white and hybrid mulberry co-exist we hypothesize that red mulberry may be experiencing establishment disadvantage. Because suitable habitat for red mulberry establishment is limited we suspect that white and hybrid mulberry are occupying sites that would normally be occupied by red mulberry. To determine if the conditions for establishment disadvantage are occurring in red mulberry, we established 3 transplant experiments. The first two are in a natural environment at Rondeau Provincial Park and the third is in a controlled environment at Ridgetown College. In the natural environment we established a seedling experiment in which 5 month old plants were planted into two environments representing red and white mulberry parental habitats, shade and sun respectively. In this experiment three cross-types (Red mulberry X Red mulberry (RxR); White mulberry X White mulberry (WxW); and each of the reciprocal crosses RxW and WxR (grouped as Hybrids) were randomly planted (N=36, 0.5m apart) in each of eight plots (four in sun habitat and four in shade habitat).

A similar design was used to establish a juvenile transplant experiment in a natural environment at Rondeau Provincial Park. In this experiment 11 month old plants of varying cross-types (RxR, WxW, and Hybrid) were planted into each of eight plots (four in sun habitat and four shade habitat).

The third experiment was established at Ridgetown College in a controlled environment. This is a common garden experiment in which RxR, WxW, and Hybrid seedlings were planted into one plot (N=333).

We then measured survival and height for each on these experiments over a period of two years and calculated cumulative fitness measures (survival of seedlings X survival of juveniles X height of juveniles) for each of the cross-types in each experiment. We then related the most fit cross-type in each environment to other cross-types in that same environment to calculate relative measures of fitness.

As seen in Figure 7, results from the seedling and juvenile experiments showed that hybrid and white mulberry significantly out-performed red mulberry in both sun and shade habitats. RxR mulberry had 13% and 6 % of the fitness of hybrid mulberry in shade and sun habitats respectively. Similar results were found in the common garden experiment where RxR mulberry had lower fitness measures than both WxW and Hybrid mulberry (Figure 8).

Figure 7. Relative fitness (seedling survival X juvenile survival X height of juveniles) of RxR, WxW and hybrid mulberry in shade and sun habitat. RxR mulberry had significantly lower relative fitness than both hybrid and WxW mulberry in both habitat types.

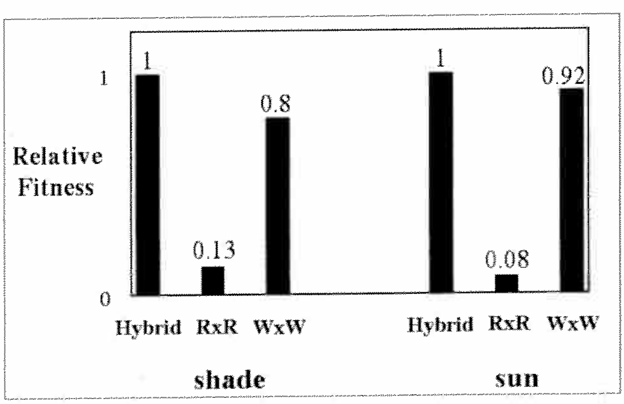
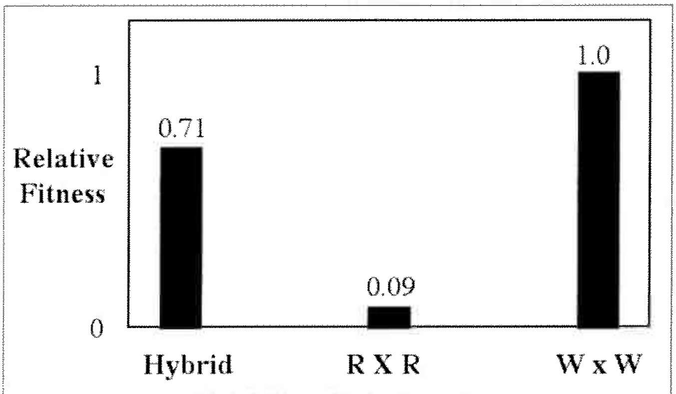


Figure 8. Relative fitness (seedling survival X juvenile survival X height of juveniles) of RxR, WxW and hybrid mulberry in a common habitat. RxR mulberry had significantly lower relative fitness than both hybrid and WxW mulberry.



The significance of the results from these transplant experiments is once again two fold. From a conservation perspective, the results provide a basis for a transplant protocol for red mulberry restoration as well as predictions on the success of such efforts. From a scientific perspective these results confirm that the conditions for establishment disadvantage are occurring in red mulberry.

Conclusion

In summary the red mulberry research program provides a balance of descriptive and experimental approaches to measure the magnitude and impact of hybridization. This not only allows us to gain new insights into the mechanisms of hybridization in rare populations but also provides the research necessary to implement and assess the conservation initiatives outlined in the National Recovery Plan for this species.

Statement of work in progress

We reiterate that this is work in progress and is part of the Ph.D. thesis of the primary author. The concepts and results presented in this paper will be also published in whole or in part in the peer review literature upon completion.

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