Habitat differentiation and the ecological costs of hybridization: the effects of introduced mulberry (Morus alba) on a native congener (M. rubra)

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Summary

1 The effects of hybridization on the abundance of parental taxa depends on their relative frequency, the viability of hybrid offspring and the degree of ecological differentiation among parental and hybrid taxa. Habitat overlap can facilitate competition for suitable sites and threaten the persistence of parental taxa, especially those in small populations.

2 Here we examine ecological differentiation between the endangered North American red mulberry (Morus rubra), introduced white mulberry (M. alba) and red × white hybrids in a reciprocal transplant experiment. Fitness of red, white and hybrid mulberry was estimated as survival and above-ground biomass of seedling and juvenile life stages, transplanted into open (white habitat) and shaded (red habitat) forest environments. In addition, all taxa, including reciprocal hybrids (R × W, W × R), were compared in a common garden in full sun.

3 In the reciprocal transplant study, red mulberry was consistently less fit than white and hybrid mulberry regardless of transplant habitat; F1 hybrids were as fit as white mulberry. In the common garden, red mulberry and hybrids with red mothers had lower fitness than white mulberry and hybrids with white mothers. Reciprocal hybrid crosses differed significantly with respect to survival and cumulative fitness, but not biomass.

4 Red mulberry is not ecologically differentiated from white or hybrid mulberry in the three transplant environments examined; rather, it is consistently the least fit taxon. Therefore, all else being equal, hybridization with white mulberry and the subsequent presence of hybrids will place red mulberry at a strong disadvantage during establishment.

5 The results highlight the potential effect of hybridization by introduced species on the abundance of native species, particularly at the northern extremes of its geographical range, where populations are small and native environments may be degraded or at the limits of ecological tolerance.

Key-words: demographic swamping, establishment disadvantage, fitness, habitat differentiation, hybridization, introduced species, Moraceae, Morus alba, Morus rubra, small populations.

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Introduction

In addition to its genetic consequences, hybridization can have demographic effects that influence the rate of formation and persistence of the parental taxa (Levin et al. 1996). Parental taxa may experience a reduction in fecundity when hybrid fertilizations usurp ovules that otherwise would give rise to non-hybrid (con-specific) offspring, a process referred to as interspecific ovule discounting (Levin et al. 1996; K.S. Burgess, M. Morgan & B.C. Husband, unpublished data). Hybridization can also influence the recruitment and establishment of parental taxa by increasing competition for suitable habitat (Levin et al. 1996; Rhymer & Simberloff 1996). The effects of hybridization on both fecundity and establishment will vary depending
on the relative abundance of parental taxa. Plants in small populations (e.g. rare species or those in sympatry with invasive congeners) will be particularly vulnerable and may be driven to local extinction through these frequency-dependent processes (Levin et al. 1996).

Theoretical studies of the evolutionary dynamics of hybrid zones have shown that establishment and persistence of rare parental taxa depend, in part, on the magnitude of habitat differentiation. Models by Huxel (1999), Wolf et al. (2001) and Buerkle et al. (2003) show that competition with more abundant congeners and hybrids for establishment sites can lead to the exclusion of rare taxa. If establishment sites are limited, the rare parent may experience a disadvantage based on its frequency only, even if all taxa are equally fit (Levin et al. 1996; Huxel 1999). The demographic effects may be further reinforced if the more abundant parental taxon and hybrids are competitively superior (Huxel 1999; Wolf et al. 2001; Buerkle et al. 2003). Alternatively, rare species can counter the frequency-dependent disadvantage and even persist in a stable hybrid zone when their fitness exceeds that of the hybrids in some portion of the ecological range (Wolf et al. 2001).

Despite the importance of habitat differentiation to the persistence of hybridizing taxa, only a few studies have investigated the degree of habitat overlap between congeners and their hybrids (Arnold & Hodges 1995; Rieseberg 1995; Arnold et al. 1999, 2001, 2003; Buerkle et al. 2003). For example, reciprocal transplant studies of hybrid and parental *Iris* species into their respective habitats showed that, although parental performance was highest in their home sites, hybrids performed as well as or better than their parents (Emms & Arnold 1997). Although competition was not directly measured, the results suggest that parental taxa will experience a disadvantage when competing with hybrids for establishment sites. Similar mechanisms have been demonstrated to have significant impacts on the outcome of hybridization with non-native congeners (Huxel 1999; Ellstrand & Schierenbeck 2000; Vilà et al. 2000; Abbott et al. 2003; Allendorf & Lundquist 2003; Potts et al. 2003) and crop relatives (Ellstrand et al. 1999; Ellstrand 2001, 2003; Haygood et al. 2003; Meseguer 2003); however, there are few empirical investigations involving taxa in small populations.

Red mulberry (*Morus rubra* L., Moraceae) is a wind-pollinated, dioecious, understory tree species that is native to eastern North America. At the northern edge of its range in southern Ontario, Canada, it is restricted to six populations (Ambrose & Kirk 2004), four of which contain the introduced and more common white mulberry (*Morus alba* L.) (Burgess et al. 2005). White mulberry was introduced to North America from East Asia in the 1600s and has since become naturalized in eastern North America (Rehder 1940; Gleason 1952). Like many North America – East Asian disjunct taxa (Wen 1999), red and white mulberry are only weakly genetically differentiated (Awasthi et al. 2004) and highly interfertile. In North America, red mulberry occurs in shaded understorey environments, whereas white mulberry is found under more open canopies (Gleason 1952; Wunderlin 1997). Hybrid abundance ranges from 43% to 67% in sympatric populations and hybrids tend to be introgressed toward white mulberry parents (Burgess et al. 2005). A white/hybrid mulberry removal study showed that hybridization causes a significant reduction in the number of red mulberry offspring produced by red mulberry trees (K.S. Burgess, M. Morgan & B.C. Husband, unpublished data). However, the magnitude of habitat differentiation and the potential for competitive interactions among red, white and hybrid mulberry are not known.

The objective of this study was to assess habitat differentiation by estimating the relative fitnesses of red, white and hybrid mulberry transplanted into open and shaded forest habitats, as well as in a common garden under conditions of full sun. In the reciprocal transplant experiment, fitness was assessed as survival and biomass at two life stages: seedlings (1 month of growth) and juveniles (5.5 months of growth). We predicted that, if red, white and hybrid mulberry are ecologically differentiated, each species should exhibit the highest relative fitness in at least one environment. Based on their current distribution, red mulberry transplants should outperform white mulberry in shaded habitats, whereas the reverse would be true in full sun.

**Materials and methods**

**SOURCE MATERIAL**

Seeds of red, white and hybrid mulberry were generated in spring 1999 through controlled pollinations in the field. Ten red mulberry (five female, five male) and ten white mulberry (five female, five male) trees were identified in each of two locations: Rondeau Provincial Park (=UTM coordinates NAD (North American Datum) 83: 429702 N, 4682792 E) and Fish Point Provincial Reserve, Pelee Island (=UTM 369965 N, 4621020 E) in southern Ontario, Canada. Thirty inflorescences on each female and male tree were bagged prior to anthesis to prevent pollen contamination. At anthesis, pollen was collected from the inflorescences on each male tree, pooled and transferred to the stigmas of each inflorescence on female trees using fine sterilized paintbrushes. Red and white mulberry were pollinated in all possible combinations [red female × red male (R × R); red female × white male (R × W); white female × red male (W × R); white female × white male (W × W)]. Each pollination treatment was replicated using five different pairs of trees and, for each pair, pollen was transferred to 30 inflorescences per maternal plant. In July 1999, fruits were collected and stored dry at 4 °C until the plant material could be prepared and transplanted into controlled and natural environments.
Reciprocal transplant experiment

To increase the range of life-history stages assessed, plants were transplanted as seedlings (1 month of growth) and juveniles (5.5 months of growth). To generate seedlings, we wetted fruits from all crosses for 24 h, removed the pulp, sowed seeds onto moist filter paper in Petri dishes (maximum of 25 seeds per dish) and incubated them in a growth chamber for 2 weeks (12 h daylight at 26°C and 12 h dark at 22°C). After germinating in October 2000, seedlings were grown in the glasshouse during November, over-wintered in cold storage (4°C) without light until May 2001, and then placed outdoors to initiate bud growth. All seedlings were transplanted into field plots on 22 May 2001 and grown for 15 months.

Juvenile plants were generated in the same way, but the seeds were germinated in early September 1999, grown until mid-December in a glasshouse and then stored cold (4°C) until April 2000. Plants were then grown in the glasshouse for 1 month, planted in 1-litre pots and transferred to a common garden until they were transplanted into the field on 4 June 2000. Juveniles were grown for 27 months. When raising plants to the seedling and juvenile stage class in the glasshouse, individuals were grown in 10-cm pots containing perlite, surface and ProMix (1:1:6), randomly assigned to positions on the glasshouse bench and grown under sodium vapour lights (16 h daylight and 8 h dark at 21°C).

Seedlings and juveniles were transplanted into eight sites within Rondeau Provincial Park. Four sites were open, sunny habitats typically associated with white mulberry, and four sites were forested, shaded habitats associated with red mulberry. Separate, adjacent plots were established for seedlings and juveniles at each site. Nine seedlings of each cross-type (generally three progeny per family) were randomly selected and planted 0.5 m apart in each plot (36 plants per plot) in a 2.5 x 2.5 m grid. Juveniles were transplanted into a plot containing 20 plants, five of each cross-type (generally two progeny per family), randomized in a 4 x 5 m grid and separated by 1 m. Supplemental watering was provided initially to minimize transplant shock. To account for mortality resulting from transplant shock, seedlings and juveniles that died within the first 2 weeks of transplanting were replaced. All plots were fenced to a height of 2 m to keep out herbivores and park visitors. Ground cover was initially cut back to facilitate transplanting but then left undisturbed. For both the juvenile and the seedling plots, plants of randomly chosen parentage (of the same plant stage) were also planted around the outside of each grid to ensure that outer plants within the plots did not experience an edge effect.

Shade and sun sites were characterized using two light (%PAR, %canopy cover) and nine soil (Ca, K, P, NH₄-N, NO₃-N, pH, organic matter, moisture, texture) variables. Percentage available photosynthetically active radiation (PAR) in each plot was measured on 18 June 2000, between 12:00 and 14:00 h using an Li-190S quantum light sensor (Li-Cor, Lincoln, NB, USA). Light intensity (µmol m⁻² s⁻¹) was estimated at 1 m above ground level in the middle and four corners of each plot and expressed as a percentage of light intensity in a neighbouring location with no canopy (Mattes et al. 2001). During the same time period, percentage canopy cover was measured at the same five points in each plot using a spherical crown densiometer (Forestry Suppliers, Inc., Jackson, MO, USA). Soil samples from the top 20 cm of soil (below leaf litter) were also collected from each of the five positions per plot. Two soil samples were randomly selected from each plot and analysed for plant-available Ca (mg kg⁻¹), K (mg kg⁻¹), P (mg kg⁻¹) as well as soil NH₄-N (mg kg⁻¹), NO₃-N (mg kg⁻¹) and pH (Page et al. 1982). Percentage organic matter (%OM), soil moisture and particle size distribution (percentage sand, silt and clay) were also calculated (McKeague 1979). The means of all light and soil characteristics for sun vs. shade habitats were compared using a one-way ANOVA.

Common garden comparison

Seeds from all four pollination treatments were germinated and cultured as in the methods described for juvenile plants. Plants were then transplanted into a cultivated plot and randomly located 1 m apart in an 11 x 30 m grid, and grown for 27 months in the field.

Fitness components

In September 2002, all transplants (field and common garden) were evaluated for two fitness components: survival and above-ground biomass. The health of each plant (alive or not) was noted and living plants were harvested, dried for 144 h at 60°C and their above-ground biomass weighed. Cumulative fitness for each pollination treatment in reciprocal transplants was calculated as: seedling survival x juvenile survival x above-ground biomass, for juveniles. Seedling and juvenile survival were combined because they represent survival probabilities for different life stages. Cumulative fitness in the common garden was calculated as mean survival x mean biomass. Because we were interested in fitness variation in particular field environments, we restricted our focus to seedling and juvenile performance and did not include rates of germination from the glasshouse. The effects of cross-type on germination rates under controlled glasshouse conditions were reported previously (Burgess & Husband 2004).

Analysis

For the reciprocal transplant experiment, variation in survival was analysed using a logistic regression, with habitat-type, plot (nested within habitat), cross-type (R x R, R x W, W x R and W x W) and habitat
× cross-type interaction as the sources of variation. Analyses of variance (ANOVA) were used to examine the fixed effects of habitat, plot and cross-type on family means of biomass and cumulative fitness. Cross-type means were compared with a Tukey’s HSD. Biomass and cumulative fitness values were log- and arcsinh-transformed, respectively, and reported as back-transformed means. For the common garden experiment, we tested for variation in fitness among cross-types with respect to survival, biomass and cumulative fitness, using family means. Cross-type means were evaluated using a one-way ANOVA and compared using a Tukey’s HSD. A logistic regression was used to analyse variation in survival among the cross-types. All statistical analyses were performed using JMP statistical software Version 5.0 (SAS Institute 2002).

Results

RECIPROCAL TRANSPLANT EXPERIMENT

Shade and sun plots differed significantly with respect to percentage PAR and percentage canopy cover. PAR in sun plots was 12 times higher than in shade plots, while percentage canopy cover was 3.5 times higher in shade plots than in sun plots (Table 1). Significant differences in soil moisture, percentage clay, NH4-N and NO3-N were also found between shade and sun plots with values being higher in the former (Table 1). There were no significant differences between sun and shade plots for Ca, K, P, pH and soil texture.

Seedlings

In total, 288 seedlings were transplanted into eight plots (36 plants per plot). Because of low seed germination and transplant-related mortality, there were 41 W × R plants and 15 R × W plants available, fewer than the 72 of each originally planned. Therefore, these cross-types were pooled and treated as an inclusive ‘hybrid’ category. Empty positions within the plots were filled with extra W × W plants to create uniform initial densities for all plants. Across all plots, 56 hybrid (19.4%), 75 R × R (26.1%) and 157 W × W (54.5%) plants were used for this life stage.

Of the 288 plants initially transplanted, 143 (50%) survived to harvest. In the logistic regression ($R^2 = 0.39, P < 0.0001$), survival was not significantly different among cross-types ($\chi^2 = 0.89, P > 0.5$) (Fig. 1a). There was no significant difference in survival between sun (29.3%) and shade habitats (67.5%) ($\chi^2 = 0.06, P > 0.5$) (Fig. 1b), nor was there a significant cross-type/habitat-type interaction ($\chi^2 = 0.06, P > 0.5$), although there were differences among plots within a habitat-type ($\chi^2 = 23.2, P < 0.001$). Biomass did not differ among cross-types ($F_{2,133} = 1.31, P > 0.1$) (Fig. 2a) but was significantly higher for plants in sun habitats (mean = 3.19 g) than for shade habitats (mean = 1.41 g) ($F_{1,133} = 43.51, P < 0.001$) (Fig. 2b). There were no significant differences among plots within habitat-types ($F_{4,133} = 2.12, P > 0.05$) nor a significant interaction between habitat-type (sun or shade) and cross-type ($F_{2,133} = 1.30, P > 0.1$).

Juveniles

In total, 137 plants were transplanted into eight plots (approximately 17 plants per plot). As with the seedling stage, sample sizes for reciprocal hybrids were low and

Table 1  Mean values (SE) for two light and four soil variables from plots in sun and shade habitats ($n = 4$ plots per habitat-type). Habitats differed significantly with respect to all variables.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>PAR (%)</th>
<th>Canopy (%)</th>
<th>NH4-N (mg)</th>
<th>NO3-N (mg)</th>
<th>Moisture (%)</th>
<th>Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shade</td>
<td>7.0 (7.6)</td>
<td>89.1 (0.9)</td>
<td>5.2 (0.6)</td>
<td>60.6 (6.1)</td>
<td>37.9 (6.9)</td>
<td>10.0 (0.6)</td>
</tr>
<tr>
<td>Sun</td>
<td>85.5 (4.2)</td>
<td>25.5 (9.7)</td>
<td>3.5 (0.3)</td>
<td>25.3 (6.7)</td>
<td>12.1 (2.5)</td>
<td>6.6 (1.2)</td>
</tr>
<tr>
<td>$F_{1,6} = $</td>
<td>81.30***</td>
<td>42.31**</td>
<td>6.19*</td>
<td>15.21*</td>
<td>12.34*</td>
<td>6.40*</td>
</tr>
</tbody>
</table>

* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$. 

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were pooled into a single 'hybrid' category. The empty positions in each plot were filled with \( W \times W \) plants to create uniform initial densities for all plants. In total, 48 hybrid (35.0%), 38 \( R \times R \) (27.7%) and 51 \( W \times W \) (37.2%) plants were included in this stage.

Of the initial 137 plants, 93 (68%) survived to harvest. In the logistic regression (\( R^2 = 0.50, P < 0.0001 \)), survival was significantly different among cross-types (\( \chi^2 = 32.3, P < 0.0001 \)) (Fig. 1a). Specifically, \( R \times R \) plants had lower survival (18.4%) than both hybrids (81.3%; \( R \times R \) vs. hybrid, \( \chi^2 = 36.168, P < 0.001 \)) and \( W \times W \) plants (92.2%; \( R \times R \) vs. \( W \times W \), \( \chi^2 = 54.94, P < 0.001 \)) (Fig. 1a). Hybrids and \( W \times W \) plants had similar survival (\( \chi^2 = 2.63, P > 0.1 \)). There was no significant difference in survival between plants in sun (57.8%) and shade (72.1%) habitats (\( \chi^2 = 12.59, P < 0.05 \)) and \( W \times W \) plants (92.2%; \( R \times R \) vs. \( W \times W \), \( \chi^2 = 54.94, P < 0.001 \)) (Fig. 1b). Hybrids and \( W \times W \) plants had similar survival (\( \chi^2 = 2.63, P > 0.1 \)). There was no significant difference in survival between plants in sun (57.8%) and shade (72.1%) habitats (\( \chi^2 = 12.59, P < 0.05 \)) and \( W \times W \) plants (92.2%; \( R \times R \) vs. \( W \times W \), \( \chi^2 = 54.94, P < 0.001 \)) (Fig. 1a). No significant differences between sun and shade habitats were found (\( \chi^2 = 12.59, P < 0.05 \)).

Plant biomass differed significantly among cross-types (\( F_{2,80} = 11.93, P < 0.001 \)) (Fig. 2a). On average, \( R \times R \) plants had less biomass (mean = 9.6 g) than both hybrid (mean = 34.2 g) and \( W \times W \) (mean = 33.1 g) plants. No significant differences between hybrid and \( W \times W \) plants were found. Plants in sun habitats had significantly more biomass (mean = 53.98 g) than those in shade habitats (mean = 13.96 g) (\( F_{1,80} = 5.38, P < 0.05 \)) (Fig. 2b) and there were significant differences among plots within a habitat-type (\( F_{6,80} = 6.94, P < 0.001 \)). The interaction between habitat-type (sun or shade) and cross-type was not significant (\( F_{2,80} = 0.65, P > 0.10 \)).

Cumulative fitness

Overall, cumulative fitness differed significantly among cross-types (\( F_{2,16} = 9.07, P < 0.01 \)) (Fig. 3a). On average, \( R \times R \) plants had significantly lower cumulative fitness (mean = 2963) than both hybrid (mean = 201 572) and \( W \times W \) (mean = 166 504) plants, but there were no significant differences between hybrid and \( W \times W \) plants. Hybrids had the highest relative fitness, and white and red mulberry had 82.6% and 1.5% the fitness of hybrids, respectively. Plants in sun habitats had significantly higher cumulative fitness (mean = 185 216) than those in shade habitats (mean = 62 116) (\( F_{1,16} = 44.11, P < 0.0001 \)) (Fig. 3b). Significant differences among plots within a habitat-type were found (\( F_{2,16} = 19.34, P < 0.0001 \)) (Fig. 3c),
although the interaction between habitat-type (sun or shade) and cross-type was not significant ($F_{2,16} = 0.88, P > 0.1$).

**COMMON GARDEN**

In total, 315 plants ($n = 44 \text{ } R \times R; n = 40 \text{ } R \times W; n = 104 \text{ } W \times R; n = 127 \text{ } W \times W$) were evaluated over a 36-month period; 161 (51%) survived to harvest. Survival was significantly different among cross-types ($\chi^2 = 78.4$, $P < 0.0001$) (Fig. 4a). Specifically, $R \times R$ and $R \times W$ plants had significantly lower survival (11.4% and 12.5%, respectively) than $W \times R$ (61.5%); $R \times R$ vs. $W \times R$, $\chi^2 = 34.75$, $P < 0.0001$; $R \times W$ vs. $W \times R$, $\chi^2 = 30.65$, $P < 0.0001$) and $W \times W$ plants (68.5%; $R \times R$ vs. $W \times W$, $\chi^2 = 46.67$, $P < 0.0001$; $R \times W$ vs. $W \times W$, $\chi^2 = 41.39$, $P < 0.0001$). No significant differences were found between $R \times R$ and $R \times W$ ($\chi^2 = 0.03$, $P > 0.1$) or between $W \times R$ and $W \times W$ ($\chi^2 = 1.22, P > 0.1$) cross-types.

Biomass differed significantly among cross-types ($F_{3,23} = 4.34, P < 0.05$) (Fig. 4b). On average, $R \times R$ plants had less biomass (mean = 5.5 g) than both $W \times R$ (mean = 87.49 g) and $W \times W$ (mean = 86.13 g) plants. However, $R \times W$ (mean = 42.76 g) plants did not differ from any other cross-type.

Cumulative fitness differed significantly among cross-types ($F_{3,23} = 8.65, P < 0.001$) (Fig. 4c). Both $R \times R$ (mean = 53.8) and $R \times W$ (mean = 534.6) plants had significantly lower values than $W \times R$ (mean = 5464.9) and $W \times W$ (mean = 6307.5) plants, although cross-types with the same maternal parent species did not differ. $W \times R$ plants had 86.6% of the fitness of $W \times W$ plants, which had the highest relative cumulative fitness.

**Discussion**

The scarcity of red mulberry in Canada has led to speculation that it is vulnerable to local extinction from hybridization with the introduced white mulberry. Theory suggests that the impact of hybridization in such small populations will depend, in part, on the degree of habitat differentiation between red, white and hybrid mulberry (Buerkle *et al.* 2003). Our study finds no evidence for habitat differentiation between these taxa. Red mulberry did not outperform the other taxa in any single environment (nor their native habitat), and, in fact, was consistently less fit than white and hybrid mulberry in all environments. With limited habitat remaining, our results suggest that red mulberry will experience reduced establishment and is at risk of local extirpation.

The weak ecological differentiation between red and white mulberry is surprising given the extensive evidence for local differentiation in plants (Clausen *et al.* 1940; Bradshaw 1984) and the fact that many plants with an Asian – North American disjunct distribution have been geographically separated for 2–25 million years (Wen 1999). Furthermore, ecological differentiation has been observed between a number of other hybridizing plant taxa (*Iris* – Emms & Arnold 1997; *Artemisia* – Wang *et al.* 1997; *Prunella* – Fritsche & Kaltz 2000). It is possible that red and white mulberry are more differentiated than we observed, but that we were unable to detect these differences for various reasons. For example, the transplant plots may not have been completely representative of red and white mulberry habitats, although they were qualitatively comparable and in close proximity to the respective species. Alternatively, ecological differences may be expressed at seed germination or in later stages in the life history (older juveniles, reproductive adults) not examined here. We cannot exclude this possibility, although glasshouse studies of germination indicate that the performance of red mulberry seeds is uniformly low and parallels establishment of seedlings or juveniles observed in this study (Burgess & Husband 2004). The ecological similarity between red and white mulberry is also typical of many other North American – East Asian disjunct plant species (Wen 2001; Milne & Abbott 2002).
Low relative fitness of red mulberry in sun and shade habitats, and lack of local adaptation, may reflect the fact that our sites are located at the northern limit of its geographical range. Red mulberry is found throughout most of the eastern USA; however, in Canada, populations are restricted to the Carolinian deciduous forest zone of southern Ontario. Consequently, these habitats may be marginal for red mulberry with respect to length of growing season and minimum temperatures. By contrast, the adventurous range of white mulberry extends further north, indicating a greater cold tolerance in this species (Dirr 1997, 1998). Low red mulberry fitness may simply reflect the fact that it is occurring at the extremes of its ecological tolerances. Here, populations may have lower vital rates, increased inbreeding, reduced genetic variation and, as a result, may be more ephemeral and less likely to reflect the effects of local selection. These attributes may also increase its sensitivity to transplantation. Low fitness of red mulberry may also reflect the recent history of disturbance in forest habitats in southern Ontario. Red mulberry is largely restricted to a small number of parks located along the northern shore of Lake Erie. These refuges are increasingly isolated owing to encroachment of agriculture and urban development around their margins. In addition, extensive recreational use of these parks has exposed much of the red mulberry habitat to disturbance through road allowances, opened canopies and invasive species. Although the sun and shade habitats used in this study differed for a number of light and soil characteristics, recent disturbance throughout these forest habitats may have reduced the original distinction between the understory habitats of red mulberry and open, disturbed white mulberry habitats. Anderson (1948) first discussed such ‘blurred’ habitats as being ‘hybridized habitats’ that favour the establishment of hybrid taxa over that of their parents. Transplant studies, e.g. on Artemisia (Wang et al. 1997), have confirmed this idea, showing that hybrids perform best in hybrid habitats and are less fit in parental habitats. In the case of mulberry, disturbance may simultaneously reduce the fitness of red mulberry and favour white and hybrid mulberry establishment.

The strong performance of mulberry F₁ hybrids parallels results from transplants of Ipomopsis (Campbell & Waser 2001) and Iris species (Arnold & Hodges 1995; Rieseberg 1995; Emms & Arnold 1997; Arnold et al. 1999, 2001, 2003), where the fitness of F₁ progeny exceeded that of at least one of the parents in parental home sites. Historically, F₁ hybrids were expected to have low fitness relative to their parents owing to incompatibilities between parental genomes and sterility; however, other evidence shows that some hybrids exhibit heterosis and can thrive in environments that are distinct from parental habitats (Burke & Arnold 2001).

The implications of F₁ heterosis for mulberry are complicated by the fact that natural hybrid zones of mulberry mostly contain later generation hybrids (Burgess et al. 2005). It is not clear how the fitness of F₁ mulberry will differ from F₂ and backcross hybrids, but research on other species indicates that fitness can vary among hybrid classes (Rieseberg & Carney 1998; Milne et al. 2003; Erickson & Fenster 2005) and will depend on the strength of epistasis, dominance and maternal effects. In a previous glasshouse study and from the common garden study reported here, hybrid fitness was largely explained by the identity of the maternal parent (Burgess & Husband 2004). Those with white mothers consistently had lower fitness than those with white mothers. The strength of these maternal effects has two implications. First, in the reciprocal transplants, reciprocal hybrids were bulked into a single class but W × R hybrids were over-represented owing to differential germination. This may account for why fitness of hybrids was more similar to fitness of white mulberry. Second, because fitness is the product of the maternal parent, it is conceivable that strong hybrid performance may persist well beyond F₁s into later backcross generations, as long as they are derived from white or near-white mothers. This may explain the predominance of hybrids with white morphology and genetics in natural populations.

Hybridization is widely considered to be a significant threat to rare species (Abbot 1992; Levin et al. 1996; Rhymer & Simberloff 1996; Ellstrand et al. 1999; Huxel 1999; Ellstrand & Schierenbeck 2000; Vilà et al. 2000; Abbot et al. 2003; Allendorf & Lundquist 2003; Ellstrand 2003); however, there are few empirical studies testing this. Our results lead to clear predictions as to the fate of the endangered red mulberry in the face of hybridization with white mulberry. Assuming that seeds are dispersed similarly and germination rates in the field are similar among taxa, then the decline in red mulberry seems imminent. Its fitness was low in all three different environments examined and thus it is vulnerable to competitive displacement by hybrid and white mulberry. Admittedly, we have not examined the outcome of competitive interactions directly, but the fitness measures predict this decline simply based on the sorting of genotypes through differential survival and reproduction. This expectation is further reinforced by other disadvantages to red mulberry, such as reduced conspecific mating (K.S. Burgess, M. Morgan & B.C. Husband, unpublished data) and relatively low germination rates (Burgess & Husband 2004).

This study highlights the potential effect that hybridization with introduced plants can have on the establishment and persistence of rare native species. However, the effect of white mulberry may not be representative of introduced species in general. Morus is an example of a genus with members in eastern North America and eastern Asia. Despite their historical geographical separation, many species pairs with this distribution have high interfertility and ecological similarity (Ricklefs & Latham 1992; Wen 1999), which may predispose them to hybridization when in secondary
contact. It is also noteworthy that the negative effects of hybridization on red mulberry are attributable to interactions with hybrids, as much as with white mulberry itself. This is similar to the case of exotic Spartina alterniflora, in which the hybrids of the native S. foliosa are spreading rapidly into the native habitat (Ayres et al. 1999). Investigation of similar exotic–native species pairs will be important for predicting the extent of hybridization and the importance of hybridization compared with other ecological interactions to native species.

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References


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Hybridization and establishment of red mulberry


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