
A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction

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Tansley review

A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction

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Contents

Summary	378	IV. Societal challenges for successful reintroduction of <i>Castanea dentata</i>	388
I. <i>Castanea dentata</i> as a model at the intersection of reintroduction biology and restoration ecology	379	V. Conclusions	389
II. Technological approaches for recovery of <i>Castanea dentata</i>	381	Acknowledgements	389
III. Ecology of the historically dominant <i>Castanea dentata</i> in contemporary forests	385	References	389

Summary

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Key words: biotechnology, *Castanea dentata*, ecosystem restoration, genetic engineering, introduced pathogen, reintroduction, socio-ecological system, tree improvement.

We propose a conceptual framework for restoration of threatened plant species that encourages integration of technological, ecological, and social spheres. A sphere encompasses ideas relevant to restoration and the people working within similar areas of influence or expertise. Increased capacity within a sphere and a higher degree of coalescing among spheres predict a greater probability of successful restoration. We illustrate this with *Castanea dentata*, a foundation forest tree in North America that was annihilated by an introduced pathogen; the species is a model that effectively merges biotechnology, reintroduction biology, and restoration ecology. Because of *C. dentata*'s ecological and social importance, scientists have aggressively pursued blight resistance through various approaches. We summarize recent advancements in tree breeding and biotechnology that have emerged from *C. dentata* research, and describe their potential to bring new tools to bear on socio-ecological restoration problems. Successful reintroduction of *C. dentata* will also depend upon an enhanced understanding of its ecology within contemporary forests. We identify a critical need for a deeper understanding of societal influences that may affect setting and achieving realistic restoration goals. *Castanea dentata* may serve as an important model to inform reintroduction of threatened plant species in general and foundation forest trees in particular.

*The first two authors are co-first authors. All three authors contributed equally to the development of the conceptual framework.

I. *Castanea dentata* as a model at the intersection of reintroduction biology and restoration ecology

Since the discovery of chestnut blight caused by *Cryphonectria parasitica* (Murr.) Barr (= *Endothia parasitica* (Murr.) Anderson & Anderson) in the early 1900s, many groups have worked to develop blight-resistant American chestnut (*Castanea dentata* (Marsh.) Borkh.); and a large-scale reintroduction program is being considered for implementation (Steiner & Carlson, 2006). The establishment of blight-resistant *C. dentata* in eastern forests resides at the intersection of two young disciplines: reintroduction biology and restoration ecology. Reintroduction biology has a single species focus (often a charismatic mammal or bird) and is used as a conservation tool to prevent extinction by re-establishing populations of a target species within its historical range (Seddon *et al.*, 2007a; Armstrong & Seddon, 2008). Restoration ecology has often been botanically focused and emphasizes community and ecosystem structure and function (Young, 2000). *Castanea dentata* represents the reintroduction of a single, charismatic species that, because it is also a foundation species, may simultaneously restore community and ecosystem function. Recent calls for a more systematic, scientific approach to reintroduction biology and for a merging of reintroduction biology and restoration ecology (Lipse & Child, 2007; Seddon, 2010; Polak & Saltz, 2011) can both be demonstrated in the case of *C. dentata*. In addition, reintroduction of *C. dentata* can serve as a catalyst for public discourse about the appropriate use of biotechnology (including genetic engineering

(GE)) for restoration and commercial applications. Collectively, these aspects implicate *C. dentata* as an informative model that merges biotechnology, reintroduction biology, and restoration ecology (Lipse & Child, 2007; Seddon *et al.*, 2007b; Seddon, 2010). Below, we introduce *C. dentata* and its devastation by the blight. We then present a conceptual framework for restoration that merges societal, technological, and ecological spheres of expertise and influence. Lastly, we describe the objectives of this review and how we will use the conceptual framework to synthesize *C. dentata* biology, genetics, and ecology to create a foundation for identifying restoration goals and strategies for implementation.

1. Loss of a foundation species caused by an introduced pathogen

Before the accidental introduction of chestnut blight, *C. dentata* was a dominant tree species throughout much of its 800 000 km² range in eastern North America (Fig. 1), sometimes comprising the majority of biomass within an exceptionally diverse forest community (Braun, 1950; Stephenson, 1986; Russell, 1987; Foster *et al.*, 2002). Particularly in the Appalachian region, *C. dentata* filled an important ecological niche and has been called a 'foundation species' because of its influence over population, community, and ecosystem processes (Youngs, 2000; Ellison *et al.*, 2005). For example, *C. dentata* was a major seed resource, producing large and consistent seed crops that drove consumer population dynamics (Dagleish & Swihart, 2012). Because of its

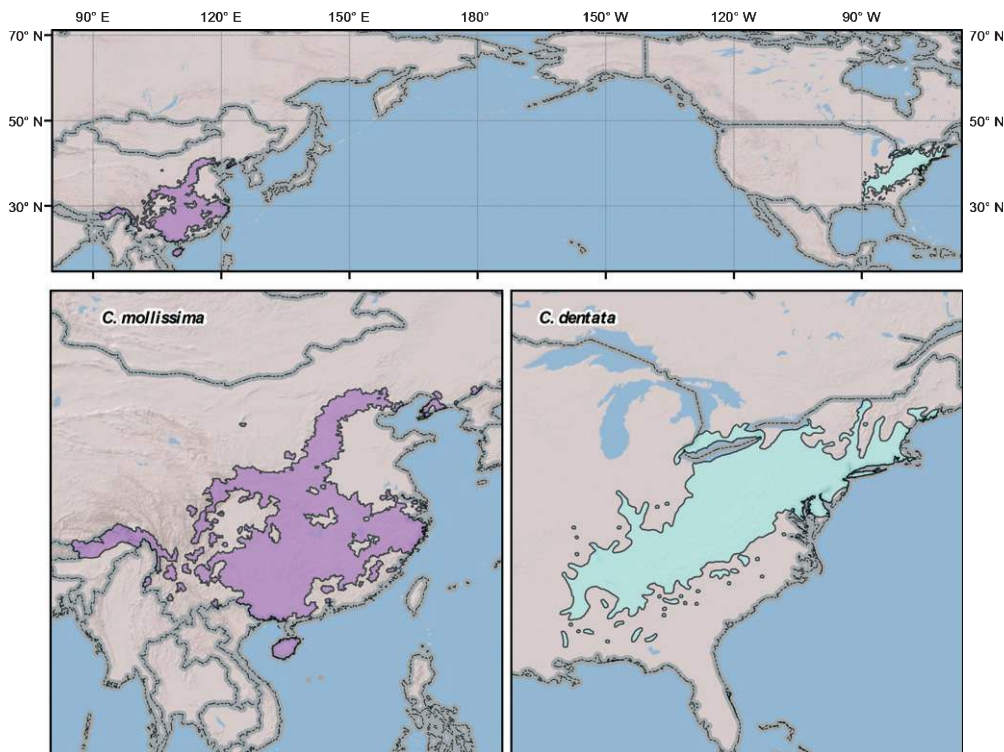


Fig. 1 Native ranges of American chestnut (*Castanea dentata*) and Chinese chestnut (*C. mollissima*), the primary source of blight resistance genes. Frost sensitivity (Parker *et al.*, 1993) may have limited the proliferation of *C. dentata* at higher latitudes (Russell, 1987). An outbreak of the introduced soil-borne oomycete pathogen *Phytophthora cinnamomi* during c. 1825–1875 may have permanently retracted the southern portion of its range, which once extended as far south as Florida (Anagnostakis, 2001). By the late 1800s, most *C. dentata* in the Piedmont region of North Carolina had disappeared, while its natural range was still expanding before the introduction of the blight in other areas, for example, into Michigan (Russell, 1987; Brewer, 1995).

fast growth, high-tannin wood, and low carbon (C) : nitrogen (N) ratio leaves, *C. dentata* affected ecosystem functions such as productivity, decomposition, and nutrient cycling (Ellison *et al.*, 2005). *Castanea dentata* also filled an important economic and social niche. The strong wood of *C. dentata* is highly decay resistant and was used for telephone poles, posts, and railroad ties, as well as for furniture, construction lumber, siding, and roofing (Smith, 2000; Youngs, 2000). The wood and bark were used to produce tannin for leather production. The nuts, which are edible raw or roasted, were collected for household consumption and to sell (Youngs, 2000).

Chestnut blight caused by *C. parasitica*, a filamentous ascomycete fungus, rapidly annihilated *C. dentata* throughout its range (Anagnostakis, 1987) in the first half of the 20th Century (Hepting, 1974). The necrotrophic pathogen, first discovered in 1904 in New York City (Murrill, 1906; Anderson & Rankin, 1914), enters chestnut trees through stem wounds as germinating conidia or ascospores and colonizes the cambial zone, causing cell and tissue death, vascular dysfunction, and stem dieback (Beattie & Diller, 1954). Because it forms a perennial canker, the fungus has ample opportunity to sporulate. Conidia (asexual spores transported by water or animal movement) can form within 1 month of infection and ascospores (wind-disseminated sexual spores) within 4 months, resulting in rapid disease spread. Indeed, in < 50 yr the disease spread from New York throughout the range and killed aboveground stems of nearly every mature tree (Hepting, 1974). *Castanea dentata* is still a common component of eastern forests, but nearly all individuals are root-collar sprouts of blight-killed trees, which mostly originated from trees that were seedlings or saplings when blight arrived (Stephenson *et al.*, 1991; Paillet, 2002). Cycles of sprouting, infection, dieback, and re-infection may persist for decades (Paillet, 1984), yet sprouts rarely grow out of the understory or to reproductive maturity (Paillet, 2002). Since the discovery of chestnut blight, many groups have worked to develop blight-resistant *C. dentata* through diverse strategies including biocontrol of the fungus with mycoviruses, breeding and selection of large surviving *C. dentata* trees, hybrid breeding with resistant Asian chestnut species, and GE. Efforts to develop blight resistance have largely evolved separately, but are coming to fruition such that a large-scale reintroduction program is being considered for implementation (Steiner & Carlson, 2006).

2. A conceptual framework for restoration

In this review, we present *C. dentata* as a model species and use it to illustrate a general conceptual framework for restoration (Fig. 2a), which contains three major spheres: *society*, *technology*, and *ecology*. We use 'sphere' to describe similar ideas relevant to restoration and the people working within similar areas of interest, influence, or expertise. The *society* sphere includes structures such as governmental policy and regulation, collaborative networks, and cultural or economic valuation. The *technology* sphere encompasses solutions to mitigate deficiencies in a given socio-ecological system. The *ecology* sphere includes targeting optimal habitats for application of the technology as well as forecasting

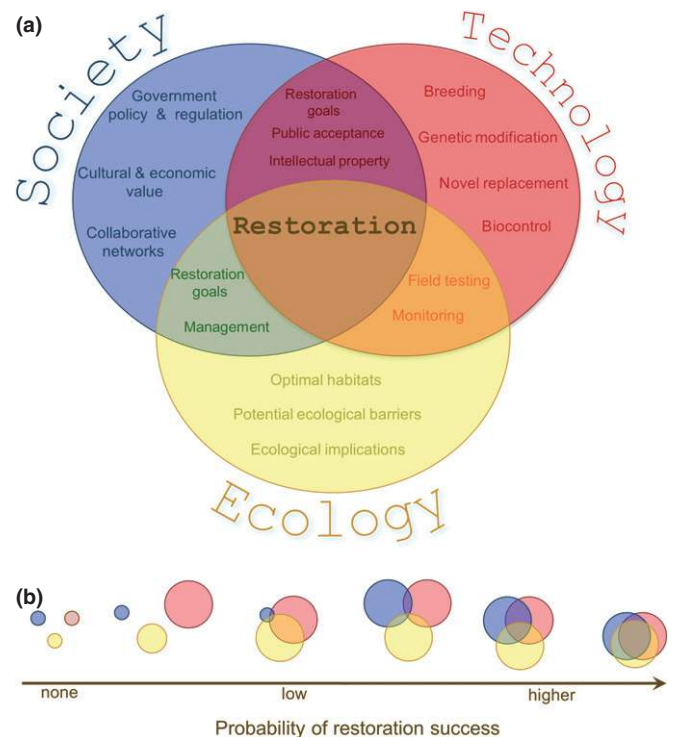


Fig. 2 Conceptual framework for restoration. (a) We identify three key spheres of expertise that underpin the process of using (re)introduction of a species as a tool in restoration: the society (blue), technology (red), and ecology (yellow) spheres. The area corresponds to the capacity of each sphere. Within each sphere and area of overlap (green, orange, and purple areas), we illustrate several key roles. The degree of overlap among all three spheres, illustrated in brown, represents the probability of achieving restoration success, which we define as meeting the restoration goals set through collaboration among members in all three spheres. (b) The framework predicts that restoration success increases as the capacity of the spheres and the degree of overlap (collaboration) among the spheres increase. If only one of the spheres has large capacity, for example, restoration success remains low because the degree of overlap is low. Similarly, restoration success will be low even when all spheres are large if the degree of overlap is low.

community and ecosystem effects. We identify areas that require collaboration among spheres, perhaps most critically the setting of realistic, attainable goals that are central to defining restoration success (Hobbs & Norton, 1996). *Castanea dentata*'s vast cultural and ecological significance has driven development of multiple technologies in pursuit of reintroduction (e.g. biocontrol, breeding, and GE) along with a recent initiative to improve ecological knowledge of the species.

Our framework predicts that the larger the capacity of each sphere (represented by the size of the circle) and the higher the degree of integration and collaboration among spheres (overlap of the circles), the greater the probability of successful restoration (Fig. 2b). Restoration programs generally are comprised of diverse groups addressing specific facets of the restoration process, sometimes covering more than one sphere, and thereby yielding a degree of overlap. We argue, however, that, for complex restoration problems to be successfully addressed at a large scale, all three spheres must be pursued collectively, simultaneously, and ideally in

a coordinated manner. In the case of *C. dentata*, several groups are currently working within subsets of our conceptual model. For example, the Forest Health Initiative (FHI), building on the technical successes of the National Science Foundation's Fagaceae Project, is using *C. dentata* as a 'test tree' to create a roadmap for evaluating the use of biotechnology (including GE) in forests through an approach that combines consideration of scientific, social, regulatory and environmental issues (<http://foresthealthinitiative.org/>). The American Chestnut Foundation (TACF) has primarily focused on the use of breeding technologies to produce a blight-resistant tree and reflects a network of devoted members aimed at reintroducing *C. dentata*. Use of our conceptual framework enables a more holistic approach to restoration that integrates collaborative networks such as FHI and TACF, and underscores the centrality and necessity of explicitly stated common goals. Although *C. dentata* is among the best-known examples of virtual extirpation caused by an exotic pest, numerous other tree species face a similar fate (e.g. emerald ash borer (*Agrilus planipennis* Fairmaire) on ash (*Fraxinus* spp. L.) in North America; hemlock woolly adelgid (*Adelges tsugae* (Annand)) on eastern hemlock (*Tsuga canadensis* (L.) Carr.) in North America; and Dutch elm disease (*Ophiostoma ulmi* (Buism.) Nannf.) on elm (*Ulmus* spp. L.) in Europe and North America). Through effective merging of biotechnology, reintroduction biology, and restoration ecology, *C. dentata* may serve as an important model to inform reintroduction of threatened plant species in general and foundation forest trees in particular.

3. Objectives of this review

Using our conceptual framework as a guide, we synthesize *C. dentata* biology, genetics, and ecology to serve as a foundation for identifying reintroduction goals and strategies for implementation. Recent reviews have detailed the *C. dentata* inter-species backcross breeding program as well as social and management considerations toward *C. dentata* restoration (Steiner & Carlson, 2006; Jacobs, 2007) and genetic implications of the backcross breeding program for restoration (Worthen *et al.*, 2010). Others have highlighted the potential for the use of GE technologies in reintroduction programs (Merkle *et al.*, 2007), a call for a broader use of species introductions as a tool for restoration (Seddon, 2010), and the need for a stronger connection between socio-economic research and restoration practice (Collier, 2011). By merging ideas from across diverse literature, we place *C. dentata* reintroduction within the broader context of biotechnology, reintroduction biology, and restoration ecology. We trace the paths of the three main technologies at the leading edge of developing blight-resistant *C. dentata* (the *technology* sphere) and examine the key ecological changes in post-blight deciduous forests that may influence the role of *C. dentata* in contemporary forests (the *ecology* sphere). We then present some of the critical challenges remaining for *C. dentata* reintroduction, the majority of which reside within the *social* sphere of our conceptual framework. These three facets should be well developed individually, yet must effectively converge to ensure successful restoration. The importance of restoration as a conservation tool is increasing as ecosystem

degradation as a consequence of anthropogenic influences becomes more prevalent; while our framework is based upon the model of *C. dentata*, we also suggest how it applies to reintroduction of threatened plants in general.

II. Technological approaches for recovery of *Castanea dentata*

Scientists have pursued three main approaches for mitigating chestnut blight: (1) inoculation of chestnut blight infections with hypovirulent strains of *C. parasitica*, (2) breeding *C. dentata* using both intra- and inter-species methods, and (3) GE of *C. dentata*. Below, we review these approaches and outline how they inform and are enhanced by genomic technologies. Though these efforts have largely developed independently, their ability to coalesce through technological development as well as social structures has the potential to increase the probability of restoration success (Fig. 2a).

1. Biological control with hypovirulence

Hypovirulence is the reduction in virulence caused by a mycovirus (family Hypoviridae) infection of the blight fungus (Milgroom & Cortesi, 2004). The reduced virulence causes sublethal or healing cankers (Griffin, 2000; Milgroom & Cortesi, 2004). In many areas of Europe, hypovirulence has effectively controlled blight (Griffin, 2000; Milgroom & Cortesi, 2004). Hypoviruses in Europe have spread both naturally and through artificial inoculations of cankers, though it remains unclear whether human-aided deployment has significantly increased spread (Milgroom & Cortesi, 2004). The incidence of blight infection, hypovirulence, and tree mortality all vary with management, environmental conditions, and the age of the trees (Milgroom & Cortesi, 2004).

The discovery of hypoviruses within blight cankers in *C. dentata* populations established in Michigan (outside the *C. dentata* native range) fueled hopes for using hypovirulence to control blight throughout North America (Jaynes & Elliston, 1980; Fig. 3). In some Michigan populations, trees grow large and reproduce via seed with healing cankers (Milgroom & Cortesi, 2004). Michigan populations are the exception, however, and not the rule: in experimental trials in Virginia, West Virginia, Connecticut, and Wisconsin, hypoviruses failed to spread among trees and sometimes even among cankers within a tree, severely limiting the use of hypovirulence for biocontrol (Griffin, 2000; Milgroom & Cortesi, 2004). Vegetative incompatibility (*vic*) is commonly observed between blight fungus cultures and several *vic* genes have been identified and mapped (Anagnostakis, 1982; Cortesi & Milgroom, 1998; Kubisiak & Milgroom, 2006). When any one of the *vic* genes does not match between cultures, anastomosis and, therefore, hypovirus transfer, is largely prevented (Liu & Milgroom, 1996; Cortesi *et al.*, 2001). Although *vic* is considered a leading cause of the failure of hypovirus spread in North America, questions remain concerning the effects of environmental and biological conditions on the establishment and spread of hypoviruses (Milgroom & Cortesi, 2004). It appears that biocontrol with hypovirulence alone is unlikely to control blight in North America, but it may be used as a tool in concert with host genetic resistance and optimized

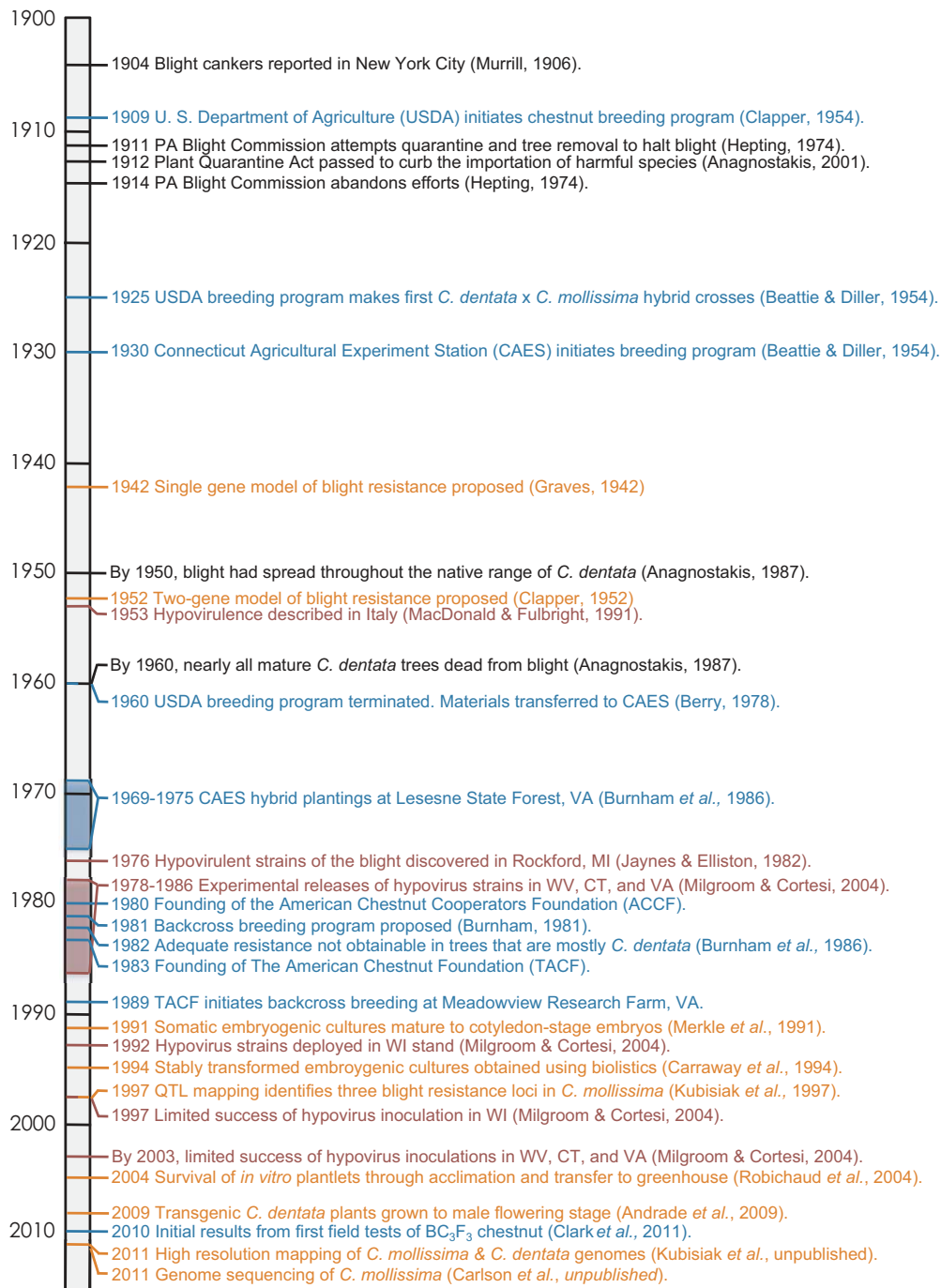


Fig. 3 Timeline showing key events in the development of blight resistance for *Castanea dentata*. Brown, blue, and orange text indicates events in the biocontrol, breeding, and genetic engineering approaches, respectively.

silviculture to achieve a high degree of blight control (Griffin, 2000).

2. Breeding for blight resistance

Breeding for blight resistance has focused on (1) intra-species breeding to increase the very low frequency of naturally occurring blight resistance in populations of *C. dentata* or (2) inter-species breeding primarily with *Castanea mollissima* (Blume) (Fig. 1) to introgress Asian resistance into *C. dentata*. The American Chestnut

Cooperators Foundation (ACCF) is actively pursuing intra-species breeding to complement their hypovirulence and silviculture programs. TACF is leading a large group of partners focusing on the inter-species strategy using backcross breeding while supporting research on biocontrol, silviculture, and GE methods. Breeding programs focusing primarily on inter-species approaches began shortly after the discovery of blight and have been conducted by the US Department of Agriculture (USDA; 1909–1960), the Connecticut Agricultural Experiment Station (CAES; 1930–present), and TACF (1983–present).

The USDA breeding program began in 1909 (Fig. 3) with the goal of producing blight-resistant forest trees for timber, tannins, and wildlife as well as horticultural trees for nut production (Clapper, 1954). By 1925, the USDA program made *C. dentata* × *C. mollissima* hybrid crosses utilizing materials collected in Asia (Beattie & Diller, 1954; Diller & Clapper, 1965). In 1960 the USDA program was discontinued and some materials were transferred to the ongoing breeding program at CAES (Berry, 1978). Work at CAES was highly collaborative with the USDA program during the years when both programs were active, using similar strategies of species hybridization and resistance testing in anticipation of finding and cloning the ideal combination of resistance from Asian chestnut species and fast growth and forest tree form from *C. dentata*. In 1983, TACF was founded to breed *C. dentata* using the backcross breeding method (Burnham, 1981; Burnham *et al.*, 1986). Several selected genotypes from both the USDA and the CAES breeding programs formed the basis of TACF's program.

The specific steps of the backcross breeding method include making three backcross generations with selection for resistance at each generation to ensure retention of Asian resistance genes (Fig. 4), intercrossing the selected BC₃F₁ trees to produce BC₃F₂ populations fully segregating for resistance, selecting in the BC₃F₂ populations for high resistance, and establishing the selections in seed orchards to produce seeds (BC₃F₃ and beyond) for forest planting (Hebard, 1994, 2006). As widely recognized in plant breeding, it is important to use many unrelated *C. dentata* trees at each generation to properly sample the native species alleles (Hebard, 2006; Worthen *et al.*, 2010) and parent trees should originate within the region where the progeny trees will be planted to promote local adaptation. Thus, throughout the original *C. dentata* range, an extensive network has developed consisting of partnering institutions, *C. dentata* mother trees, and breeding and seed orchards to help ensure production of locally adapted *C. dentata* germplasm for reintroduction (Fig. 5). Ideally, sources of resistance should include parent trees of *C. mollissima*, *Castanea seguinii*

(Dode), and *Castanea crenata* (Siebold & Zucc.), for example, because it is likely that trees within and among species will carry different resistance genes. To achieve these goals, TACF is using multiple genotypes of primarily *C. mollissima* as resistance sources and breeding lines should contain at least 20 *C. dentata* parents, providing necessary genetic diversity (Hebard, 2006). Putatively blight-resistant BC₃F₃ seed was first harvested by TACF in 2005 (Diskin *et al.*, 2006); germplasm availability from the breeding program should increase exponentially with time (Jacobs, 2007).

3. Genetic engineering

The first application of genetically engineered (GE) forest trees will probably be for reintroduction of species decimated by invasive pathogens or pests (Adams *et al.*, 2002; Merkle *et al.*, 2007). An important consideration for GE trees is the source and tissue specificity of resistance genes as well as their regulatory elements. Both transgenic and cisgenic approaches draw from the same recombinant DNA toolbox, but they differ in the source of the pest resistance genes. Transgenic approaches use genes from nonplant organisms or from plants that are not closely related (i.e. sexually incompatible) to the target plant. Cisgenic approaches use genes from closely related or even the same species and cisgenics has been noted for its similarity to inter-species backcross breeding (Fig. 6; Schouten & Jacobsen, 2008; Han *et al.*, 2010). GE technologies have progressed rapidly (Fig. 3) and may offer new opportunities for restoring species on the verge of extirpation. The exotic hemlock woolly adelgid, for example, is devastating *T. canadensis* and no crossable species with resistance exists; however, congeners with resistance do exist (i.e. *Tsuga chinensis* (Franch.) Pritzel ex Diels.; Montgomery *et al.*, 2009), opening the possibility for cisgenics. Both transgenic and cisgenic approaches are viable options for



Fig. 4 (a) BC₃F₁ *Castanea dentata* 2.5 yr after inoculation with chestnut blight showing canker response indicating susceptibility; tree is dead above point of inoculation. (b) The first moderately blight-resistant BC₃F₁ *C. dentata* produced by the Indiana chapter of the American Chestnut Foundation also shown 2.5 yr after inoculation (IN-96-01A, a cross from IW2 × GR226). Photographs courtesy of James McKenna.

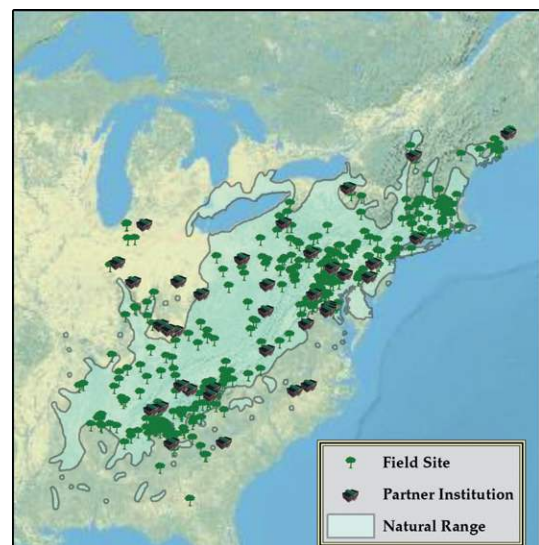


Fig. 5 Distribution of field sites (*Castanea dentata* mother trees, breeding plantations, orchards, and research sites) and major partnering organizations (university, government, and corporate) contributing toward *C. dentata* reintroduction. Data were provided by Sara Fitzsimmons (The American Chestnut Foundation).

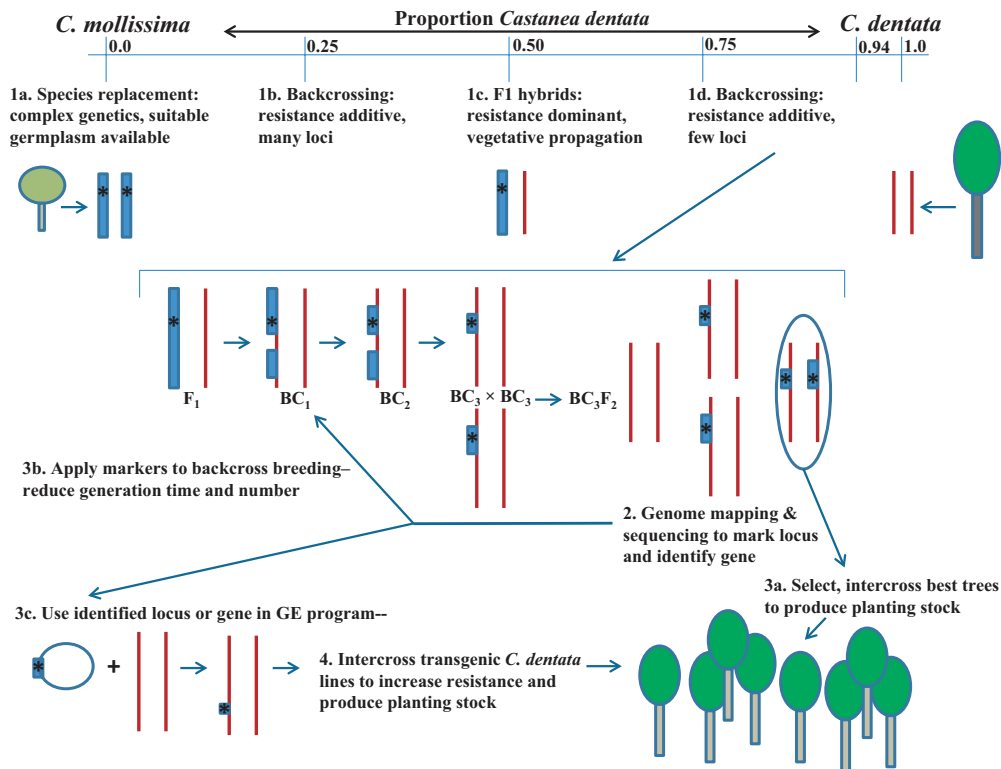


Fig. 6 Approaches for attaining resistance to chestnut blight, ranging from species replacement to various forms of hybridization and selection (1a to 1d). These methods represent a continuum in proportions of donor genome in new or recipient species, ranging from 100% under species replacement (1a) to c. 6% in backcross breeding to the BC₃F₂ generation (1d). The backcross breeding program provides optimal materials for genetically mapping blight resistance gene loci (2). Selected BC₃F₂ trees can be used in seed orchards as relatively true breeding parents to produce seeds for forest planting (3a). Once genetically mapped, markers can be used to facilitate backcross breeding in other crosses for increasing genetic diversity of the foundation species (3b). In addition, fine mapping and quantitative trait locus (QTL) sequencing can identify candidate genes for testing in genetic engineering (GE) lines of the foundation species (3c). Resistant GE lines can be further propagated and intercrossed to increase diversity and provide seeds for forest planting (4).

C. dentata and make it a valuable test case for the use of these GE technologies for forest restoration.

Substantial progress has been made using transgenic approaches to developing blight resistance in *C. dentata*. Several small proteins with antimicrobial activity, including antifungal activity, show little or no toxicity to *Castanea*, *Malus*, or *Salix* spp. pollen (Powell *et al.*, 1995, 2000, 2006). The oxalate oxidase (*OxO*) gene provides another promising lead for chestnut blight resistance (Polin *et al.*, 2006; Welch *et al.*, 2007). When inserted into poplar (*Populus × euramericana* (Dode) Guinier), *OxO* increases tissue tolerance to oxalate and enhances resistance to the fungal pathogen *Septoria musiva* (Liang *et al.*, 2001). Co-transformation of three genes is routine in *C. dentata* – a visual selectable marker (e.g. *GFP*), antibiotic resistance (e.g. *npII*, neomycin phosphotransferase II) for selection in culture, and the candidate resistance gene (Newhouse *et al.*, 2010) – further facilitating work in this species. In this case, because the marker and selection genes are not linked to the resistance gene, they can be removed from the segregating breeding population while retaining progeny that contain resistance genes. Co-transformation has limitations, however, including high variation in gene expression and gene silencing (Halpin *et al.*, 2001), although the co-expression of multiple genes in a single open reading frame may provide a solution to this problem (Liang *et al.*, 2005).

A cisgenic approach relies on the identification of resistance genes in closely related species. The most definitive research on blight resistance genetics in *Castanea* supports a three gene model as detected by quantitative trait locus (QTL) mapping in a *C. mollissima × C. dentata* cross (Kubisiak *et al.*, 1997). The three-locus model accounted for c. 70% of the genetic variation, implicating a combination of major and minor genes contributing to resistance. New higher density maps using short sequence repeat (SSR or microsatellite) and single nucleotide polymorphism (SNP) markers developed from large-scale expressed gene sequencing (Barakat *et al.*, 2009) have confirmed and refined genomic locations of blight resistance QTLs (Kubisiak *et al.*, in press). Such maps enable increased precision in locating blight resistance loci and greater sensitivity in finding smaller effect loci. At present, candidate genes for cisgenic GE applications are identified by their presence in genomic regions identified as QTLs for resistance, their presence or absence in suppressive subtraction hybridization (SSH) libraries (Baier, 2009), and their differential expression in inoculated vs noninoculated *C. mollissima* stems or in noninoculated *C. mollissima* vs *C. dentata* stems (Barakat *et al.*, 2009, 2012). For example, one promising candidate resistance gene, a laccase gene, is highly expressed in *C. mollissima* stem tissues, expressed at very low levels in *C. dentata*, and appears to map to a blight resistance QTL (Baier, 2009).

4. Genome-informed genetics

Molecular markers have improved our understanding of *C. dentata* genetics by delineating patterns of genetic diversity and dissecting quantitative trait variation (Pigliucci *et al.*, 1990; Kubisiak *et al.*, 1997; Huang *et al.*, 1998; Casasoli *et al.*, 2006). Isozyme studies show that *C. dentata* contains low to moderate levels of genetic diversity relative to other species with large geographic ranges and similar life history traits (Dane *et al.*, 2003), while DNA markers show *C. dentata* having similar levels of diversity to these species (Kubisiak & Roberds, 2006). It is unclear whether the low genetic diversity as observed using isozyme markers predisposed *C. dentata* to rapid population decline in response to the blight epidemic or whether it is a consequence of blight-induced population decline (Dane *et al.*, 2003). Most of the neutral genetic variation observed in *C. dentata* resides within populations (>~90% for isozymes; >~95% for DNA markers), with evidence of clinal trends in overall allele diversity and allele frequencies for some loci (Huang *et al.*, 1994; Kubisiak & Roberds, 2006). Relatively early in the DNA marker era, Bernatzky & Mulcahy (1992) and Ellingboe (1992) suggested using markers to map resistance genes in *C. mollissima* and thereby facilitate their introgression into *C. dentata* through backcross breeding. Conceptually, this is an excellent idea that has been shown to be effective in numerous systems (Collard & Mackill, 2008; Moose & Mumm, 2008), yet early markers (e.g. restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplification fragment length polymorphism (AFLP), and isozymes) proved to be suboptimal and cost prohibitive for operational use in chestnut breeding programs.

The recent development of large sets of SSR and SNP markers (Kubisiak *et al.*, in press) is likely to provide the practical application envisioned (Fig. 6; Nance *et al.*, 1992; Wheeler & Sederoff, 2009). SSRs and SNPs are codominant and have much higher sequence specificity (providing data on the same loci across parents), effectively overcoming the two major problems encountered with RAPD and AFLP. The candidate genes are being isolated from *C. mollissima* and *C. seguinii* and used to transform *C. dentata* to directly test their effectiveness in providing blight resistance. Additionally, the highly informative maps enable tracking of introgressed *C. mollissima* genes within inter-species backcross families as well as estimating the remaining *C. mollissima* genome at various generations, facilitating the dual selection for resistance (from *C. mollissima*) and recurrent type (from *C. dentata*). The use of markers in selection for recurrent type can greatly improve its recovery (Tanksley & Rick, 1980; Soller & Beckmann, 1983). Many backcross programs plan for six backcross generations (Allard, 1960); however, with informative markers two generations provide similar results (Visscher *et al.*, 1996). Markers may reduce the three backcross generations of the TACF program to only one, allowing additional resistance sources to be introgressed with a similar level of effort. Such integration and collaboration among technological developments will expand the capacity of the technological sphere with the potential to increase restoration success (Fig. 2).

III. Ecology of the historically dominant *Castanea dentata* in contemporary forests

To ensure that efforts associated with development of blight-resistant *C. dentata* are fully realized, silvicultural management based on a thorough ecological understanding of the species is essential (Griffin, 2000). Our incomplete understanding of the basic biology and ecology of *C. dentata* (Paillet, 2002; Jacobs, 2007) originates primarily from historical observations, growth of stump sprouts, and studies of a few extant populations that were initiated by plantings outside the native range (Paillet, 1982, 1984; Paillet & Rutter, 1989). Collectively, this information has provided important insight into the historical role of *C. dentata*. However, over the past *c.* 100 yr, during which *C. dentata* has been functionally extirpated from the canopy, several major landscape-level changes have occurred across the species range. Eastern deciduous forests were heavily impacted by extensive land clearing and large-scale fires during the late 19th to early 20th Centuries, which has since been replaced by management favoring fire suppression and clearcutting over progressively smaller units, selective cutting, or single tree selection (Abrams, 2003). Concurrent with a decrease in disturbance, dramatic increases in white-tailed deer (*Odocoileus virginianus* Zimmermann) populations have intensified herbivory (Abrams, 2003; McEwan *et al.*, 2011). Analysis of climatic data for the period from the early 16th Century onwards suggests that the 20th Century exhibited increased soil moisture availability and decreased severity of drought (McEwan *et al.*, 2011). The result is a distinctly different forest composition in contemporary forests, characterized by decreased regeneration of oaks (*Quercus* spp.) and an increase in the importance of maples (*Acer* spp.) (Abrams, 1998, 2003; Fei *et al.*, 2011). Considering these broad-scale changes in land-use patterns and forest dynamics within the context of a recent burst of research into *C. dentata* ecology can improve our ability to forecast the niche of blight-resistant *C. dentata* in modern-day forests. Within our conceptual framework, ecological knowledge informs both where and how to reintroduce blight-resistant *C. dentata*, and the capacity to forecast ecological barriers to and implications of reintroduction (Fig. 2a).

1. Identifying optimal reintroduction habitats

Current insight into the physiological ecology of *C. dentata* combined with evidence of its historical range and dominance suggests that the species is an opportunistic generalist, well adapted to conform to a broad range of environmental and climatic conditions. However, *C. dentata* is absent from or grows poorly on high-pH, limestone-derived soils (Russell, 1987; Tindall *et al.*, 2004). *Castanea dentata* exhibits characteristics of both a late-successional (relative shade tolerance and extended longevity) and pioneer species (aggressive stump sprouting and juvenile competitiveness), thereby distinguishing *C. dentata* from oaks and other co-occurring species.

Castanea dentata exhibits high plasticity to varying light conditions and has been classified as both an intermediate shade-tolerant (Joesting *et al.*, 2007, 2009) and a shade-tolerant (Wang *et al.*, 2006) species. Shading produces either a neutral (Wang *et al.*, 2006;

Rhoades *et al.*, 2009) or a positive (McCament & McCarthy, 2005; Anagnostakis, 2007) effect on germination and/or juvenile survival. Once established, seedlings and saplings may persist for years under low light conditions beneath the canopy (Paillet & Rutter, 1989; McEwan *et al.*, 2006), exhibiting plasticity by increasing leaf mass per unit area with greater light availability (King, 2003; Wang *et al.*, 2006; Joesting *et al.*, 2009). *Castanea dentata* seedlings, saplings, and mature trees exhibited light compensation points, quantum efficiency, leaf mass per unit area, and nitrogen concentrations similar to those of shade-tolerant species (Joesting *et al.*, 2009). Understory trees, however, have high maximum rates of photosynthesis representative of fast-growing, shade-intolerant species such as yellow-poplar (*Liriodendron tulipifera* L.) and eastern cottonwood (*Populus deltoides* Bartram ex Marsh.) (Joesting *et al.*, 2009). Furthermore, *C. dentata* growth and photosynthesis are promoted by increased light availability (McCament & McCarthy, 2005; Wang *et al.*, 2006; Joesting *et al.*, 2007), exceeding or equaling those of many shade-intolerant pioneer species (Boring *et al.*, 1981; Griffin, 1989; King, 2003). Although *C. dentata* may survive for prolonged periods as stump sprouts or seedlings beneath the canopy, it retains the ability to rapidly respond to release following disturbance (Paillet & Rutter, 1989; Paillet, 2002; McEwan *et al.*, 2006). Sprout growth may exceed that of any other hardwood species following clearcutting (Mattoon, 1909) and radial growth rates approach 5 mm yr⁻¹ in plantation or natural stand settings, with maximum reported values of 10–12 mm yr⁻¹ (Zeigler, 1920; Paillet & Rutter, 1989; Jacobs & Severeid, 2004).

The former dominance of *C. dentata* in upland habitats suggests greater drought tolerance compared with co-occurring species (Jacobs, 2007). *Castanea dentata* exhibited higher instantaneous water use efficiency under controlled drought conditions relative to several species of upland oaks and dry-site red maples (*Acer rubrum* L.) (Bauerle *et al.*, 2006) and *C. dentata* sprouts had higher leaf water potential than several species of upland oaks during an early summer drought (Abrams *et al.*, 1990). In spite of its capacity to persist on dry sites, *C. dentata* was reported to have comprised 25–40% of the basal area in some riparian zones in the southern Appalachians (Vandermast & Van Lear, 2002). Although *C. dentata* has been shown to increase leaf, shoot, and root biomass with greater availability of specific nutrients, including nitrogen, potassium, and magnesium (Latham, 1992; Rieske *et al.*, 2003; McCament & McCarthy, 2005), responses to specific nutrient limitations or nutrient interactions are less well studied. In glasshouse experiments, however, *C. dentata* outperformed all other species tested in traits associated with competitive ability over a wide range of nutrient and light combinations (Latham, 1992). In addition, leachate from *C. dentata* litter may have allelopathic properties (Vandermast *et al.*, 2002). In combination, the evidence regarding eco-physiological parameters and historical occurrence suggests that *C. dentata* should perform well over a broad range of sites targeted for reintroduction.

2. Management to promote establishment and spread

Based on the apparent generalist character of *C. dentata*, Jacobs (2007) outlined several promising paths toward reintroduction,

namely through afforestation of degraded sites, such as former minelands, and reforestation by underplanting in extant forests. Despite the competitiveness of juvenile *C. dentata*, aggressive silvicultural management will probably be necessary to ensure vigorous establishment of high-value blight-resistant seedlings (Griffin, 2000; Rhoades *et al.*, 2009). Specific recommendations for underplanting (Wang *et al.*, 2006), thinning and burning (McCament & McCarthy, 2005), and herbicide application (Selig *et al.*, 2005; Robertson & Davis, 2011) have been proposed to promote establishment in natural stands and field plantations. The co-occurring species, yellow-poplar, is a particularly serious potential competitor that may necessitate use of prescribed fire to promote establishment of *C. dentata*, particularly for reforestation in large openings on mesic sites (Griscom & Griscom, 2012), along with consistent silvicultural management through the stem exclusion stage of stand development (Morrissey *et al.*, 2008). Reforestation of *C. dentata* in the Appalachian Mountains may also be hindered by competitive exclusion from dense thickets of understory ericaceous shrubs, such as *Rhododendron maximum* L. and *Kalmia latifolia* L. (Vandermast & Van Lear, 2002), which may require mechanical removal to facilitate seedling establishment and to ensure safe implementation of prescribed fire. Mycorrhizal colonization has been shown to increase *C. dentata* seedling survival and total biomass in afforestation plantings on mine reclamation sites (Bauman *et al.*, 2012). Management actions, such as plowing or disking, increased the percentage colonization of ectomycorrhizas in planted *C. dentata* seedlings, and seedling survival was enhanced with nursery inoculation of mycorrhizas before planting (Bauman, 2010). A broad-scale restoration program will probably combine reforestation and afforestation approaches, and variable management techniques will be required to promote initial establishment of chestnut populations in different settings.

Ultimately, however, all reintroduction approaches will rely on effective seed dispersal and natural regeneration to promote population growth and spread of blight-resistant *C. dentata*. The little we know about natural regeneration dynamics of *C. dentata* comes largely from an isolated stand of *C. dentata* in southwestern Wisconsin located over 600 km from the native range that developed in the absence of blight. In 70 yr, nine original planted *C. dentata* trees supplied sufficient regeneration to spread the species over 1 km, outcompeting oaks and hickories (*Carya* spp.) (McEwan *et al.*, 2006). Within c. 0.5 km from the source trees, *C. dentata* comprised at least 25% of total canopy basal area and predominated among advanced saplings entering the canopy (Paillet & Rutter, 1989). Regeneration and dispersal of *C. dentata* in this stand was a multi-step process, including (1) establishment of pioneer trees following seed dissemination in light gaps, (2) development of advance regeneration in the understory, and (3) persistence of advance regeneration underneath the established canopy until released by disturbance to assume canopy dominance (Paillet & Rutter, 1989; Jacobs, 2007). Reintroduction of blight-resistant chestnut will probably rely upon management techniques that promote success at each of these three steps. Selecting appropriate sites for reintroduction and applying effective silvicultural management will minimize environmental stresses, thereby enhancing the expression of blight resistance (Jones *et al.*, 1980;

Griffin, 2000). As discussed above, open pollination and natural seeding, facilitated by silvicultural treatments, should allow for reproduction in stands of reintroduced *C. dentata* and may also provide complementary materials for the current breeding programs.

3. Potential ecological barriers

Unfortunately, chestnut blight is not the only pathogen or pest that threatens *C. dentata* reintroduction. Emerging as perhaps the most serious obstacle in the southern portion of its range, the introduced soil-borne Oomycete pathogen *Phytophthora cinnamomi* Rands causes ink disease lesions that form on roots (i.e. root rot) and inhibit water and nutrient uptake (Maurel *et al.*, 2001a,b), leading to reduced tree vigor and eventual mortality (Anagnostakis, 2001; Rhoades *et al.*, 2003, 2009). Strategies to mitigate effects of *P. cinnamomi* include careful site selection (e.g. well-drained sites), identification of ectomycorrhizas that confer protection to roots, and additional resistance breeding using Asian chestnuts (Anagnostakis, 2001; Rhoades *et al.*, 2003). The recently imported oriental gall wasp (*Dryocosmus kuriphilus* Yasumatsu) forms galls on actively growing chestnut shoots that may lead to severe damage or mortality (Anagnostakis, 2001), although the severity of gall wasps may be regulated by their own pests such as parasitoid wasps or pathogens (Cooper & Rieske, 2007). Additional pests that may hamper *C. dentata* reintroduction include gypsy moth (*Lymantria dispar* L.), and ambrosia beetles (*Xylosandrus crassiusculus* Mot. and *Xylosandrus saxeseni* Blandford); the magnitude of these threats is still unclear (Oliver & Mannion, 2001; Rieske *et al.*, 2003). Compared with blight resistance, however, less is known about the genetics of resistance to other pathogens and pests. Chestnuts (*C. crenata*, *C. dentata*, and *C. mollissima*) are apparently more susceptible to gall wasps relative to the chinkapins (*Castanea pumila* and *Castanea pumila* var. *ozarkensis*) and *Castanea henryi* (Anagnostakis *et al.*, 2009). Thus, resistance to chestnut blight, ink disease, and gall wasps is available through other *Castanea* spp. and much of the variation seems to be controlled by only a few genes. Whether these resistance responses are controlled by the same or similar genes in the different host species remains to be seen, but emerging genomic technologies (Wheeler & Sederoff, 2009) should facilitate the manipulation of the effective genes in breeding and GE programs as restoration goals dictate.

Herbivores and seed predators may limit both the establishment and spread of reintroduced populations of *C. dentata*. White-tailed deer densities have increased greatly since chestnut was a dominant species in eastern forests, and both enclosure and modeling studies have shown that deer herbivory can have a profound effect on the structure and composition of forest tree communities (Rooney & Waller, 2003; Côté *et al.*, 2004; Didion *et al.*, 2009). Deer browse has been implicated in limiting establishment of many tree species including northern red oak (*Quercus rubra* L.), yellow birch (*Betula alleghaniensis* Britt.), northern white cedar (*Thuja occidentalis* L.), and eastern hemlock (*Tsuga canadensis* (L.) Carrière) (Rooney & Waller, 2003). For *C. dentata* and the oaks that largely replaced them, scatterhoarding small mammals and birds are the major agents of seed dispersal (Steele *et al.*, 2005). Scatterhoarding

animals, however, may also function as seed predators, particularly in low-mast years, when all cached seeds are recovered and consumed (Steele *et al.*, 2005). While acorns, hickories, and walnuts all contain a higher percentage of lipids, *C. dentata* nuts have a higher percentage of carbohydrates and much lower levels of tannins, which may lead to preferential consumption of *C. dentata* (Steele *et al.*, 2005). Other potentially significant seed predators for reintroduced *C. dentata* include weevils (Dalglish *et al.*, 2012), turkey, deer, and black bear.

Because the native range of *C. dentata* spans five US climatic zones (Fig. 1), reintroduction must also be locally adapted in order to promote successful establishment (Worthen *et al.*, 2010). Kubisiak & Roberds (2006) found low but positive correlations between genetic and geographic distances, suggesting that *C. dentata* was a single metapopulation established by high gene flow and genetic drift. Neutral DNA markers support a southwest to northeast clinal trend in decreasing genetic diversity with no indication of regional boundaries (Kubisiak & Roberds, 2006). Although these trends are important to consider in breeding and reintroduction planning, new research to better understand adaptive trait variation is needed to clearly delineate zones of optimal adaptation. Recent evidence of uncertainty regarding cold tolerance of hybrid-backcross *C. dentata* used for reintroduction (Gurney *et al.*, 2011) re-emphasizes the need for local adaptability. To achieve local adaptability, final crosses within backcross breeding and/or GE approaches must utilize *C. dentata* germplasm specifically adapted to the planting environments (Fig. 5). In addition to being locally adapted, it is necessary to ensure that reintroduced *C. dentata* has and maintains sufficient genetic diversity, which will be challenged by the continually declining vigor of extant sprouts (Huang *et al.*, 1998). Furthermore, given that a relatively narrow range of genotypes has served as resistance donors for backcross breeding (Hebard, 2006), additional sources of resistance should be continuously integrated to protect against adaptation of the pathogen to the resistance genes (Ellingboe, 1992).

4. Ecological implications of reintroduction

Because *C. dentata* was a foundation species in eastern forests, successful reintroduction has the potential to influence population, community, and ecosystem dynamics in these forests. For consumer populations, *C. dentata* may increase and stabilize the available seed resource (Dalglish & Swihart, 2012). Previous research has shown that the size and frequency of the seed resource have far-reaching community effects: increasing small mammal populations can increase predation pressure on songbirds, decrease gypsy moth outbreaks, and increase Lyme disease risk to human populations (Jones *et al.*, 1998; Ostfeld *et al.*, 2006). While successful *C. dentata* reintroduction may offset seed losses due to limited oak regeneration, it also may exacerbate the oak recruitment problems as acorns compete with *C. dentata* for dispersers and establishment sites.

Successful reintroduction of *C. dentata* may also alter ecosystem functions such as productivity, decomposition, and nutrient cycling as a consequence of its fast growth, high tannin content of the wood, and low C : N ratio of the leaves (Jacobs & Severeid,

2004; Ellison *et al.*, 2005). Jacobs *et al.* (2009) found that *C. dentata* had greater aboveground biomass and bole C than other interplanted species and compared favorably in carbon sequestration ability with fast-growing species commonly used for forestry plantations. Furthermore, *C. dentata* wood is decay resistant: Wallace *et al.* (2001) found that >24% of the large woody debris sampled in an Appalachian stream was *C. dentata*, although all the large *C. dentata* trees in the area had been dead for 70 yr. The leaves of *C. dentata*, however, decay more quickly and have a higher nutritional quality for stream macroinvertebrates than do oak leaves (Smock & MacGregor, 1988). The loss of *C. dentata* has probably altered nutrient cycling in Appalachian streams by changing the foliar inputs that form the energy base in these systems (Ellison *et al.*, 2005).

Deployment of blight-resistant *C. dentata* is intended by TACF and partners to be targeted toward the original *C. dentata* range and most activity is concentrated within this zone (Fig. 5). Nevertheless, in the wake of a rapidly changing climate the original native range may no longer reflect the zone to which *C. dentata* is optimally adapted (Harris *et al.*, 2008; Potter & Hargrove, 2012). Additionally, much of the large-scale hardwood afforestation plantings in the USA for C sequestration, conservation, wildlife, and timber occur in the Midwest and along the Mississippi River Alluvial Valley, which encompass a limited portion of the original *C. dentata* range. *Castanea dentata* has demonstrated its ability to thrive when introduced far outside of its native range (Jacobs & Severeid, 2004; McEwan *et al.*, 2006; Jacobs *et al.*, 2009), raising ecological considerations regarding its potential to suppress indigenous vegetation (Jacobs, 2007). Additional research into the biotic interactions between chestnut and its dispersers, seed predators, plant competitors, and herbivores within the context of the current forest ecosystems is required to adequately forecast the ecological consequences of chestnut restoration, whether inside or outside the native range.

IV. Societal challenges for successful reintroduction of *Castanea dentata*

Recently, the importance of understanding the connections and feedback between social and biological systems has come to the forefront in conservation (Pretty & Smith, 2004) and restoration (Aronson *et al.*, 2010; Collier, 2011). While barriers to integrating sociological and ecological knowledge can be significant, effective restoration will require both (Stanturf, 2005; Hobbs, 2007; Miller & Hobbs, 2007). Socio-economic research, however, can serve to simultaneously gather and disseminate information among different stakeholders, and thereby increase cohesion and participation in restoration activities (Collier, 2011). In the case of *C. dentata* reintroduction, the social sphere within our conceptual framework remains, perhaps, the least well understood (Fig. 2a). We detail three key areas for further socio-ecological study, including understanding the social acceptability of different technologies for developing blight resistance, current policy issues that may influence reintroduction, and the crucial task of setting restoration goals.

An integral question is whether hybrid and GE *C. dentata* trees will prove socially acceptable for reintroduction (Jacobs, 2007;

Merkle *et al.*, 2007). The backcross breeding program, which produces a backcross hybrid, relies upon traditional plant breeding techniques that are widely used in production agriculture, but may prove less publically acceptable for use in forest restoration (Jacobs, 2007). The efficacy of backcross breeding for forest trees also remains uncertain, which could necessitate a shift toward integration of GE technologies in *C. dentata* restoration. Whether the public will accept a GE *C. dentata* to ensure restoration of the species and its ecosystem is unclear (Merkle *et al.*, 2007). The case of *C. dentata* (and other threatened forest tree species targeted for restoration) is further complicated by the notion that any GE tree deployed in North America must be sterile or have some other means to control flowering (Merkle *et al.*, 2007), which conflicts with the underlying premise of repopulation through sexual reproduction. While pursuit of both backcross breeding and GE simultaneously may be the soundest strategy to obtain effective blight resistance, ultimately, public perception and acceptance may dictate which types of trees may be used for restoration, regardless of efficacy (Strauss *et al.*, 2009).

Although public concerns regarding application of GE technologies have been increasing, many leading scientific groups espouse that the plant trait and not the GE method of obtaining the trait, as well as its associated ecological impacts, should determine whether and in what contexts GE trees should be used (Strauss *et al.*, 2009). In addition, because of the diversity of GE methods, desired outcomes, potential applications, costs, and benefits, each use of GE trees should be evaluated on a case-by-case basis (Strauss *et al.*, 2009). For example, recent advances in GE technology may allow the development of cisgenic *C. dentata* (Han *et al.*, 2010), whereby the expressed genes along with their genetic control/regulatory elements are from *Castanea* species. The relative similarity of cisgenics to backcross breeding (Schouten & Jacobsen, 2008) suggests that this approach may garner the broadest social support, and ultimately prove to be the most biologically viable method to introduce a trait such as blight resistance in a GE context. Restoring a culturally important species, such as *C. dentata*, for the public good may be viewed as an acceptable use of GE. Conversely, allowing GE for restoration of *C. dentata* could be seen as a 'slippery slope' for the broader commercial use of GE trees (Strauss *et al.*, 2009). Further education on GE methods, benefits and risks, as well as a better understanding of public opinion and concerns is needed (Bublea *et al.*, 2009).

Governmental and regulatory policies relevant to restoration are shaped by both political and financial interests (Collier, 2011). GE technologies are regulated by multiple governmental agencies when the modified trait is pest resistance and the organism produces food for wildlife and human consumption (i.e. USDA Animal and Plant Health Inspection Service (APHIS)-Biotechnology Regulatory Service, Environmental Protection Agency (EPA), and Food and Drug Administration (FDA)). Societal influences may also drive silvicultural management policy options for reintroduction as public opinion regarding harvesting, fire, and other forms of disturbance exert direct influence on management on public lands (Jacobs, 2007). While targeting private land for reintroduction overcomes many of the political and policy restrictions specific to silvicultural manipulations on public lands, it creates other social

obstacles. For example, the increasingly parceled nature of private forest land ownership may limit silvicultural options that promote establishment of *C. dentata* (McEwan *et al.*, 2006; Jacobs, 2007). In addition, Knoop *et al.* (2010) document a lack of support among private landowners for management that favors oak regeneration (e.g. disturbance), raising the question of whether similar management strategies will be supported for *C. dentata*. The reluctance of private forest landowners to use prescribed fire because of air quality issues and the risk of fire escape at the expanding wildland–urban interface (Stanturf & Goodrick, in press) has been identified as a serious obstacle for restoration of the fire-adapted longleaf pine (*Pinus palustris* Mill.) in the southeastern USA (Brockway *et al.*, 2005). Ultimately, the success of different management options to promote *C. dentata* on varying land types will also be balanced against their cost to implement (Stanturf *et al.*, 2009).

Restoration resides at the center of our conceptual framework as the overarching and central common goal among the three spheres. The Society for Ecological Restoration defines ecological restoration as the ‘process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed’ (SER, 2004). Identifying the source of the ecosystem degradation and setting realistic, attainable goals are critical to the success of any restoration endeavor (Hobbs, 2007). In the case of *C. dentata* reintroduction, it is the loss of a foundation species that is the cause of degradation. Setting realistic and attainable goals for *C. dentata* reintroduction is arguably among the most critical and difficult tasks. Static reliance upon the historical distribution and abundance of *C. dentata* as the sole criterion for success may be neither feasible nor desirable (Hildebrand *et al.*, 2005). We posit that some of the major determinants of *C. dentata* reintroduction goals will be (1) the ability to confer blight resistance, effectively and sustainably, in a socially acceptable manner, (2) the availability of sufficient blight-resistant plant material at an acceptable cost, (3) prioritization of restoration targets (locations and site types, e.g. urban landscapes, public forests, and private land) with capable partners and at appropriate scales, and (4) the capacity to accurately assess the viability of reintroduced populations (e.g. growth and spread) within the varied levels of management intensity. The large geographic scale, immense number of diverse interest groups involved (Fig. 5), and potential to influence many aspects of eastern forest ecology will require setting national, regional, and local goals that engage stakeholders as active participants (Hobbs & Cramer, 2008; Collier, 2011); ambiguity will set the stage for failure.

V. Conclusions

In the case of *C. dentata*, an introduced pathogen reduced a major component of the eastern forest canopy to an understory shrub within a single generation, illustrating how the rapid loss of a foundation species can dramatically change an ecosystem across a landscape. Because of its strong ecological and cultural significance, developing blight resistance has been aggressively pursued by a diverse contingency of supporters over several decades. The ultimate goal of producing blight-resistant *C. dentata* has advanced the fields of tree breeding and biotechnology, offering the

possibility to bring new tools to bear on socio-ecological problems. We are now arguably on the cusp of perhaps the largest reintroduction effort to date for a plant species.

Castanea dentata restoration provides an effective model for restoration. Based upon *C. dentata*, we have proposed a conceptual framework, whereby maximizing restoration success requires increasing the capacity and integration among social, ecological, and technological spheres (Fig. 2a). While the technological and ecological spheres have been the primary targets of emphasis and are the areas for which the majority of breakthroughs have been realized for *C. dentata*, the critical need for progress in the social sphere has only recently been fully recognized; development in this area is in its infancy and remains perhaps the weakest link toward achieving restoration success.

Although *C. dentata* was among the first eastern deciduous tree species threatened with extirpation as a result of introduced pathogens or pests, many other tree species face a similar fate. Our conceptual framework can be broadly applied because the three spheres (technological, ecological, and social) are common to all restoration challenges, yet with the common denominator that restoration success is limited by the size and degree of overlap among spheres (Fig. 2b). Through effective merging of genetic technologies, reintroduction biology, and restoration ecology, *C. dentata* may serve as an important model to inform reintroduction of threatened species in general and foundation forest tree species in particular.

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