

Should hybrids be protected by listing; *Betula* × *sandbergii* and *Botrychium minganense* in Vermont¹

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BARRINGTON, D. S. (Pringle Herbarium, Plant Biology Department, University of Vermont, Burlington, VT 05405). Should hybrids be protected by listing; *Betula* × *sandbergii* and *Botrychium minganense* in Vermont. J. Torrey Bot. Soc. 138: 465–471. 2011.—Recent consideration of two taxa, a fern (*Botrychium minganense* Vict.) and a birch (*Betula* × *sandbergii* Britton), for addition to the list of protected plants by the Vermont Scientific Advisory Group on Flora revealed the need for an assessment of hybrids in the context of the listing of rare taxa for protection. Though both are hybrids in that they are the product of secondary contact between divergent lineages, the two lie at opposite ends of the hybrid continuum. A consideration of the known array of hybrid biologies in the context of the conservation literature leads to the argument that the fern should be listed, but not the birch. *Botrychium minganense* is reproductively competent, fully isolated from its progenitors, and evidences a genetic heritage unique relative to them. However, *Betula* × *sandbergii* does not have the integrity or genetic uniqueness typical of the rare hybrid lineages that have been listed for protection, either at the state or national level. It lacks a unique gene pool vulnerable to extinction. The two candidates for listing in Vermont present contrasting biologies that together inform a substantive understanding of the issues relating to the conservation biology of plant hybrids.

Key words: conservation, endangered species, hybrid, listing, Vermont.

In some groups of plants—such as in the ferns, oaks, birches, and willows—hybrids are common. Taxa within these groups are often the most difficult to identify and thus create problems not only for taxonomists but also for ecologists and conservationists. Specialists in these groups become preoccupied with the detection and characterization of hybrids. In addition, the substantial role of hybridization in the origin of new species has become clear, especially in recent years (Mallet 2007). Some hybrids are extremely rare; conservation biologists and others have asked whether these hybrids should be protected in the same way as other rare plant taxa (i.e., rare species, subspecies, and varieties).

Recently, the Vermont Scientific Advisory Group on Flora considered the addition of two taxa, a fern (*Botrychium minganense* Vict.) and a birch (*Betula* × *sandbergii* Britton), to

the list of protected plants for the state. Both of these taxa are rare in Vermont, meet the definition of hybrid, and present complex evolutionary histories. Historically, the plants pertaining to *B. minganense* were included in a broadly defined *B. lunaria* (L.) Sw.; they were already protected in Vermont by the listing of the latter species name. However, recent insights into the species delineation of these moonworts have led to the consideration of *B. minganense* for listing as a distinct species. In this case, the nomenclatural change from *B. lunaria* to *B. minganense* has predicated a new review of the Vermont plants' status. In contrast, the Vermont plants pertaining to *Betula* × *sandbergii* passed as *Betula pumila* L., one of the progenitor species of the hybrid. The realization that the Vermont plants are in fact hybrids has led to their consideration for listing, this time as a hybrid rather than as a species. In this case, the underlying problem was the misidentification of the plants.

This contribution addresses these taxa in the context of the biology of plant hybrids with the goal of informing decisions about the conservation status of hybrids in general and the two candidates in particular. Where possible, I have drawn examples from plants common in Vermont as a local basis for understanding the conservation status of the candidate hybrids. A comparative analysis of the two hybrids yields insights into the complex problems relating to hybrid conservation.

¹ This work is supported in part by a Vermont Agricultural Experiment Station Grant to DSB (VT-H01708).

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³ I thank Arthur Gilman for his close review and critical suggestions for development of this essay; Marc Lapin also contributed to its improvement. The Vermont Scientific Advisory Group on Flora solicited this contribution; substantial discussions at three different meetings of the group contributed to its development.

Received for publication June 7, 2011, and in revised form September 21, 2011.

What is a Hybrid? Addressing hybrids and the endangered species act, Haig and Allendorf (2006) defined hybrids as the result of "interbreeding of individuals from genetically distinct populations, regardless of their taxonomic status." Their use of the term *genetically distinct populations* is problematic, because there is no non-arbitrary criterion for deciding what constitutes *genetically distinct*. Nevertheless a useful first approach to the problem of defining *hybrid* is to characterize species as the product of primary divergence and hybrids to be the product of secondary contact between divergent lineages. This approach is useful because it allows for the observed diversity among hybrids in 1) levels of divergence between progenitors, 2) reproductive biologies, 3) vegetative persistence of individuals and lineages, and consequently 4) degree of evolutionary integration and autonomy of the hybrids as a lineage. There is an outer limit to the divergence of hybridizing lineages. Members of different plant families never hybridize. Intergeneric hybrids are rare enough to be striking exceptions; they commonly have highly irregular morphology and present strong evidence of genetic incompatibility, such as complete sterility or inability to form reproductive structures. Most hybrids are between species in the same genus.

Proposing and Testing Hypotheses for Hybrids. The common morphological criterion used in proposing a plant as a hybrid is that it be morphologically intermediate between two candidate progenitors. A broad array of classical work has established the typical patterns of morphological character intermediacy in hybrids (e.g., Anderson 1936 & 1949). Characters of hybrids can be medial (i.e., quantitatively at the midpoint) between those of the progenitors, but in a number of cases individual characters can approach one or the other progenitor, or they can even be transgressive (lying outside the range of variation between the progenitors). Because of this complexity, there has been much attention to hybrid indices, i.e., the quantitative scoring of intermediacy in a set of characters (e.g., Anderson 1936 & 1949). In simple cases, though individual characters of hybrids may be diverse in relation to their progenitors, Anderson's hybrid index reveals the mean of a character set to be medial between the progenitors (Anderson 1949). However, the mean of a hybrid's character set need not be

medial between those of the progenitors, a feature that becomes important in the consideration of hybrid birches later in this article.

A number of genetic and biochemical tools have been powerful in the testing of candidate hybrids first proposed based on morphology. These tools, including chromosome number and meiotic pairing behavior (Dancik and Barnes 1972, Barrington 1986, Wagner et al. 1992), flavonoid analysis (Powell et al. 1991), isozyme analysis (Wagner et al. 1992), random amplification of polymorphic DNA (RAPDs, Padgett et al. 1998), and DNA sequences (Barrier et al. 1999) all use summation of characters that are different in the proposed progenitors as the test for hybridity. Analyses of hybrids using these tools often prove remarkably complex, as consideration of an array from the recent literature demonstrates.

Hybrids Vary in Their Fertility. The salient variable in hybrids is their level of fertility. As de Queiros (1997) says, "reproductive incompatibilities are of central importance to biologists who study hybrid zones." Considering interspecific hybrids, in some groups, such as the ferns, hybrids are almost never fertile. The sterile hybrids, being all F_1 s, are morphologically similar to each other and distinct from their progenitors. So, for instance, *Polystichum* \times *potteri* Barrington, the hybrid between *P. acrostichoides* (L.) Roth and *P. braunii* (Spencer) Fée, has an array of character states that allow diagnosis of the hybrid as distinct from either progenitor (Barrington 1986).

However, a broad array of hybrids is partially fertile. When no other factors intervene, these hybrids breed regularly with each other and their progenitors. A prominent Vermont example is the hybrid between *Quercus macrocarpa* Michx. and *Q. alba* L., which is widespread in the Champlain Valley of New York, Québec, and Vermont. This hybrid is so variable that it is difficult to find pure *Q. macrocarpa* in the area, and Whittimore and Schaal (1991) found evidence of introgression (i.e., transfer of genetic traits between the progenitor species) between the hybrid's progenitors.

A fertile hybrid relevant to conservation and listing is *Echinocereus* \times *lloydii* Britton and Rose of Texas and New Mexico. Powell et al. (1991) demonstrated with an extensive artificial breeding program that this hybrid was not only fertile, but also easily backcrossed to its progenitors *E. coccineus* Engelm. and

E. dasyacanthus Engelm. Summation of flavonoid profiles was used to test hybridity as predicted from morphological intermediacy. They summarized the implications of their work as follows:

“Despite [*Echinocereus* × *lloydii*’s] localized persistence, we have found no demonstrable evidence of independent evolution following its origin. Thus, we suspect that *E.* × *lloydii* merely manifests unusual gene recombinations of the common parental species, and is not a unique gene pool that could be lost through extinction.”

So, *Echinocereus* × *lloydii* presents no evidence of evolutionary integration or autonomy. The Powell group’s findings contributed to the removal of this taxon from the list of federally endangered species.

Fertile hybrids can be sufficiently isolated and genetically integrated to qualify as species. The reproductive isolation of *Helianthus* × *paradoxus* Heiser, a federally endangered species, from its progenitors (*Helianthus annuus* L. and *H. petiolaris* Nutt.) is ecological as well as genetic. Backcrosses to the progenitors do show reduced pollen viability and virtually complete failure of seed set. Unlike the hybrid, the progenitors are unable to occupy salt marshes characterized by heavy clay soils and brackish water. In this way, the hybrid is also ecologically isolated from its progenitors – and Lexer et al. (2003) argued that additive allelic effects (for calcium uptake, sodium ion exclusion, and leaf succulence) were in fact transgressive, lying outside the range of variation in either progenitor. These transgressive features were critical in providing the ecological isolation that led to the hybrid speciation event that yielded *Helianthus* × *paradoxus*.

Hybrids Vary in Their Vegetative Persistence.

Some sterile hybrids are remarkably persistent through vegetative spread and division, others are not. A striking example of longevity comes from *Osmunda* × *ruggii* R. M. Tryon (the hybrid of *O. regalis* L. and *O. claytoniana* L.). At its best-documented station in West Virginia this hybrid presents a set of ramets over a contiguous area of 16.5 by 6.1 m (Wagner et al. 1978). Based on a typical *Osmunda regalis* rhizome growth rate of 0.7 cm/year (Klekowski and Berger 1976), this colony appears to be a clone at least 1100 years old.

Hybrids in the clubmoss genus *Huperzia* persist vegetatively, but with an extra level of complexity. These hybrids, such as *H.* × *buttersii* (Abbe) Kartesz & Gandhi, (*H. lucidula* [Michx.] Trev. × *H. selago* [L.] Bernh. ex Schrank & Mart.), produce gemmae that detach from the parent at an abscission zone and are dispersed. In the case of *Huperzia* hybrids, the persisting fragments of the original hybrid are separate individuals that can be moved as propagules over long distances, especially by water, leading to widespread presence in a flora without any sexual reproduction.

Apomixis. Sterile and partially fertile hybrids can restore full reproductive competence through apomixis (formerly referred to as apogamy, especially in the ferns), an alternative to sexual reproduction (Asker and Jerling 1993). Ferns and angiosperms are different in relation to apomixis. In the ferns, which typically yield sterile hybrids, apomictic lineages show no tendency to sexual reproduction (Manton 1950). On the other hand, many apomictic lineages among the angiosperms arise from partially fertile hybrids, and there is a variable frequency of sexual reproduction along with the apomictic alternative. Apomictic ferns of hybrid origin, as a result, tend to be clearly demarcated morphological taxa. In a Vermont context, *Phegopteris connectilis* (Michx.) Watt., is an example. In contrast, the combination of sexual and apomictic reproduction typical of the angiosperm lineages yields morphological chaos. The infamous genera *Hieracium*, *Rubus*, *Crataegus*, *Antennaria*, and *Taraxacum* are all rich in apomictic lineages. A prominent anomaly in a conservation context is that apomictic plants, but not hybrids or polyploids, are suggested for listing on the IUCN Red List (IUCN Standards and Petitions Subcommittee 2010).

Polyploidy. Sterile hybrids can resume sexual reproduction through polyploidy. In this process, a single cell of a hybrid individual goes through an event including chromosome replication but no cell division, leaving the cell’s descendants with two full sets of chromosomes from each progenitor species. Pairing of the duplicated progenitor chromosomes during meiosis enables the restoration of fertility; the hybrid individual can reproduce sexually if it can self-fertilize. These plants behave much like their sterile progenitor hybrids

in that they maintain morphological integrity; backcrosses to progenitor species are usually sterile because of strong reproductive barriers imposed by failure of chromosome pairing. Recent molecular-genetic inquiries have revealed that the origin of allopolyploids (i.e., polyploids derived from hybrids) is sometimes accompanied by major alterations in genome organization and gene expression (Paun et al. 2007), which contributes to the evolutionary autonomy of the polyploid lineage. Thus, fertile allopolyploids present the attributes of species, including the ability to form interbreeding populations, to occupy unique niches, and to spread across distinct geographic ranges, so they are generally recognized as species in the biological literature.

In regions affected by Pleistocene glaciation, allopolyploids are more common than in known Pleistocene refugia for north-temperate flora (Brochmann et al. 2004). The rich array of recently originated polyploids in the flora of Northeast North America adds to the species diversity in the region. Most prominent in a Vermont context is *Adiantum viridimontanum* Paris, an allopolyploid derived from the common eastern maidenhair *A. pedatum* L. and the common western maidenhair *A. aleuticum* Rupr., disjunct on ultramafic soils in Vermont and other eastern states (Paris and Windham 1988, Paris 1991). This species is currently protected in Vermont, as it is known from a limited set of sites in the state and is found elsewhere only in a few sites in adjacent Québec and Maine. Prominent among rare allopolyploids is the fertile tetraploid race of *Asplenium* × *ebenooides*, restricted to Hale County, Alabama (Wagner 1954; Walter et al. 1982). In this rare instance, both the sterile hybrid and a derivative allopolyploid are known; the sterile hybrid is much more common. Named as *Asplenium tutwilerae* B. R. Keener and L. J. Davenport, the tetraploid cytotype is listed as critically imperiled by the State of Alabama (Alabama Natural Heritage Program, 2007). Allopolyploids have independent histories and a significant role to play in diversification. An example from the charismatic megafauna is *Argyroxiphium kauense* O. Deg. & I. Deg., a federally endangered species, part of the Hawaiian silversword alliance. The alliance comprises a set of 30 species in three genera, all endemic to the Hawaiian archipelago; all are tetraploid (Barrier et al. 1999). Analysis of the component genomes of the Hawaiian species

reveals that they have a single common ancestor, which was an allotetraploid originating by hybridization between species of southwestern North American tarweeds (the *Madia/Railardiopsis* group, Barrier et al. 1999). Hence, the hybrid in the ancestral history of *Argyroxiphium kauense* is geographically, evolutionarily, and chronologically remote from the Hawaiian lineage. So, although allopolyploid lineages are hybrid in origin, they are species, with all the integrity and genetic isolation expected of species lineages. They certainly merit conservation action if they are rare.

The Biological Integrity of Hybrids and Conservation. The endangered species act of 1973, as originally enacted, did not include the word *hybrid*, nor did it address hybrids implicitly. As of 2006, although there had been a history of advocacy for protecting species originating from hybrids (e.g., O'Brien and Mayr 1991), there remained no language relating to the protection of hybrids (Haig and Allendorf 2006). As Powell et al. (1991) articulated, the key variable to consider in deciding whether to seek protection for lineages with a hybrid heritage is whether the lineage has a unique gene pool that could be lost by extinction. To be endowed with this unique set of genetic attributes a lineage needs to be reproductively competent and genetically isolated from other lineages, so that it has a discrete evolutionary fate. Some hybrids have these characteristics and some do not. Sterile hybrids clearly do not even if individuals persist for millennia. They may be repeatedly formed from their progenitor species, but they do not have the capacity to develop their own genetic profile or to establish a lineage. Similarly some fertile hybrids do not meet the criteria to merit protection; like *Echinocereus* × *lloydii* they have no independence from their progenitors, because there is no reproductive isolation from the parents. They lack the genetic isolation to be an integrated lineage with a unique genetic heritage even though they can reproduce sexually.

However, we have seen in this review that there are a number of hybrids that meet the reproductive integrity and autonomy criteria: allopolyploids, though hybrid in origin, are isolated from their progenitors and evidence the capacity to evolve independently (e.g., Barrier et al. 1999). Similarly, some fertile hybrids, like *Helianthus* × *paradoxus*, are ecologically and genetically autonomous even

though they are not genetically isolated from their progenitors, since they have limited ability to interact with their progenitors. It may be possible to include some apomictic taxa of hybrid origin on the list of hybrids that meet this criterion, if they are isolated from their progenitors, as is common in the ferns.

Vermont Candidate: *Botrychium minganense*. Common across boreal North America with a southern extension in the Western cordilleras, *Botrychium minganense* is a fertile allotetraploid with the clear imprint of two progenitors in its genetic profile (Hauk and Haufler 1999). The species is at the southern limit of its range in Vermont, where it is known from three stations. Recent work reveals that *B. lunaria* (L.) Sw. in the broad sense was one of the progenitors. The contribution from this lineage is complex. *Botrychium lunaria* comprises at least four taxa including a North American and a circumpolar lineage that have diverged enough to be recognized as separate species (Stensvold 2007). *Botrychium minganense* includes genetic markers for both of these two taxa. Stensvold's work revealed what she called introgressants, i.e., *B. lunaria* s.l. plants that included alleles from both the North American and circumpolar species in spite of the routine observation of abortive spores in hybrids between these two taxa. The *B. lunaria* s.l. progenitor of *B. minganense* may be one of these introgressed plants. The second progenitor is not so well understood; among species so far surveyed it would be closest to *B. pallidum* W. H. Wagner, a diminutive species of the North American boreal region. In sum, *Botrychium minganense* combines the genetic heritage of three different diploids, and *Botrychium* hybrids as a rule are sterile (Wagner and Wagner 1983). Thus, it meets the criteria of genetic isolation and unique genetic heritage that provide a sound basis for recognizing it as a distinct lineage. This is the sort of lineage with a hybrid ancestry that should have species status and be listed when of conservation concern.

Vermont Candidate: *Betula* × *sandbergii*. An equally complex problem is presented by *Betula* × *sandbergii*, the hybrid between *Betula papyrifera* Marsh. and *B. pumila*. This hybrid is best known from populations of the progenitors and the hybrid in North Dakota, Minnesota, Wisconsin, and Michigan, near

the southern limit of *B. pumila*'s range (Clausen 1962). It is known from one historical station in Vermont, though the more northern *B. pumila* is not known from the state. Both of the progenitor species are polyploids (presumably allopolyploids): *B. pumila* is tetraploid, while *B. papyrifera* can be either pentaploid or hexaploid. Ordinarily, the hybrids between species with different ploidies are sterile (unless they become polyploid or apomictic). However, in an exhaustive morphological analysis, Clausen (1962) found that mean pollen viability of *B. × sandbergii* was 58% (range, 28.1 to 94.5%), reduced from progenitor levels (both with means over 90%) but still partially fertile. Morphometric work revealed a strong pattern of intermediacy in the hybrids using several analytic approaches. The viability of the pollen and the retrieval of some individuals with subsets of characters favoring *B. papyrifera* suggested to Clausen that there were backcrosses in the sample, and that introgression had occurred between the two progenitor species.

Clausen did not include any genetic analysis of his hybrids beyond scoring pollen viability; his criterion for including an individual in his hybrid class is unclear. Dancik and Barnes (1972), in addressing the hybrid between the hexaploid *Betula alleghaniensis* Britton and *B. pumila*, cast doubt on Clausen's claim that *B. × sandbergii* presents evidence of introgression (and that the Minnesota population he studied was a hybrid swarm). Their work included chromosomal as well as pollen analysis of the hybrids and progenitors in their field sample; it revealed that though progenitor and hybrid pollen viabilities were similar to Clausen's, in fact all of the hybrids were f_1 s. Their morphological analysis of the chromosomally documented plants in their sample demonstrated that a subset of the hybrids more closely resembled *B. alleghaniensis*, the progenitor with the higher ploidy. Their review of the then-recent literature on *Betula* hybrids showed that crosses between species of different ploidies all yielded hybrids tending to resemble the higher-ploidy progenitor. Their conclusion was that morphological skew towards the higher-ploidy progenitor was often mistaken for evidence of backcrossing and even introgression. Dancik and Barnes's insights suggest that *Betula* × *sandbergii* also comprises a system of independently derived f_1 hybrids.

The possibility remains that this hybrid is part of a hybrid swarm that is breeding and perhaps introgressing with its progenitors, because of recent work demonstrating extensive introgression in European birches using an array of modern genetic tools (Thorsson et al. 2001, Palmé et al. 2004). Hybrid swarm or a population system comprising solely f_1 hybrids, *Betula* × *sandbergii* is not a cohesive evolutionary lineage with its own genetic heritage independent of and isolated from other lineages. The hybrid lies in the nebulous ground between lacking reproductive viability and lacking genetic autonomy from its progenitors. Nationally and within Vermont, hybrids like *B.* × *sandbergii* are not listed as threatened or endangered species, nor should they be.

Conclusions. The two candidates for listing in Vermont present contrasting biologies that together inform a substantive understanding of the issues relating to the conservation biology of plant hybrids. *Botrychium minganense* lies at one end of the spectrum: reproductively competent, fully isolated from its progenitors, and evidencing a unique genetic heritage relative to them, this allopolyploid of hybrid origin meets the criteria for species status in the conservation literature (e.g., O'Brien and Mayr 1991, Powell et al. 1991, Haig and Allendorf 2006). On the other hand, the work on hybrid birches, though without a clear resolution of the status of *Betula* × *sandbergii*, demonstrates that this hybrid does not have the integrity or genetic uniqueness typical of the rare hybrid lineages that have been listed, either at the state or national level. Rather, like *Echinocereus* × *lloydii*, it presents no evidence of independent evolution and does not evidence a "unique gene pool that could be lost through extinction" (Powell et al. 1991).

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