Journal of the Torrey Botanical Society 146(4): 278–290, 2019.

Hybridization between *Asclepias purpurascens* and *Asclepias syriaca* (Apocynaceae): A cause for concern?[1]

Steven B. Broyles[2,4] and Geordie Elkins[3]

[2]Biological Sciences Department, SUNY Cortland, Cortland, NY 13045 and

[3]Highstead, 127 Lonetown Road, Redding, CT 06896

Abstract. Rare plant species can be at risk of hybridization, reduced genetic variation, and genetic assimilation when a numerically abundant congener co-occurs in or invades their habitat. We investigated hybridization between a species in decline, *Asclepias purpurascens* L., and its common and widespread congener, *Asclepias syriaca* L. A total of 40 morphological traits were measured on 60 flowering plants from a mixed population in Connecticut. Cluster and principal component analyses identified two distinct clusters of parental species taxa and a third cluster representing putative hybrids. Although leaf traits of putative hybrids were more similar to *A*. syriaca, floral traits and the morphological space on the principal component analysis were more similar to *A. purpurascens*. This suggests that the population contains a mix of F1 and advanced generation hybrids with the possibility of introgression into *A. purpurascens*. Although putative hybrids are intermediate for most traits, pollen counts reveal reduced fertility of presumed hybrid that might influence mating behaviors and increase the likelihood of backcrossing with *A. purpurascens*. We suggest a combination of morphological traits that better identify the two parental species as well as hybrids in the field. Conservationists should consider options of managing *A. syriaca* where *A. purpurascens* needs protection from competition to prevent genetic assimilation of the latter.

Key words: *Asclepias*, conservation, hybridization

Angiosperms abound with natural and anthropogenic hybrid taxa as a result of circumventing pre- and postzygotic reproductive barriers (Baack *et al*. 2015). Hybrid taxa can become bridges for transgressing genetic traits between species and affect the process of ecological adaptation and evolution in one or both parental species (Arnold 1992). Hybridization between small endemic or rare populations with those numerically abundant, widespread, and common, can promote more rapid evolution by genetic introgression than would occur through other natural processes such as mutation, genetic drift, and recombination (Harrison and Larsen 2014). Hybridization can impose a conservation dilemma to land managers, because hybrid taxa in some countries do not hold conservation status in environmental laws (Ellstrand *et al*. 2010; Jackiw, Mandil, and Hager 2015). Hybridization can threaten the rare species through the loss of genetic diversity and the breakdown of coadapted gene complexes (Reiseberg, 1991). On the other hand, in extreme cases, hybridization can serve as a rescue method for reinvigorating a threatened species with an infusion of new genetic variation (Hamilton and Miller 2015; Suarez-Gonzalez, Lexer, and Cronk 2018).

Natural hybridization was initially regarded as rare between milkweed species (Moore 1946; Woodson 1954), but a growing body of evidence suggests that hybrid formation (Wyatt and Broyles 1994) and perhaps introgression (Broyles 2002) does occur between some sympatric taxa. For example, the widespread, weedy *Asclepias syriaca* has been reported to hybridize with the prairie species, *A. sullivantii* Engelm. ex A. Gray, in Ohio (Klips and Culley 2004) and *Asclepias speciosa* Torr. (Adams, Toomb, and Price 1987) in prairie states, as well as the infrequent woodland species, *Asclepias exaltata* L. Authority (Kephart, Wyatt, and Parrella 1988). In the latter case, pollinator sharing (Broyles, Vail, and Sherman-Broyles 1996; Stoepler *et al*. 2012) gives rise to hybrid formation and interspecific gene flow between *A. syriaca* and *A. exaltata* (Broyles 2002).

For several decades, milkweeds were regarded as an exemplar for mechanical reproductive isolation in plants. The size and depth of the stigmatic chamber appears to be adapted to the size of conspecific pollinia. A pollinium of large

---

[1] We thank Jesse Hubbard and Amanda Hewes for providing field assistance, and Tessa Brown, Mia DiMartino, Sarah Jensen, and Sophie Whittington for laboratory assistance. Sue Sherman-Broyles provided useful comments on the manuscript. This research was supported by NSF grant number 1645256, G. Jander, PI.

[4] Author for correspondence: steven.broyles@cortland.edu

doi 10.3159/TORREY-D-18-00050.1

©Copyright 2019 by The Torrey Botanical Society

Received for publication September 21, 2019, and in revised form March 22, 2019; first published October 23, 2019.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

TABLE 1. Characters used to distinguish *A. syriaca* and *A. purpurascens* in floras of the eastern United States.

	A. syriaca	*A. purpurascens*
Manual of the Vascular Flora of the Carolinas (Radford, Ahles, and Bell 1968)	"Hood margin with single median triangular tooth; follicles muricate"	"Hood margin without a median tooth; follicles smooth"
Flora Novae Angliae (Haines 2011)	"Corona hoods 4–5 mm long, light purple, each with prominent marginal lobe near the center; follicles covered with conical processes; corolla purple green to light purple"	"Corona hoods 5–7 mm long, red-purple to purple, without marginal lobes; follicles smooth; corolla red-purple to purple"
Manual of Vascular Plants of Northeastern United States and Adjacent Canada (Gleason and Cronquist 1991)	"Hoods with a sharp, triangular, ascending or inflexed lateral lobe at or near the middle of the margin; cor greenish-purple to nearly white"	"Hoods without lateral lobes, or merely slightly widened at or near the middle; cor commonly purple"
Additional features to consider	Hoods without lateral protuberance and median margins with an ascending tooth; pedicels thin resulting in loose globose inflorescence; abaxial surface of petals pubescent.	Hoods with lateral protuberance and lacking median margin tooth; pedicels thick resulting in erect flowers forming a semihemispherical inflorescence; abaxial surface of petals glabrous.

proportions is unlikely to be deposited in small stigmatic chambers of other milkweed species, and the small pollinia with many fewer pollen grains are less likely to successfully sire a fruit with complete seed set on larger species. This "lock and key" hypothesis lacks support because examination of field pollinations has illustrated that interspecific pollinations frequently occur between congeneric milkweeds (Kephart and Heiser 1980; Broyles, Vail, and Sherman-Broyles 1996; Stoepfler *et al.* 2012). Furthermore, morphological, isozyme, biochemical, and genetic evidence have demonstrated that hybridization is more common than originally proposed in milkweeds (Kephart, Wyatt, and Parrella 1988; Wyatt and Broyles 1992; Broyles 2002; Klips and Culley 2004).

Hybridization between the declining *Asclepias purpurascens* and common *A. syriaca* has been suggested using flavonoid chemistry in Virginia (Wyatt and Hunt 1991) and a report on a single specimen in Missouri (Rintz 2014). Field identification of hybrids is difficult using morphological traits. Many eastern floras and identification guides (Table 1) use flower hood length and the presence/absence of marginal teeth along the hood as features to distinguish the two species, but hood length is highly variable for *A. syriaca*, and finding hood teeth is difficult even with a good hand lens. For example, Rintz's (2014) report from Missouri is based on a single, unmeasured specimen. Rintz published a color photograph of the putative hybrid plant, but the hoods appear elongated and it is difficult to determine if the flowers had median hood teeth. The flower and fruit photographs of Rintz's putative hybrid actually bear many characteristics (*i.e.*, stiff pedicels with erect flowers, elongated hoods, distinct lateral hood protuberances, and smooth fruits) more reminiscent of *A. purpurascens* than *A. syriaca*. A more thorough analysis of morphological traits in a sympatric population of the two species is warranted.

Biochemical evidence from leaf tissue of plants in central Virginia (Wyatt and Hunt 1991) suggests that hybridization can occur between *A. purpurascens* and *A. syriaca*. Wyatt and Hunt pooled leaf material from several putative hybrids in central Virginia and demonstrated that leaf flavonoids of the hybrids represented an admixture of the two parental species. In addition, they found two novel flavonoids that were absent in the parents. This biochemical study does suggest that hybridization with *A. syriaca* is possible, but it does not provide guidance in the recognition of hybridizing populations.

The ranges of *Asclepias purpurascens* and *A. syriaca* overlap in the midwestern and eastern United States. *Asclepias syriaca* is considered a weedy species found in urban waste areas, roadsides, and agricultural areas, as well as prairies and sand dunes. *Asclepias purpurascens* occurs in dry and moist prairies, meadows, and woodland edges in the Midwest and eastern North America.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

Asclepias purpurascens is a declining species in the east where it is a species of concern, threatened, or extirpated in 11 of 20 states with historical records (Farnsworth and DiGregorio 2002; USDA Forest Service, Eastern Region 2003). It has been extirpated from Maine, Rhode Island, Delaware, and Minnesota. At least in New York State, the small remaining populations of *A. purpurascens* are usually located in the vicinity of *A. syriaca* plants.

The focus of the current study was to investigate variability in leaf and flower morphologies in a population in Connecticut where both species co-occur. Specifically, we were interested in determining whether morphological variation supports the hypotheses for hybridization between *A. purpurascens* and *A. syriaca*. Finally, we will discuss the implications of hybridization from a taxonomic and conservation perspective for the small populations of *A. purpurascens* in the range of the more common *A. syriaca* in eastern North America.

Methods. We took advantage of a newly discovered population of *Asclepias purpurascens* at Highstead in Redding, CT (Fairfield County; population centroid lat. 41.32715, long. 73.39223). The population occurred in two small, mesic meadows (0.50 and 1.4 ha) on a west facing-slope dominated by *Agrostis capillaris* L. Additional *A. purpurascens* and *A. syriaca* were found on a higher meadow (11.3 ha) dominated by *Agrostis* and other forbs. Milkweed populations in the upper and lower meadow are about 80–150 m apart, separated by a 30–40 m hedgerow of shrubs and small trees. The meadows are managed to promote nesting of grassland birds, such as bobolink, through seasonal mowing in early September.

On June 30, 2017, a systematic survey of the *A. purpurascens* population was completed by Geordie Elkins, William Moorhead, and Jesse Hubbard. This survey resulted in a map illustrating the locations and relative densities of *A. purpurascens* flowering stems in both meadows. The survey also discovered plants that appeared intermediate between *A. syriaca* and *A. purpurascens*. On June 25, 2018, we performed a survey of the entire population discovered in the 2017 survey. A total of 60 flowering milkweed plants from across the upper and lower meadows were sampled on June 25 or July 9, 2019. A single midstem leaf adjacent to the first flowering node was removed and dried in an herbarium press. A few flowers were removed from each plant and preserved in 70% ethanol for laboratory measurements. While collecting plants in the field, we recorded an initial taxonomic identification based on the key differences described in Gleason and Cronquist (1991).

Nine leaf traits were measured on dried leaves collected in the field. Leaves were photographed using a digital camera adjacent to a metric ruler. Linear measurements were calibrated against the metric rule and analyzed using Image J (Schneider, Rasband, and Eliceiri 2012; version 1.46r). These traits included: leaf length, leaf width at each quarter of the leaf length, trichome density on abaxial surface, number of main veins, and angles at the base and apex. Basal and apical leaf angles were measured using the angle tool in Image J. The abaxial leaf surface was photographed on a stereo microscope at 50 ×. Leaf trichome density was determined on the abaxial surface by conducting a single count of trichomes/mm^2 in an area of a leaf between main veins. Leaf apex and base angles were measured from the tip and base of the leaves between lines drawn tangent to the leaf blade. Four derived ratios (leaf length relative to the three width measurements and the number of main veins divided by leaf length) were calculated for all plants.

Twenty floral traits were measured on preserved and fresh flowers. Three traits were scored by the presence or absence of a character state. These included the presence of hood teeth on the hood margin facing the flower column, the presence a lateral protuberance on the lower side of the hood, and the presence of a dimple above the protuberance (Fig. 1 B–C). The remaining 18 traits (Fig. 1) were measured using a calibrated Olympus stereo microscope linked to the computer imaging software CellSens (version 2.0, Olympus Corporation, Center Valley, PA). Images of flower hoods, columns, petals, sepals, and pollinaria were captured using CellSens. A single petal and sepal were removed per flower and pressed flat using a glass slide on the microscope stage. Ovaries were removed from flowers and opened with dissecting needles under a stereo microscope. Ovules from a single ovary were split into two or three sections that were photographed at 50 × and then counted using the count feature on Image J.

Measurements of the column and anther flaps forming the stigmatic chamber were made by

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

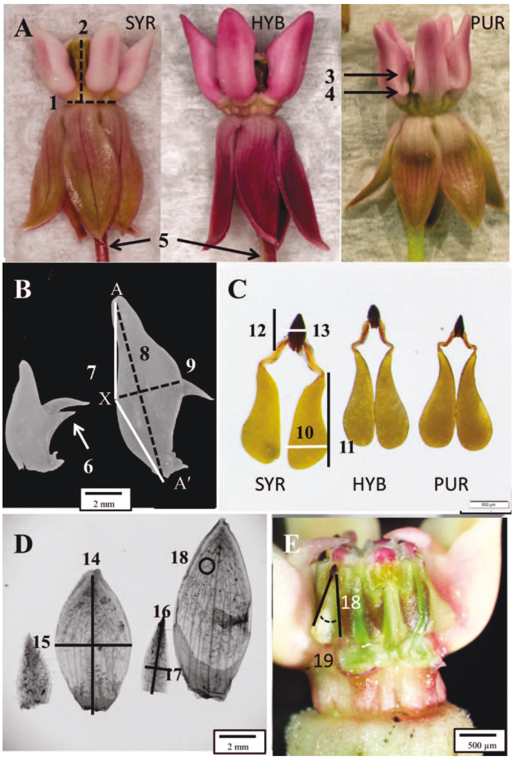

FIG. 1. Reproductive traits measured on *A. syriaca,* putative hybrids, and *A. purpurascens*. A. Flowers of *A. syriaca* (left), putative hybrid (center), and *A. purpurascens* (right). Traits shown are (1) column width, (2) column height, (4) hood lateral protuberance, and (5) pedicel width. B. Lateral view of flower hoods of *A. syriaca* (left) and *A. purpurascens*. Traits shown are (5) hood teeth, (6) hood angle as measured from points AXA′, (7) hood length, and (8) hood depth. C. Pollinaria of *A. purpurascens* (right), putative hybrid (center), and *A. syriaca*. Traits include (9) length, (10) width of pollinium, and (11) length and (12) width of corpuscular. D. Petals and sepals of *A. syriaca* (pair on left) and *A. purpurascens*. Traits shown include (13) length, (14) width of petals, (15) length, (16) width of sepals, and area where (17) trichome density was measured on abaxial surface of petals. E. Close-up of flower column showing anther flaps enclosing stigmatic chamber of *A. syriaca*. Traits shown include (18) stigmatic chamber flap length and (19) chamber flap angle. Ovule number not shown in figure.

removing a single hood and rotating the flower on the stage to capture the desired photos (Fig. 1B). Anther flaps enclose the milkweed stigmatic chamber. The anther flap angle was measured from the anther flap tip along tangent lines leading back to the column. Widths were measured at the midpoint of the length for hoods, petals, sepal, pollinia, and corpusculum (Fig. 1D–E). Six additional derived ratios (petal width:length, sepal length:width, hood width:length, column width: height, pollinium width:length, and corpusculum width:length) were calculated for all plants.

A cluster analysis was performed on a matrix of normalized variables using R Statistical Program (2015). Ward's method was used on a matrix of Euclidean distance values to assign plants to one of three clusters. The resulting cluster analysis was then used to assign plants belonging to three clusters assumed to represent *A. purpurascens*, *A. syriaca*, and putative hybrids. The Clusplot command in R was then used to perform a principal component analysis on the normalized variable data. The resulting graph of the first and second principal components also identifies points assigned to each of the three clusters. A minimum volume ellipse was drawn around the points of each cluster to assist with identifying the points assigned to each cluster. Analysis of variance was performed on the first two principal components for the three clusters using R Statistical Program (2015). Tukey's HSD was used to test for multiple comparison statistical ($P < 0.05$) differences between the three clusters.

Analysis of variance was performed on all 40 traits and number of pollen grains per pollinium for the three clusters using R Statistical Program (2015). Tukey's HSD was used to test for multiple comparison statistical ($P < 0.05$) differences between individuals in the three clusters. Boxplots were created for the variables with significant F values ($P < 0.05$) to illustrate the range of variation for each taxon.

Following the morphological and cluster analysis, pollinia of the 60 plants were reexamined. We observed that many pollinia in the hybrid cluster had a patchwork pattern of clear and opaque sections. We subsequently conducted pollen counts on pollinia. A single pollinium from preserved flowers was placed in spot plates and covered with 3–4 drops of 2-aminoethanol. The spot plate was incubated in a drying oven at 75 °C for 1 hr. This treatment softens the pollinium wall so that a dissecting needle easily frees individual pollen grains. Pollen was then stained with a 1% solution of toluidine blue. Pollen grains that stain deep purple were counted under a 10 × objective on a compound light microscope.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

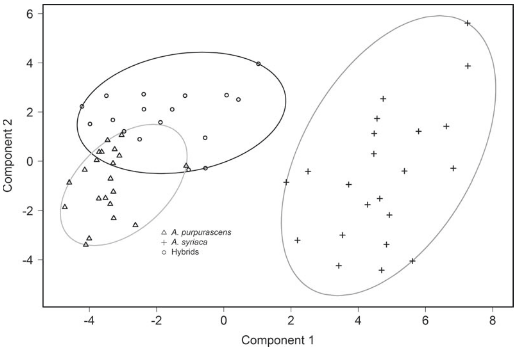

Fig. 2. Scatterplot of principal components 1 and 2 for clusters assigned to *A. syriaca, A. purpurascens,* and putative hybrids from Highstead, CT. Minimum volume ellipses are used to outline all points within a cluster.

Results. Cluster and principal component analyses revealed that the morphological spaces of the two parental species were distinct and nonoverlapping (Fig. 2). The first two principal components explained a total of 47% of the variation. Principal component 1 was significantly different for the three clusters and the putative hybrid cluster was significantly different than both *A. syriaca* and *A. purpurascens* for principal component 2 (Table 2).

Putative hybrids were most similar to *A. purpurascens* where individuals of the two clusters overlapped (Fig. 2). No individual identified in the hybrid cluster approached or fell within the morphological space of *A. syriaca*. The range of variation along the first principal component was greater for hybrids than *A. purpurascens*. These observations suggest that the putative hybrids might represent a mix of F1 and advanced generation hybrids with *A. purpurascens*. Twelve putative hybrids were found in the lower meadow and six in the upper meadow.

Cluster analysis assigned 22, 20, and 18 individuals to the clusters of *A. syriaca*, *A. purpurascens*, and putative hybrids, respectively. Our *a priori* field assignment of taxa suggested the population had as many as 11 hybrids. The cluster analysis assigned only eight of these plants to the hybrid cluster. Therefore, we missidentified 10 putative hybrids. We also identified two plants as hybrids that were assigned as *A. syriaca* by the cluster analysis. Our most common identification error was identifying plants as *A. purpurascens* that were most likely hybrids.

Statistically significant differences were observed for 33 of the 37 measured traits (Table 3; Fig. 3). The two parental and hybrid clusters were significantly different for five floral traits (hood length, stigmatic chamber length, pollinium width/length, corpusculum length, and petal trichome density). In all five cases, hybrids possessed intermediate values. For 15 of the remaining 28 traits, hybrids were statistically different from *A. syriaca*, but not *A. purpurascens*. Hybrids were statistically more similar *to A. syriaca* for five traits, and four of these were leaf traits. Hybrid leaves tended to be small like *A. purpurascens,* but trichome density, leaf apex angle, and leaf width-length ratios were more like *A. syriaca*. Hybrids had significantly greater mean values for flower column width than both parental species.

The two parental taxa had some overlap in values for nearly all morphological traits. Corpusculum length and width provide good separation of *A. syriaca* and *A. purpurascens* with the exception of a single individual of *A. purpurascens*. Hood length and width, pedicel width, stigmatic chamber length, corpusculum length and width, and the presence of trichome pubescence provided good separation of the species. *Asclepias syria4ca* had smaller hoods, thinner pedicels, longer stigmatic chambers, larger corpuscula, and more petal pubescence than *A. purpurascens*. Leaf traits were much less reliable for distinguishing *A. syriaca* and *A. purpurascens*.

Three traits scored (*e.g*., hood teeth, lateral hood proturberance, and lateral hood dimple) by their presence or absence provide good species recognition. *Asclepias syriaca* hoods have two distinct teeth on the inner margin facing the column. This

Table 2. Means and standard deviations for the first (PC1) and second (PC2) principal components of clusters assigned as *A. syriaca,* putative hybrids, and *A. purpurascens*. *F* and *P* values were determined using analysis of variance. For each principal component, means with different letters are statistically different ($P <$ 0.05) using Tukey's HSD multiple comparison test.

	A. syriaca	Putative Hybrid	*A. purpurascens*	*F*	*P*
PC1	4.45 ± 1.52a	−1.79 ± 1.52b	−3.28 ± 0.75c	204.7	0.001
PC2	−0.59 ± 2.64b	1.76 ± 1.06a	−0.94 ± 1.25b	11.8	0.001

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

TABLE 3. Means and standard deviations for leaf and floral traits of *A. syriaca, A. purpurascens*, and putative hybrids as revealed by cluster and principal component analyses. *F* and *P* values were determined using analysis of variance. For each trait, means with different letters are statistically different ($P < 0.05$) using Tukey's HSD multiple comparison test.

	A. syriaca ($N = 22$)	Putative hybrid ($N = 18$)	*A. purpurascens* ($N = 20$)	*F*	*P*
Leaf traits					
Leaf width, 1/4 from base (mm)	67.6 ± 15.4a	58.8 ± 11.7ab	49.4.0 ± 6.4b	13.1	< 0.001
Leaf width, 1/2 from base (mm)	66.03 ± 14.2a	60.8 ± 9.2a	50.13 ± 6.8.6b	12.5	< 0.001
Leaf width, 3/4 from base (mm)	52.3 ± 11.5a	46.5 ± 5.1ab	39.9 ± 9.8b	9.3	< 0.001
Leaf length (cm)	17.3 ± 2.6a	13.9 ± 2.0b	13.6 ± 1.3b	20.4	< 0.001
Leaf trichome density (no./mm^2)	58.9 ± 18.5a	57.5 ± 20.1a	35.8 ± 16.5b	10.6	< 0.001
Leaf apex angle (°)	97.9 ± 17.2ab	111.2 ± 21.5a	85.2 ± 18.4b	8.8	< 0.001
Leaf base angle (°)	130.4 ± 22.5a	121.7 ± 27.2a	115.5 ± 29.0a	1.8	0.211
Leaf area (cm^2)	97.0 ± 32.0a	65.9 ± 20.0b	52.0 ± 10.4b	18.2	< 0.001
Leaf veins	45.1 ± 6.1a	42.3 ± 4.9a	4,453 ± 6.8a	1.0	0.512
Leaf ratios					
Leaf veins/length (cm^{-1})	2.7 ± 0.4a	3.1 ± 0.4a	3.3 ± 0.5a	9.4	< 0.001
Leaf width (1/4)/length	0.39 ± 0.05ab	0.42 ± 0.03a	0.36 ± 0.04b	8.1	< 0.001
Leaf width (1/2)/length	0.38 ± 0.05b	0.44 ± 0.02a	0.37 ± 0.04b	13.8	< 0.001
Leaf width (3/4)/length	0.30 ± 0.04a	0.34 ± 0.03a	0.29 ± 0.07b	4.2	0.019
Flower traits					
Ovule number	169.8 ± 36.6a	140.6 ± 26.9b	118.5 ± 22.0b	16.8	< 0.001
Hood length (mm)	5.7 ± 0.6c	7.0 ± 0.5b	7.6 ± 0.6a	61.4	< 0.001
Hood width (mm)	2.0 ± 0.3b	2.5 ± 0.3a	2.5 ± 0.2a	30.7	< 0.001
Petal length (mm)	8.2 ± 0.8b	9.0 ± 0.6a	9.4 ± 0.5a	16.6	< 0.001
Petal width (mm)	3.9 ± 0.4a	4.0 ± 0.2a	4.0 ± 0.2a	0.4	0.660
Sepal length (mm)	5.1 ± 1.0a	4.0 ± 0.5b	4.4 ± 0.8b	9.9	< 0.001
Sepal width (mm)	1.7 ± 0.3a	1.2 ± 0.2b	1.2 ± 0.3b	21.9	< 0.001
Pedicel width (mm)	0.9 ± 0.1b	1.1 ± 0.1a	1.2 ± 0.2a	38.7	< 0.001
Column width (mm)	3.1 ± 0.3b	3.4 ± 0.3a	3.2 ± 0.3b	4.0	0.024
Column height (mm)	3.9 ± 0.3a	3.5 ± 0.3b	3.6 ± 0.5ab	5.6	0.006
Pollinium length (mm)	1.18 ± 0.05b	1.22 ± 0.04ab	1.23 ± 0.09a	3.2	0.046
Pollinium width (mm)	0.54 ± 0.05a	0.49 ± 0.02b	0.48 ± 0.02b	24.2	< 0.001
Corpusculum length (mm)	0.51 ± 0.05a	0.38 ± 0.07b	0.32 ± 0.06c	65.1	< 0.001
Corpusculum width (mm)	0.27 ± 0.03a	0.20 ± 0.04b	0.18 ± 0.04b	34.4	< 0.001
Chamber flap length (mm)	1.6 ± 0.2a	1.5 ± 0.2b	1.3 ± 0.1c	20.6	< 0.001
Hood angle (°)	144.0 ± 11.4a	134 ± 10.4b	139 ± 9.4ab	4.0	0.023
Chamber flap angle (°)	19.6 ± 4.3b	29.08 ± 4.9a	29.5 ± 5.0	29.2	< 0.001
Petal trichome density (no./mm^2)	22. ± 15.2a	1.1 ± 2.6b	0.0c	35.2	< 0.001
Flower ratios					
Petal width/length	0.48 ± 0.04a	0.44 ± 0.03b	0.43 ± 0.03b	13.4	< 0.001
Sepal length/width	0.34 ± 0.05a	0.31 ± 0.05ab	0.28 ± 0.05b	9.5	< 0.001
Hood width/length	0.36 ± 0.04a	0.36 ± 0.03a	0.33 ± 0.03b	5.1	0.009
Column width/height	0.81 ± 0.12b	0.98 ± 0.19a	0.88 ± 0.12ab	8.9	< 0.001
Pollinium width/length	0.46 ± 0.04a	0.40 ± 0.02b	0.39 ± 0.03c	13.4	< 0.001
Corpusculum width/length	0.54 ± 0.08a	0.53 ± 0.07a	0.57 ± 0.11a	1.25	0.330
Presence *versus* absence (scores represent number of individuals with trait)					
Hood teeth	21	0	0		
Lateral protuberance on hood	5	18	20		
Lateral dimple on hood	2	18	20		

characteristic was observed on 21 of 22 *A. syriaca* individuals. These teeth are prominent in dissected flowers and by examining the area adjacent to the horns. Hood teeth were absent in all hybrids and *A. purpurascens* plants. *Asclepias purpurascens* floral hoods were longer, and all 20 individuals had a distinct lateral protuberance with a distinct indentation above the protuberance (Fig. 1). These *A. purpurascens*-like traits were found in all hybrid plants and fewer than 5 of the 22 *A. syriaca* plants.

The large pollinia of *A. syriaca* contained significantly more pollen that pollinia of *A. purpurascens* and hybrids (Fig. 4; $F = 69.7$; $P < 0.001$). Pollinia of hybrid plants had a mosaic of dark and light tan patterns indicative of filled and empty pollen grains (see Fig. 1C, middle polli-

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

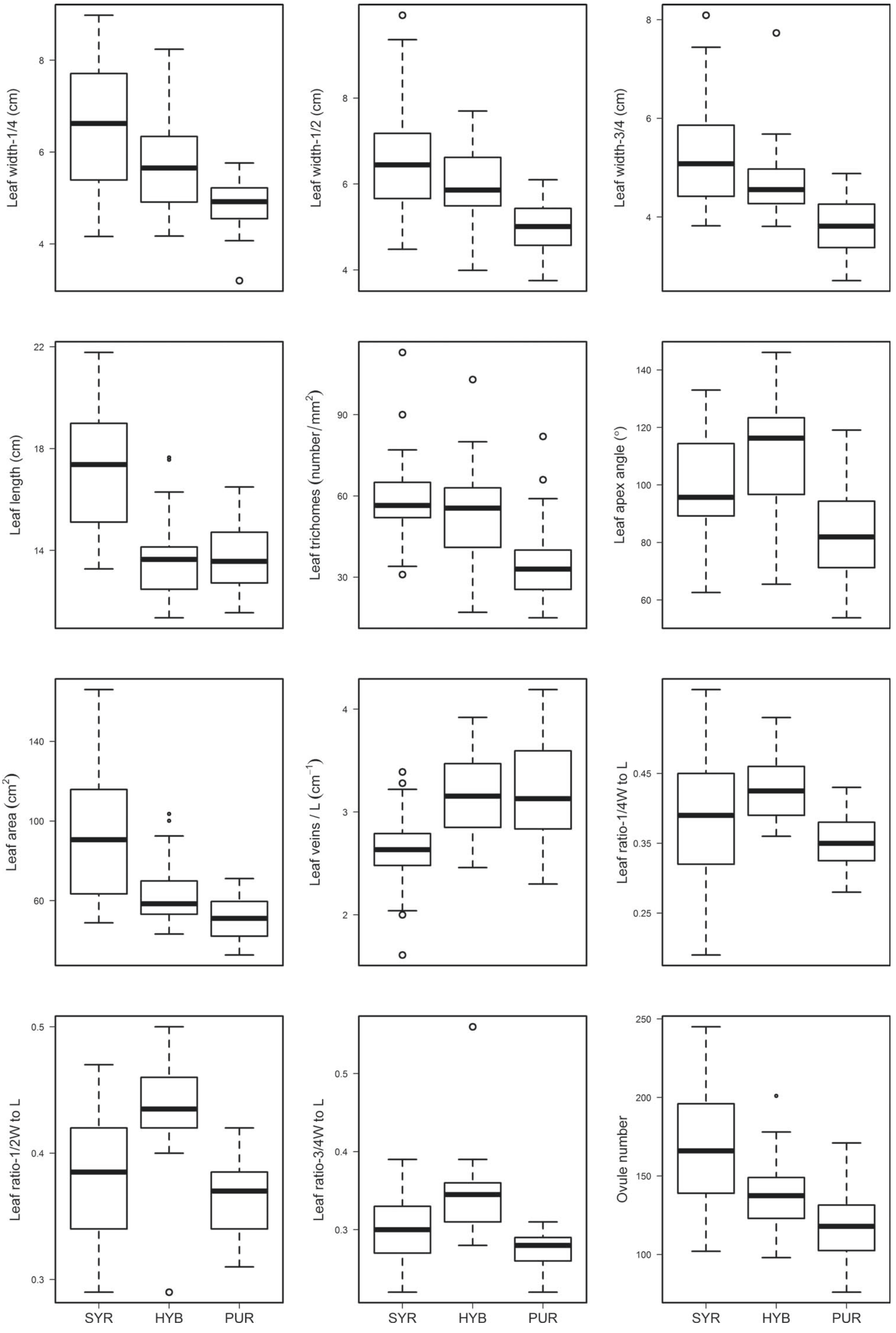

FIG. 3. Median, quartiles, and outliers (open circles) for 33 morphological traits of *A. syriaca* (SYR), putative hybrids (HYB), and *A. purpurascens* (PUR) as identified by cluster analysis from Highstead, CT.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

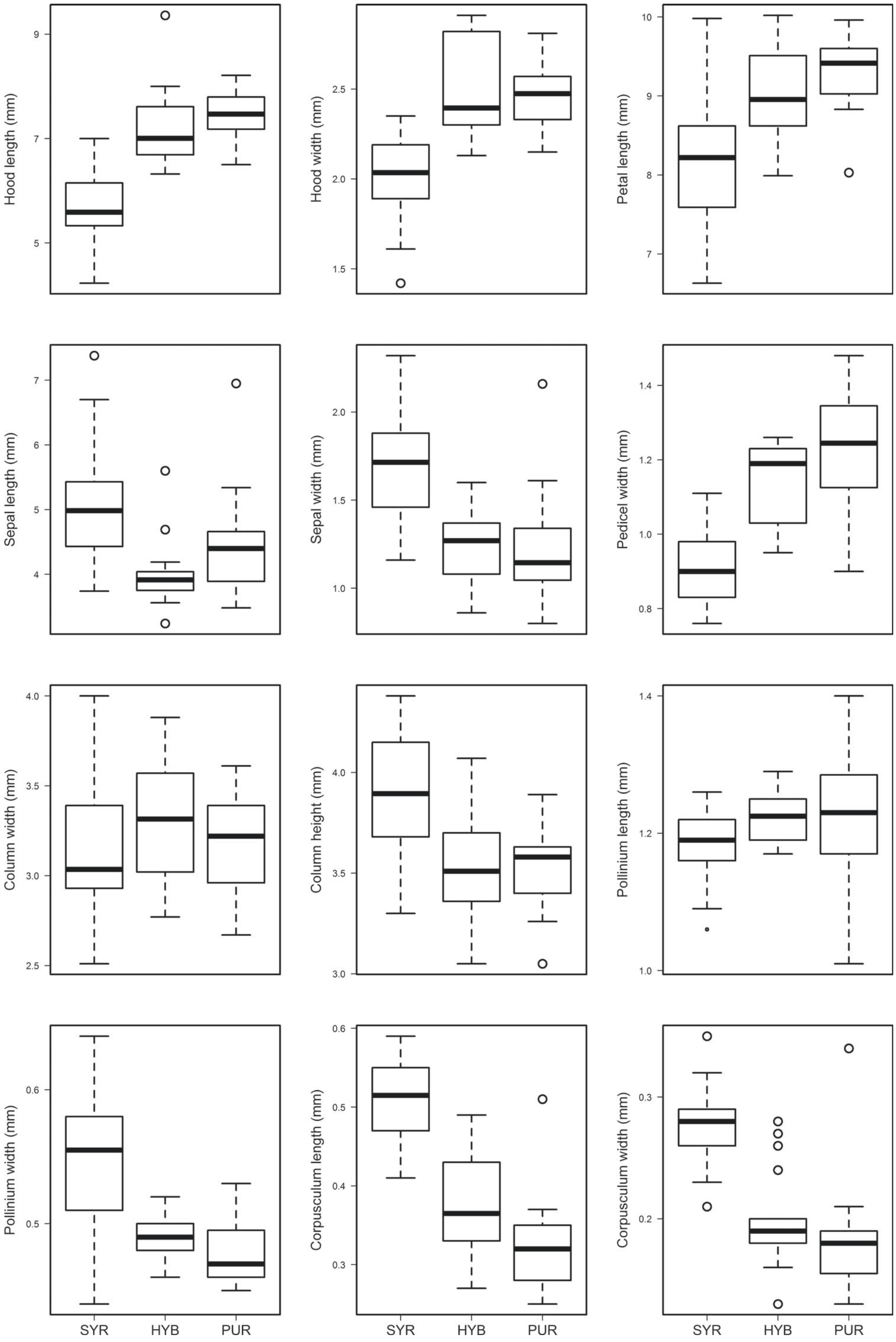

FIG. 3. Continued.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

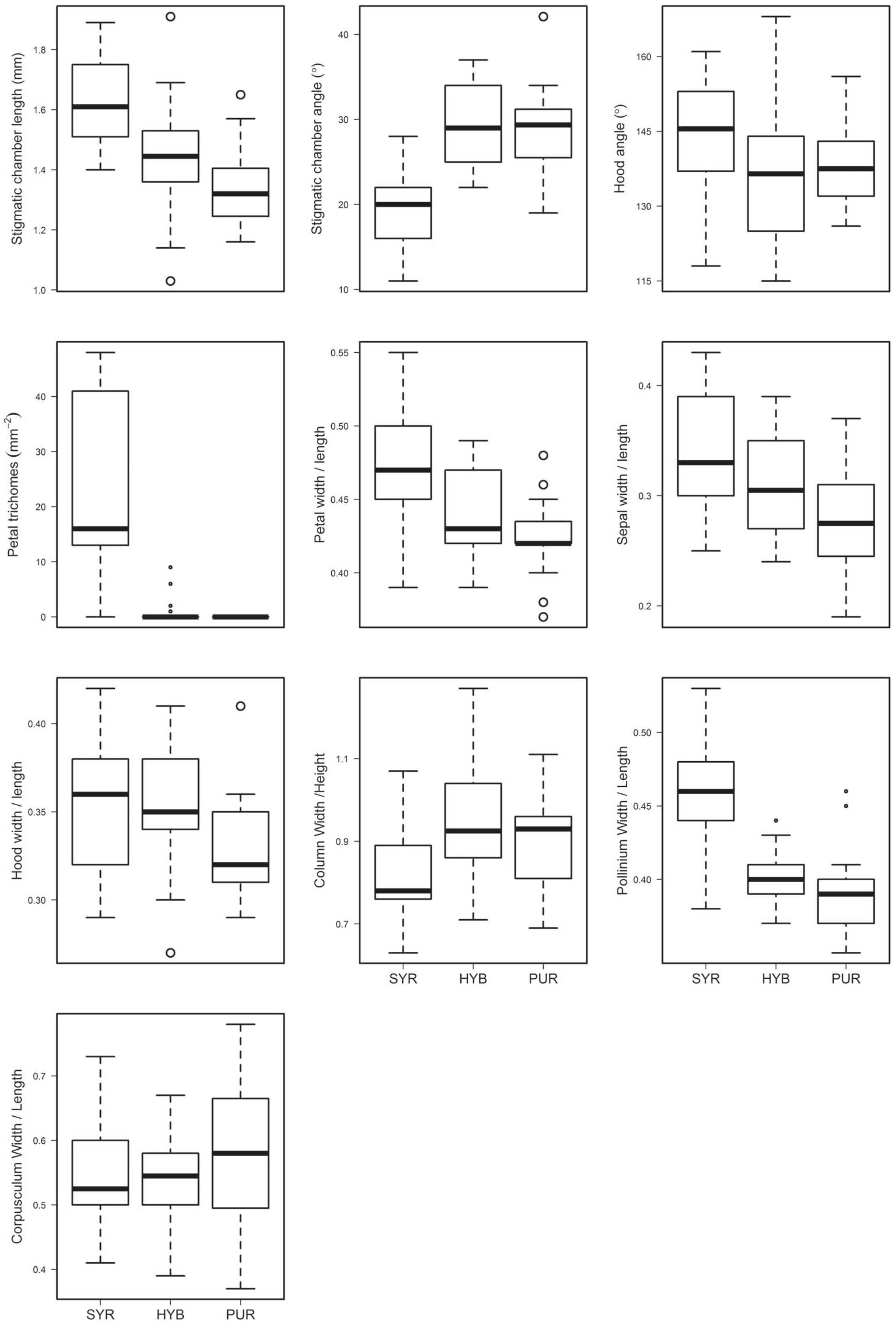

FIG. 3. Continued.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

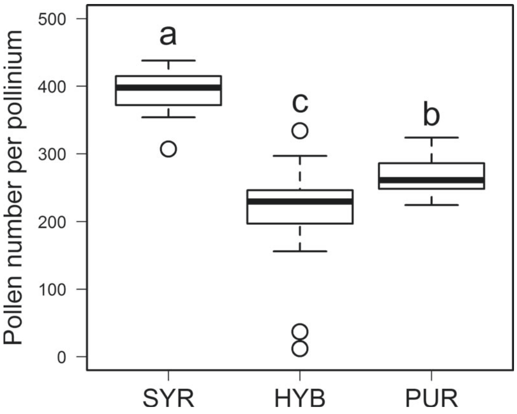

FIG. 4. Median, quartiles, and outliers (open circles) for pollen number per pollinium of *A. syriaca* (SYR)*,* putative hybrids (HYB), and *A. purpurascens* (PUR) as identified by cluster analysis from Highstead, CT. Box plots with different letters are significantly different (*A. syriaca*–hybrid*,* $P < 0.001$; *A. syriaca–A. purpurascens*, $P < 0.001$; *A. purpurascens*–hybrid, $P = 0.003$) using Tukey's HSD multiple comparison test.

narium). This pattern is likely attributed to the significantly lower numbers of pollen grains per pollinia for hybrids relative to *A. purpurascens* and *A. syriaca* (Fig. 4).

Discussion. THE CASE FOR HYBRIDIZATION. Morphological evidence presented here provides strong evidence of hybridization between the rare milkweed, *A. purpurascens,* and its widespread and common congener, *A. syriaca*. The morphological space of the hybrid cluster overlaps considerably with *A. purpurascens* (Fig. 2). Nonetheless, several of the hybrid plants are intermediate to both parental species. Hybrid plants were most similar to *A. syriaca* for leaf traits, but more similar to *A. purpurascens* for floral traits. The composition of the hybrid population at Highstead likely represents a combination of F1 and advanced generation plants (F2s and parental backcrosses with *A. purpurascens*).

Hybridization between *A. purpurascens* and *A. syriaca* should be expected in the Midwest and eastern North America for several reasons. (1) The species habitats overlap. Both species co-occur in dry to moist fields, meadows, and prairies (Woodson 1954; Farnsworth and DiGregorio 2002; USDA Forest Service, Eastern Region 2003). In addition, there is considerable overlap of flower phenology in these habitats. (2) Like many milkweeds, the flowers of these species are pollinated by generalist insects such as Hymenoptera, Lepidoptera, Diptera, and Coleoptera (Robertson, 1887). Although this study did not examine the pollinator diversity and movement between plants, our casual observations indicated that insect pollinators were shared at Highstead. (3) *Asclepias purpurascens* and *A. syriaca* possess similar sized pollinia. Although the numbers of pollen grains per pollinium were significantly different at Highstead, there is overlap in the range of pollen numbers for the two species, and in both cases the numbers of pollen grains were greater than the number of ovules in ovaries (see also Wyatt, Broyles, and Lipow, 2000). Thus, F1 hybrid seed set would be complete in fruits of both *A. syriaca* and *A. purpurascens*. It is worth noting that the numbers of pollen grains per pollinium are reduced in hybrids and this might impact the hybrid mating dynamics within the population. (4) *Asclepias purpurascens* is rare in many areas throughout its range. This creates a unique situation where a reduction in compatible conspecific genotypes in populations is likely and interspecific pollinations represent a more likely avenue for reproduction. Our data at Highstead strongly support the hypothesis that hybridization occurs under the above situations. The opportunity for advanced hybrid formation is possible and the bridge for genetic introgression is open.

Asclepias syriaca has been a focal species in many milkweed hybridization studies. Its widespread distribution and vigorous growth in disturbed habitats have contributed to its hybridization and introgression with a western prairie species, *A. speciosa* (Adams, Tomb, and Price 1987), a midwestern prairie species, *A. sullivantii* (Klips and Culley 2004), and an eastern forest species, *A. exaltata* (Kephart, Wyatt, and Parrella 1988). We can now add *A. purpurascens* to the list of milkweeds capable of hybridizing with *A. syriaca* and focused searching could yield more hybridizing populations throughout their ranges.

TAXONOMIC IMPLICATIONS OF HYBRIDIZATION. The taxonomic affinities of species hybridizing with *A. syriaca* are not restricted to close relatives. In his monograph of 107 species of *Asclepias*, Woodson (1954) recognized nine series based on flower morphologies. *Asclepias syriaca,* series Syriacae, has hybridized with three species of series Purpurascentes (*A. purpurascens, A. speciosa*,

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

and *A.* sullivanti) and one of Exaltatae (*A. exaltata*). Current and ongoing molecular studies (Fishbein *et al.* 2011; S. C. K. Straub, personal communication) will likely dissolve or modify the "series" treatment of Woodson; however, these molecular studies suggest *A. syriaca* hybridization has occurred with both closely related and more distant species.

Field Identification of Hybrids and the Parental Species. Identifying the parental species in mixed populations is straight forward. Two of the three floras often used in the eastern USA focus on the teeth (*i.e.*, lobes) along the median margin of the hoods (Table 1). The Highstead parental species display these differences consistently, but the teeth are small and difficult to examine without a hand lens. Hood length works well for distinguishing these species, although our species range values differ from those reported by Haines (2011). Hoods of *A. syriaca* were conspicuously shorter (4.2–6.3 mm) when compared to *A. purpurascens* (6.5–8.2 mm). We suspect the hood length differences with those reported by Haines (see Table 1) are the result of differences in how hoods were measured rather than underlying biological differences. Additional features that future floras could use include: the presence/absence of a lateral protuberance on hoods, thickness of pedicels. Umbel shape (globose for *A. syriaca* and semi-hemispheric/erect for *A. purpurascens*), and fruit surface traits (muricate for *A. syriaca* and smooth for *A. purpurascens*) might provide additional diagnostic traits, but neither was examined in the present study.

Hybrids are very difficult to recognize in the field. In fact, our field assignment of plants did not agree with the results of the cluster analysis for 12 of the 60 plants. Although mean values are intermediate for the hybrids, the large range of values observed in this cluster prevent us from developing a list of traits to permit their rapid identification in the field. Even hood length does not provide good hybrid definition because the range of values (6.4–9.4 mm) for the hybrid cluster encompassed all values for *A. purpurascens*. Missing pollen and the resulting patterning on pollinia is suggestive of hybrids, but this is difficult to examine without a field microscope. The problem of recognizing hybrids could be prevalent throughout the range of *A. purpurascens* where casual observations can lead to the conclusion that one is simply examining natural variation in the species. Creation of known hybrids (F1, F2s, and backcrosses) will provide a better picture for expect character states in hybrids.

Conservation Implications. *Asclepias purpurascens* has experienced a rapid decline in the eastern United States. Remaining populations are small (< 30 flowering individuals; S. Young, New York State Botanist, personal communication; Farnsworth and DiGregorio 2002). The shared habitat preferences bring *A. syriaca* and *A. purpurascens* into frequent contact and one of the authors has observed putative hybrids in two populations in Orange County NY. Small populations and shared habitat likely increase the chance of hybridization, introgression, and swamping of the natural gene pool of *A. purpurascens*. Conservation stewards of extant populations might wish to consider breeding programs to increase seed stock of genetically diverse, purebred *A. purpurascens* for reestablishment projects. Furthermore, *A. syriaca* might need to be controlled at reintroduction sites, allowing *A. purpurascens* to increase in numbers. The aggressive, vigorous growth of *A. syriaca* might also consume the resources at reintroduction sites and outcompete *A. purpurascens*.

Are there benefits to maintaining widespread, vigorous *A. syriaca* in populations of *A. purpurascens*? The presence of the large, multistemmed, flower-abundant *A. syriaca* might maintain a greater diversity of pollinators than *A. purpurascens* could alone. However, on a seasonal basis, *A. syriaca* tends to flower later, perhaps reducing the impact of pollinator competition. The idea of adaptive introgression as a conservation tool has gained support in recent years (Hamilton and Miller 2015; Suarez-Gonzales, Lexer, and Cronk 2018). Proponents argue that interspecific gene flow across semipermeable species boundaries introduces novel alleles to the rare population and offer new adaptive traits that improve long-term survival in a changing climate (Harrison and Larson 2014; Hamilton and Miller 2015). Others maintain that introgression and genetic assimilation dilute the species integrity of the rare taxon (Rieseberg 1991; Levin, Francisco, and Jansen 1996). In either case, hybridization between *A. purpurascens* and *A. syriaca* is likely and land managers should consider the relative benefits/detriments of maintaining a hybridizing population as a conservation strategy.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

Conclusions. Morphological evidence on floral and vegetative traits strongly suggests that hybridization and backcrossing has occurred in the Highstead population of *A. purpurascens* and *A. syriaca*. Hybridization between these species should be expected in eastern populations where *A. purpurascens* is an uncommon species in decline. We question whether hybridization is contributing to the decline of *A. purpurascens* or if the decline is an artifact of mating outcomes in small populations where the number of compatible mates is reduced. If hybrids are viable, fertile, and they leave more offspring than *A. purpurascens*, then *A. syriaca* genes could assimilate and ultimately swamp *A. purpurascens* in small populations. *Asclepias purpurascens* is a presumed outbreeding, self-incompatible species, as suggested by high allozyme heterozygosity in populations from New York (extirpated population in Stewart Forest) and Virginia (S. B. Broyles, unpublished data). These hypotheses warrant investigation because the information could guide management strategies for *A. purpurascens* in eastern populations.

Literature Cited

Adams, R. P., A. S. Tomb, and S. C. Price. 1987. Investigation of hybridization between *Asclepias speciosa* and *A. syriaca* using alkanes, fatty acids, and triterpenoids. Biochemical Systematics and Ecology 15: 395–399.

Arnold, M. 1992. Natural hybridization as an evolutionary process. Annual Review of Ecology and Systematics 23: 237–261.

Baack, E., M. C. Melo, L. H. Reiseberg, and D. Ortiz-Barrientos. 2015. The origins of reproductive isolation in plants. New Phytologist 207: 968–984.

Broyles, S. B. 2002. Hybrid bridges to gene flow: A case study in milkweeds (*Asclepias*). Evolution 56: 1943–1953.

Broyles, S. B., C. Vail, and S. L. Sherman-Broyles. 1996. Pollination genetics of hybridization in sympatric populations of *Asclepias exaltata* and *A. syriaca* (Asclepiadaecae). American Journal of Boany 83: 1580–1584.

Ellstrand, N. C., D. Biggs, A. Kaus, P. Lubinsky. L. A. McDade, K. Preston, L. M. Prince, H. M. Regan, V. Roriver, O. A. Ryde, and K. A. Schierenbeck. 2010. Got hybridization? A multidisciplinary approach for informing science policy. Bioscience 60: 384–388.

Farnsworth, E. J. and M. J. DiGregorio. 2002. *Asclepias purpurascens* L. (Purple milkweed) conservation and research plan. New England Plant Conservation Program, Farmington, MA, USA. 18 pp.

Fishbein, M., D. Chuba, C. Ellison, R. J. Mason-Gamer, and S. P. Lynch. 2011. Phylogenetic relationships of *Asclepias* (Apocynaceae) inferred from non-coding chloroplast DNA sequences. Systematic Botany 36: 1008–1023.

Gleason, H. A. and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. The New York Botanical Garden, Bronx, NY. XXX pp.

Haines, A. 2011. Flora Novae Angliae. A Manual for the Identification of Native and Naturalized Higher vascular Plants of New England. Yale University Press. New Haven, CT. 973 pp.

Hamilton, J. A. and J. M. Miller. 2015. Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology 30: 33–41.

Harrison, R. G. and E. L. Larson. 2014. Hybridization, introgression, and the nature of species boundaries. Journal of Heredity 10: 795–809.

Jackiw, R. N., G. Mandil, and H. A. Hager. 2015. A framework to guide the conservation of species hybrids based on ethical and ecological considerations. Conservation Biology 29: 1040–1051.

Kephart, S. and C. H. Heiser. 1980. Reproductive isolation in *Asclepias*: Lock and key hypothesis reconsidered. Evolution 34: 718–746.

Kephart, S., R. Wyatt, and D. Parrella. 1988. Hybridization in North American *Asclepias*. I. Morphological evidence. Systematic Botany 13: 456–473.

Klips, R. A. and M. Culley. 2004. Hybridization between prairie milkweeds, *Asclepias sullivantii* and *Asclepias syriaca*: Morphological, isozyme, and hand-pollination evidence. International Journal of Plant Sciences 165: 1027–1037.

Levin, D. A., J. Francisco, and R. K. Jansen. 1996. Hybridization and the extinction of rare plant species. Conservation Biology 10: 10–16.

Moore, R. J. 1946. Investigations on rubber-bearing plants IV. Cytogenic sudies in *Asclepias* (Tourn.) L. Canadian Journal of Research 24:66–73.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. The University of North Carolina Press, Chapel Hill, NC. 1183 pp.

Rieseberg, L. H. 1991. Hybridization in rare plants: insights from case studies in *Cerocarpus* and *Helianthus*, pp. 171–181. *In* D. A. Falk and K. E. Holsinger, eds. Genetics and Conservation of Rare Plants. Oxford University Press, New York, NY.

Rintz, R. E. 2014. A naturally occurring hybrid between *Asclepias syriaca* L. and *A. purpurascens* L. (Apocynaceae, Ascepiadoideae). Phytologia 96: 130–134.

Robertson, C. 1887. Insect relations of certain Asclepias. I. Botanical Gazette 12: 244–250.

RStudio Team. 2018. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA. Retrieved May 15, 2018. http://www.rstudio.com/.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671–675.

Stoepler, T. M., A. Edge, A. Steel, R. L. O'Quinn, and M. Fishbein. 2012. Differential pollinator effectiveness and its importance in a milkweed (Asclepias, Apocynaceae) hybrid zone. American Journal of Botany 99: 448–458.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University

Suarez-Gonzalez, A., C. Lexer, and Q. C. B. Cronk. 2018. Adaptive introgression: a plant perspective. Biology Letters 14: 20170688.

USDA Forest Service, Eastern Region. 2003. Conservation assessment for purple milkweed (*Asclepias purpurascens*). Retrieved September 18, 2019. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fsm91_054165.pdf.

Woodson, R. E., Jr. 1954. The North American species of *Asclepias* L. Annals of the Missouri Botanical Garden 41: 1–211.

Wyatt, R. and S. B. Broyles. 1992. Hybridization in North American *Asclepias*. III. isozyme evidence. Systematic Botany 17: 640–648.

Wyatt, R. and S. B. Broyles. 1994. Ecology and evolution of reproduction in milkweeds. Annual Review of Ecology and Systematics 25: 423–441.

Wyatt, R. and D. M. Hunt. 1991. Hybridization in North American *Asclepias*: II. Flavonoid evidence. Systematic Botany 16: 132–142.

Wyatt, R., S. B. Broyles, and S. R. Lipow. 2000. Pollen-ovule ratios in milkweeds (Asclepiadaceae): An exception that probes the rule. Systematic Botany 25: 171–180.

Downloaded From: https://bioone.org/journals/The-Journal-of-the-Torrey-Botanical-Society on 05 Feb 2020
Terms of Use: https://bioone.org/terms-of-use Access provided by Cornell University