

Morphological, chemical, and biogeographical analyses of a hybrid zone involving *Juniperus virginiana* and *J. horizontalis* in Wisconsin

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Natural hybridization between *Juniperus virginiana* (L.) and *J. horizontalis* (Moench.) has been reported in the Driftless Area in Wisconsin. We applied multivariate statistical techniques to morphological and terpene data both to document the phenomenon of hybridization and to determine more accurately the distribution of hybrids and parentals in the Driftless Area. Analysis of morphological, terpene, and electrophoretic data is consistent with the hypothesis that hybridization is occurring beyond the F₁ generation. The biogeography of hybridization resembles an archipelago of hybrid populations arrayed along the Driftless boundary. The situation is unusual in that while most hybrid populations are in contact with extensive *J. virginiana* populations, none appear to be in contact with *J. horizontalis*. The one-sided structure of the hybrid zone suggests that hybrids are favored by selection along the eastern boundary of the Driftless Area.

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La présence d'hybrides naturels entre *Juniperus virginiana* (L.) et *J. horizontalis* (Moench.) a été signalée dans le Driftless Area du Wisconsin. Nous avons analysé les données morphologiques et les terpènes à l'aide de techniques statistiques multivariées, dans le but de documenter le phénomène de l'hybridation et de déterminer plus précisément la répartition des hybrides et des parents dans le Driftless Area. L'analyse des données morphologiques, terpéniques et électrophorétiques s'accorde avec l'hypothèse que l'hybridation se poursuit au-delà de la génération F₁. La biogéographie de l'hybridation ressemble à un archipel de populations hybrides alignées le long de la frontière du Driftless Area. Tandis que la plupart des populations hybrides sont en contact avec de grandes populations de *J. virginiana*, aucune ne semble être en contact avec *J. horizontalis*. La structure unilatérale de la zone d'hybridité laisse croire que les hybrides sont favorisés par la sélection le long de la frontière est du Driftless Area.

[Traduit par le journal]

Introduction

Natural hybridization has been reported between several species of junipers (Fassett 1944a, 1945a, 1945b; Hall 1952, 1955; Hall et al. 1962; Van Haverbeke 1968; Schurtz 1971; Von Rudloff 1975a; Flake et al. 1978; Adams 1983; Comer et al. 1982). Based on morphological analysis, Hall (1952, 1955) and Hall et al. (1962) concluded that the ashe juniper, *Juniperus ashei* Buch., and the eastern red cedar, *J. virginiana* L., hybridize in the Arbuckle Mountains in southern Oklahoma. However, subsequent morphological studies and analyses of terpene profiles based on gas-liquid chromatography (GLC) resulted in the refutation of Hall's conclusion (Adams 1975, 1977; Adams and Turner 1970; Flake et al. 1973; Von Rudloff et al. 1967; Von Rudloff 1975a).

Fassett (1945b) reported hybrid swarms between *Juniperus virginiana* L. and the creeping juniper, *J. horizontalis* Moench., in the Driftless Area of south

central Wisconsin. *Juniperus virginiana* is one of the most widely distributed North American species ranging from southern Ontario to Texas and from the Atlantic coast to the Great Plains, whereas *J. horizontalis* is limited to the northern part of the United States and southern Canada. A diversity of intermediate forms occurs where the two species are sympatric. Fassett (1945b) hypothesized that *Juniperus horizontalis* survived the most recent glacial advance in the Driftless Area and that in post-Wisconsin times *J. virginiana* migrated from the south into the Driftless Area where hybridization ensued. Fassett (1945b) also reported that where the two species overlap, they may remain morphologically distinct or produce hybrids which may appear as uniformly intermediate or exhibit different combinations of parental characteristics within a population. In fact, Fassett (1945b) described some hybrids as *J. virginiana* var. *ambigens*. Ross and Duncan (1949) studied chromosomes and meiosis in the pollen mother

cells of putative hybrids and parental plants from Wisconsin. The somatic chromosome complement of *J. horizontalis* contained a heterobrachial pair of chromosomes not found in *J. virginiana*, whereas some putative hybrids had novel heterobrachial chromosomes. In addition, putative hybrids had a higher frequency of irregular meiotic figures in pollen mother cells than did "pure" plants. In most populations, pollen production was not markedly reduced, although Pine Bluff (site 4, Fig. 2; Appendix) and Grand marsh (site 39) were reported to have a lower pollen production range (Ross and Duncan 1949).

The purpose of this paper is to reexamine and extend Fassett's work on putative hybrid populations in Wisconsin. Specifically we apply multivariate statistical procedures to morphological characters of junipers from Wisconsin and Michigan to test further the hypothesis that there are hybrid populations and to determine more accurately the distribution of hybrid and parental populations. Although results of our morphological analysis are in general agreement with Fassett's inference, we felt it prudent to follow up our morphological study with a GLC analysis of terpenes and an electrophoretic analysis of peroxidases in foliage.

Materials and methods

Morphological analysis

The choice of Wisconsin populations to be sampled was based on the studies of Fassett (1945*b*), on our own preliminary reconnaissance studies, and on herbarium vouchers from the University of Wisconsin. Collecting efforts were concentrated along the Driftless boundary because the preliminary studies suggested that hybrid populations were restricted to that region. The additional samples reported here bear out that suggestion. Individual collecting sites are listed in the Appendix and mapped in Fig. 2. Sample sizes were based on the degree of variation observed in growth habit; 10 plants were sampled where variation suggested hybridity, whereas smaller samples were taken from more homogeneous stands. Sampled plants were chosen at random within populations. Three populations from Michigan, Pinckney (site 1), Mattawan (site 2), and Mackinac City (site 56), were included in the study as "controls." The two species are completely allopatric in Michigan, and hybrids have never been reported. Thus, the Michigan populations were assumed, *a priori*, to be representative of "pure" parental types (Pinckney and Mattawan = *J. virginiana*; Mackinac City = *J. horizontalis*). Fresh foliage of all the individuals for voucher specimens (morphological analysis) and biochemical analyses was collected and placed in an ice chest.

Fassett (1944*b*, 1944*c*) and Van Haverbeke (1968) reported several morphological characters that are useful for distinguishing *Juniperus virginiana* from *J. horizontalis*. The subset of these that we used is tabulated in Table 1 along with the units (if any) and ranges of the character states. The following is a brief description of each character and the criteria used to determine the character states: growth habit (HABIT), (1) upright, more or less vertical main trunk, (2)

ascending, growing obliquely upward, (3) decumbent, prostrate at base, ascending elsewhere, (4) prostrate, creeping; foliage color (COLOR), (1) dark green, (2) light green, (3) bluish green; branching character (BCHAR), (1) one main axis, (2) two or three main axes, (3) four or more; leaf tip shape (LTIP), (1) acuminate, (2) acuminate-apiculate (intermediate), (3) apiculate; average epidermal cell width (EWIDTH), the average in micrometres of 30 cells in 10 leaves; height/width ratio (HWRATIO), ratio of the height and width of the plant; female cone length (CLENGTH), the average of 10 female cones for each plant; female cone width (CWIDTH), the average of 10 female cones for each plant; number of seeds per cone (SEED), the average for 10 cones for each plant; percent of straight female cone peduncles (SPED), the percentage of straight peduncles in 20 randomly chosen peduncles per plant.

Terpene analysis

Fresh foliage samples of 89 Wisconsin junipers from 12 populations were collected between November 21 and November 24, 1980 and frozen until volatile oils could be extracted by steam distillation. The populations sampled are a subset of those sampled for the morphological analysis, and in most cases the same trees were sampled. However, Michigan trees were not sampled for terpene analysis, and trees from North Freedom and Sheboygan were substituted as "controls" for "pure" *Juniperus virginiana* and *J. horizontalis*, respectively. Ten trees from North Freedom and 15 from Sheboygan were sampled as "controls," which means that seven new plants from North Freedom and five from Sheboygan were added to the original morphological samples. The choices of North Freedom and Sheboygan were based on the morphological analysis; morphologically these two populations are indistinguishable from the respective Michigan controls. Ideally, Michigan populations should have been sampled as parental reference material. However, seasonal variation in terpene profiles compounded by our scheduling limitations made this difficult. In retrospect, we believe that the Sheboygan and North Freedom samples served as well since they proved to have very distinct terpene profiles and putative hybrids were intermediate.

Volatile terpenoids were extracted by steam distillation for 2 h and the extracts were sealed and stored at -20°C until chromatographed (Adams et al. 1970). GLC conditions were similar to those reported by Adams et al. (1981). Specific terpene components were identified by comparison with profiles previously reported for the two species (Adams et al. 1970).

Protein electrophoresis

Two grams of foliage was extracted with the grinding buffer of Koppers and Weidner (1980) and prepared for polyacrylamide gel electrophoresis, using the method in M. Palma-Otal et al. (unpublished data). Gels were subsequently stained and scored for peroxidase activity (Symeonidis et al. 1979). Sixteen samples of Michigan *J. virginiana* and 10 samples of Michigan *J. horizontalis* were run as reference. Some of the Wisconsin samples were also electrophoresed.

Statistics

The morphological and terpene data were subjected to statistical analyses (Blackrith and Reymont 1971; Cooley and Lohnes 1971; Pimentel 1979). Principal-component analyses

TABLE 1. Morphological characters that distinguish *J. virginiana* from *J. horizontalis*. Ranges and units of measure given when necessary

Characters	Character states	
	<i>J. virginiana</i>	<i>J. horizontalis</i>
Growth habit (HABIT)	1	4
Foliage color (COLOR)	1	3
Branching character (BCHAR)	1-2	3
Leaf tip (LTIP)	1	3
Epidermal cell width (EWIDTH)	9.5-11.4 μm	13.3-16.4 μm
Height/width ratio (HWRATIO)	4.7-37.2	0.004-0.007
Cone width (CWIDTH)	0.399-0.498 cm	0.564-0.681 cm
Cone length (CLENGTH)	0.457-0.546 cm	0.518-0.607 cm
Mean no. seeds per cone (SEEDS)	1.4-2.0	2.3-4.3
% straight peduncles (SPED)	80-95	0-10

were done using MIDAS (Michigan Interactive Data Systems, Statistical Research laboratory, University of Michigan). For other analyses, we used SPSS (Statistical Package for the Social Sciences (Nie et al. 1975)). Percentages and proportions were normalized through the arcsin transformation before Pearson correlations were computed (Zar 1974).

Results

Morphological analysis

Principal-component analysis (PCA) was applied to the three control populations so that the several distinguishing variables could be condensed into one or a few synthetic variables that express most of the overall difference between *Juniperus virginiana* and *J. horizontalis*. Because of the disparity in units of measure, variables were standardized (Z-transformation) (Zar 1974) and the PCA was based on the correlation matrix. Two separate analyses were performed. The first included all plants, both male and female, but did not include the female characters as variables; the second included all characters but was then restricted to only female plants. The statistics for the composite and female samples for the first three principal components are tabulated in Tables 2 and 3, respectively. The first principal component (PC1) accounts for 82.45% of the total variance in the composite sample and 82.28% in the female sample. Calculation of scores on PC1 for individual plants in the control samples showed a substantial separation of the species along this axis. PC1 scores are calculated by multiplying coefficients (values of the first eigenvector) by the value of the appropriate variable and then summing the products to give a "classification" score for an individual plant. PC1 scores for all the plants in the composite sample are plotted along the horizontal axis in Fig. 3 according to collecting site. Michigan *J. virginiana* is represented by sites 1 and 2 at the bottom of the figure, whereas site 56 at the top represents Mackinac City, MI, *J. horizontalis*. Site

TABLE 2. Coefficients for the composite principal-component analysis using the morphological data

Characters	Component		
	1	2	3
HABIT	0.442	0.134	0.019
COLOR	-0.422	-0.166	-0.382
BCHAR	0.401	0.189	-0.794
LTIP	0.439	0.112	-0.071
EWIDTH	0.420	0.096	0.464
HWRATIO	-0.311	0.947	0.050
Variance (eigenvalue)	4.95	0.581	0.268
Cumulative % total variance	82.45	92.12	96.59

TABLE 3. Coefficients for the principal-component analysis using the morphological data of female samples

Characters	Component		
	1	2	3
HABIT	0.338	0.268	0.027
COLOR	-0.338	-0.268	0.027
BCHAR	0.318	-0.362	0.234
LTIP	0.334	0.053	-0.105
EWIDTH	0.324	0.377	-0.163
CLENGTH	0.296	-0.604	0.0515
CWIDTH	0.320	0.078	0.108
SEEDS	0.307	-0.216	0.458
SPED	-0.339	-0.253	-0.061
HWRATIO	-0.237	0.314	0.824
Variance (eigenvalue)	8.238	0.718	0.664
% total variance	82.28	89.46	96.09

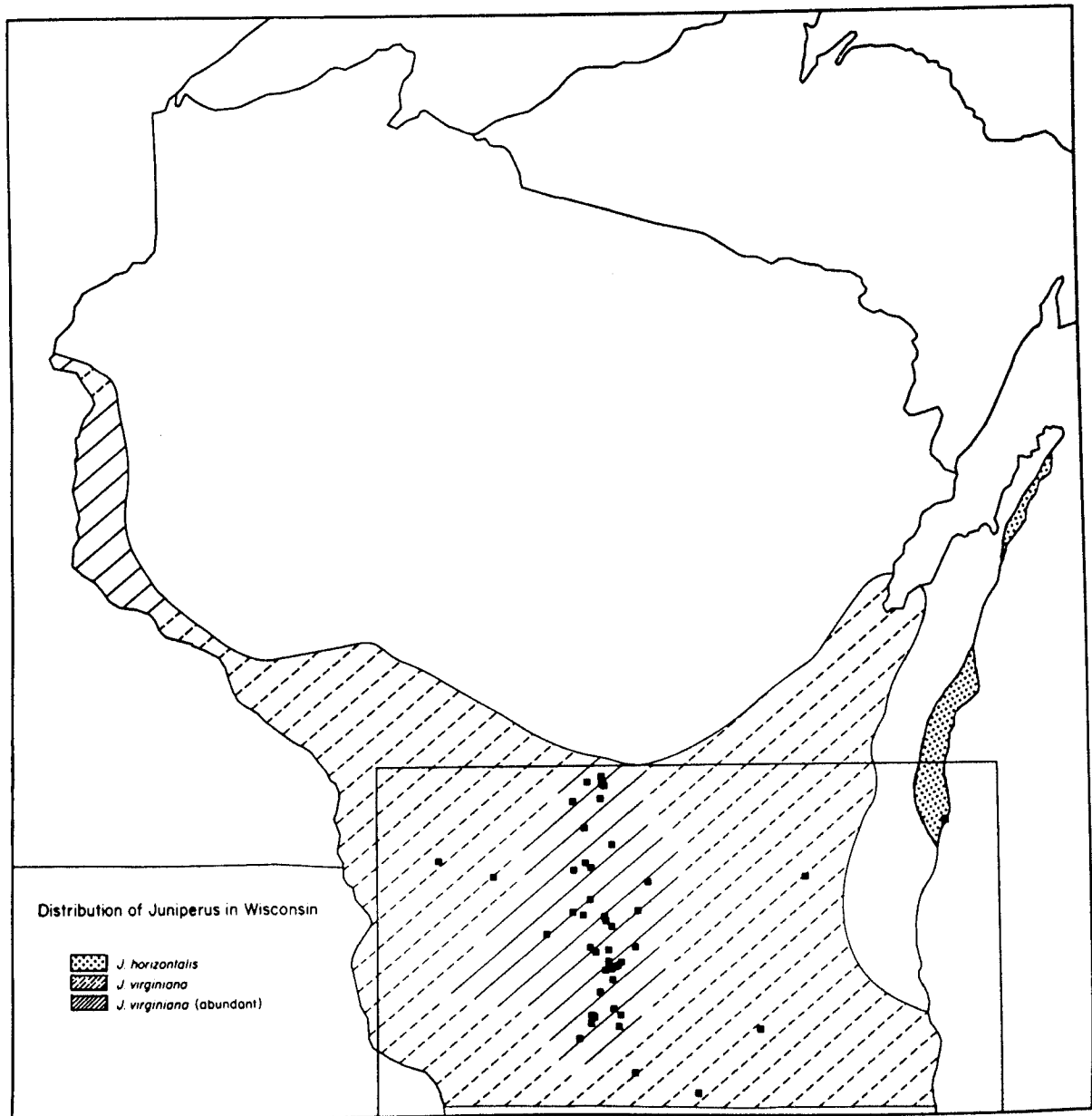


FIG. 1. The distribution of *J. virginiana* and *J. horizontalis* in Wisconsin. ■, locales sampled in this study. The map is based on Fassett (1930) and on maps being prepared for the proposed "Atlas of the Vascular Flora of Wisconsin," Herbarium of the University of Wisconsin, Madison, WI. The latter was furnished by T. Cochran, curator, Herbarium of the University of Wisconsin.

55 represents the Sheboygan, WI, population which has scores essentially identical with those of Michigan *J. horizontalis*. Although not illustrated here, similar results were obtained for the female sample.

Although morphological intermediacy is not itself conclusive evidence of hybridization, if the hypothesis is true that the peculiar populations along the Driftless boundary are indeed hybrids, then the first principal-

component scores for plants in these populations should be intermediate, generally, between those of the parental populations. Examination of Fig. 3 corroborates this prediction. The PC1 scores of these populations are represented by lines 4–55 in Fig. 3, where line number corresponds to site number. Many populations are represented by a variety of intermediate-scoring plants, but the means of other samples do not differ significantly

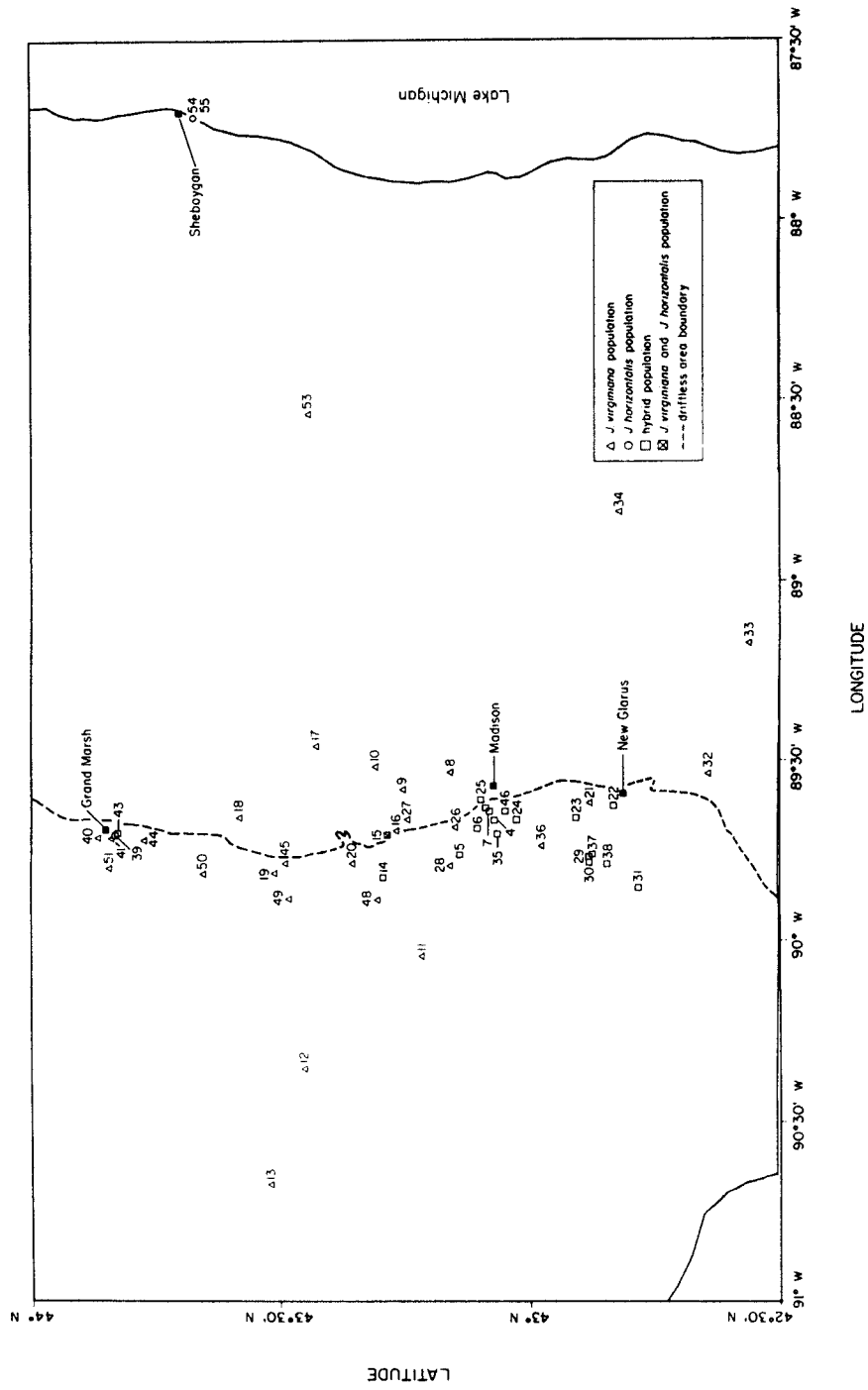


Fig. 2. Hybrid zone collection sites in Wisconsin. This map corresponds to the inset in Fig. 1. The Wisconsin-Iowa state line can be seen in the lower left-hand corner.

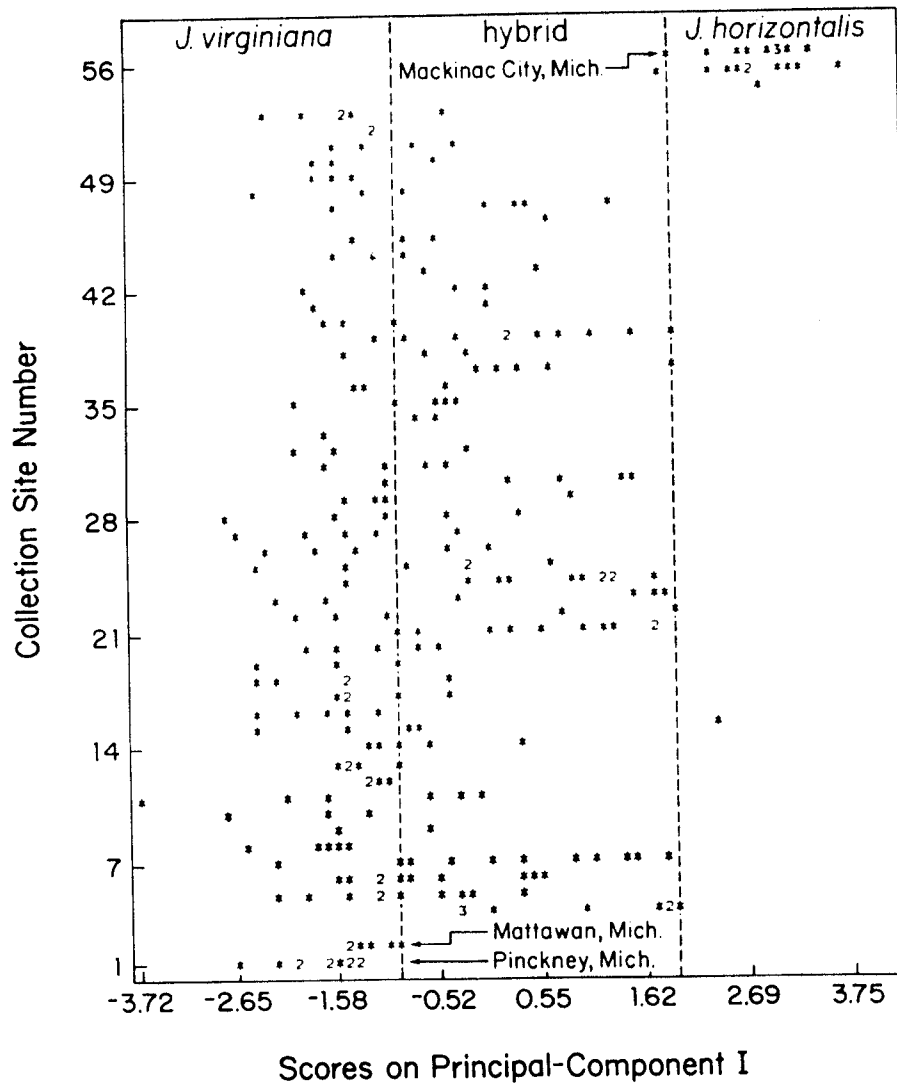


FIG. 3. Principal-component scores derived from morphological data of individual plants (x -axis) plotted against collection site number (y -axis). Collection site numbers 1 and 2 are *J. virginiana* populations in Michigan, while site 56 is a *J. horizontalis* population. Other populations represent Wisconsin localities. The broken vertical lines mark the extreme values in the "control" populations.

from the *J. virginiana* controls, and as mentioned above, the Sheboygan sample is indistinguishable from Michigan *J. horizontalis* ($P = 0.97$, Mann-Whitney U -test).

Figure 1 illustrates the overall distribution of *J. virginiana* and *J. horizontalis* in Wisconsin based on the preliminary study done by Fassett (1930). A more detailed distribution of the hybrid populations is illustrated in Fig. 2. Specific locales can be cross-indexed with either sites listed in the Appendix or Fig. 3 by collecting site number.

Individual populations were classified as *J. virginiana*, *J. horizontalis*, or containing hybrids by the

following rule. Ninety-five percent confidence intervals were constructed for the PC1 scores of the Michigan control populations. Wisconsin samples with means greater than the upper limit of *J. virginiana* and less than the lower limit of *J. horizontalis* were classified as containing hybrids. It is apparent in Fig. 2 that all of the hybrid populations occur within the Driftless Area; none is very distant from the Driftless boundary and most are in very close proximity. However, many populations along the Driftless boundary score as pure *J. virginiana* and, in fact, the distribution of hybridization would be described best as an "archipelago" of hybrid populations arrayed along this ancient glacial front.

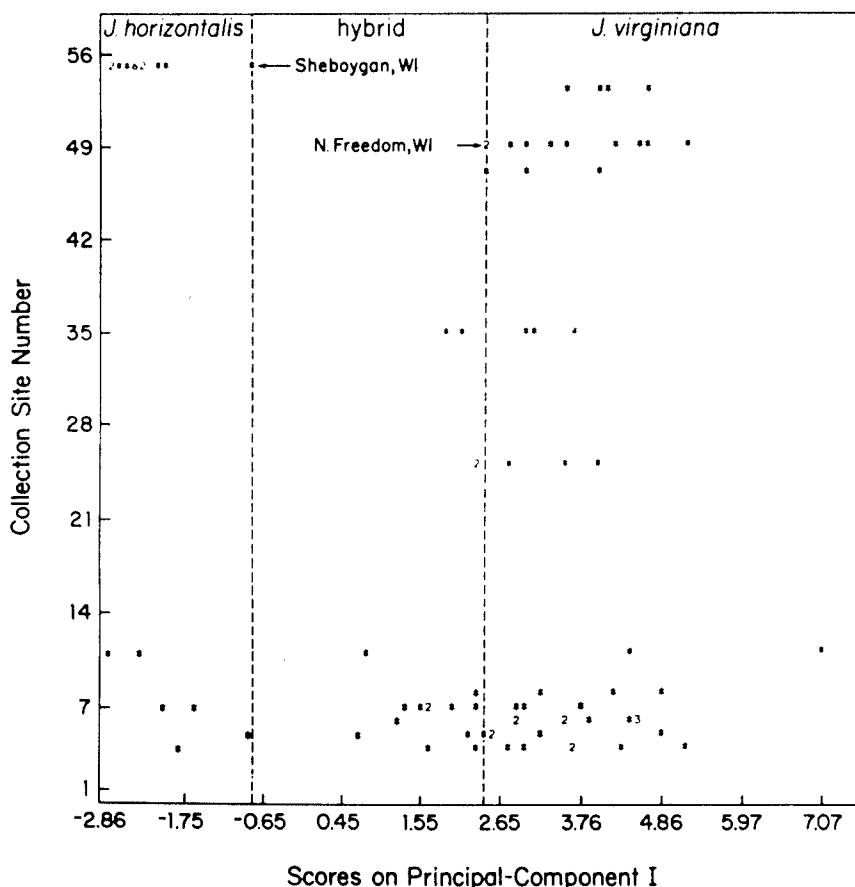


FIG. 4. Principal-component scores derived from terpene data of the individual plants (x -axis) plotted against the collection site number (y -axis). The populations sampled (in consecutive order from bottom to top) are 4, 5, 6, 7, 8, 11, 25, 35, 47, 49, 53, 55. The reference populations are North Freedom (site 49) and Sheboygan (site 55), for *J. virginiana* and *J. horizontalis*, respectively. The broken vertical lines mark the extreme values in the "reference" population.

In general, populations classified as hybrid contain a broad spectrum of morphotypes as indicated by the PC1 scores with little evidence of bimodality which would suggest sympatry without hybridization. A possible exception is Black Hawk's Lookout (site 15) where a single large male *J. horizontalis* occurs atop a stone outcropping amid an extensive population of junipers that are invariably like *J. virginiana* in growth habit. Several additional individuals scattered among the populations containing hybrids score as nearly pure *J. horizontalis*, but there are no populations of *J. horizontalis* near the Driftless Area devoid of hybrids (see Terpene analysis and Electrophoretic results, Figs. 4 and 5). Although this is not apparent in Fig. 1, juniper populations are extensive to the west of the hybrid populations but occur only as uncommon isolated trees and small stands to the east. The latter populations comprise trees which are like *J. virginiana* with regard to growth habits and the one population we studied in detail (site 53) was not significantly different from the *J.*

virginiana controls in PC1 scores, although one plant showed perhaps a hint of hybridity (Fig. 3, line 53). It is of very considerable interest with regard to gene flow that the hybrid populations are in direct contact with massive *J. virginiana* populations but are isolated by at least 160 km from the nearest pure *J. horizontalis* populations along the Lake Michigan dunes just south of Sheboygan.

An additional test of hybridity is based on the argument that if the character states used in the PCA are determined by genes that assort with some measure of independence, then the PC scores of hybrid populations should be more variable than those of parental populations. To test this prediction, the variances of all the Wisconsin populations, whether they contained hybrids or not, were compared with the pooled variance of the Michigan control populations (the variances of the Michigan samples were homoscedastic, $P \leq 0.42$, Bartlett's test for homogeneity of variances). The variances of 20 of 25 populations classified as contain-

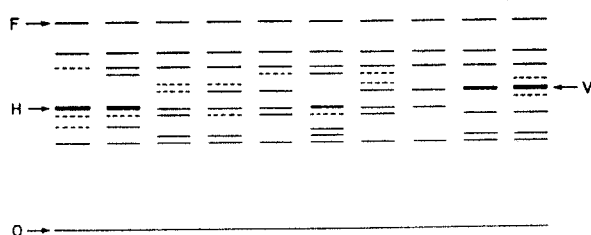


FIG. 5. Zymogram of the *Juniperus* foliage for peroxidases. The 9th and 10th lanes are *J. virginiana* samples from Pinckney (site 1) and North Freedom (site 49). The first two lanes are *J. horizontalis* samples from Mackinac City (site 56) and Sheboygan (site 55), respectively. The third to the eighth lanes are samples from Black Earth (site 5). The distinguishing fast and slow bands are denoted by V (virginiana) and H (horizontalis). O indicates the origin and F indicates the dye front.

ing hybrids were significantly greater than the control variance, whereas only 10 of 21 populations classified as parentals had greater variances than the controls. Furthermore, a chi-square test revealed a significantly higher frequency of significant variance ratio tests among populations classified as hybrid than among those classified as parentals ($0.02 \leq P \leq 0.05$). In other words, the populations containing putative hybrids are, indeed, more variable.

Terpene analysis

Initially, 104 terpene components identified by gas-liquid chromatography were compared between *Juniperus virginiana* and *J. horizontalis* controls (North Freedom, site 49, and Sheboygan, site 55, respectively) by analysis of variance (ANOVA). Since our objective was to synthesize the overall difference between the two taxa into one or a few latent variables, the less significant variables were eliminated stepwise leaving a subset of the 12 most distinctive terpene characters (Table 4). Actually, 34 of the 104 terpene components differed between the two species ($P \leq 0.05$). From several preliminary PC analyses, however, it became apparent that further reduction of this subset produced a more heuristic separation as the overall difference between the parental taxa was synthesized into a single principal component (PC1). The results of the final PCA are summarized in Table 5. Examination of Table 5 shows that the first principal component (PC1) accounts for 79.70% of the total variance and that the coefficients for the 12 variables are of similar magnitudes. A classification score based on PC1 was calculated for each plant in the manner described for the morphological analysis. PC1 used as a classification function readily distinguishes the "control" parental populations (*t*-test, $P \leq 0.001$, see Fig. 4), whereas significant separation of the parental taxa was not achieved along any other axis.

The results of the terpene PCA are summarized in Fig.

TABLE 4. Terpene oils that distinguish *J. virginiana* from *J. horizontalis*

Terpene oil	Range of oil content (%)	
	<i>J. virginiana</i>	<i>J. horizontalis</i>
Isosafrole (<i>cis/trans</i>) (ISFR)	22.8–29.5	1.41–9.72
Safrole (SFRL)	7.70–29.5	1.41–7.06
Methyl eugenol (MEUG)	4.79–7.67	1.00–2.00
Germacrene, isomer 3 (GRM3)	2.45–3.16	4.0–5.38
Calamanene (CLMN)	4.47–6.47	7.73–10.7
Elemol (ELOL)	27.3–31.6	1.41–6.77
Berm S2501, BP41, MW220 (BR12)	11.2–15.3	3.0–4.58
Cubenol (CBNL)	10.4–11.9	3.16–8.86
Gamma-eudesmol (GUDL)	16.6–18.8	1.41–2.65
Beta-eudesmol (BUDL)	12.3–14.6	1.41–2.00
Isomer of ACII, virg 2411 S2628 (IAC2)	4.12–4.9	1.41–3.00
Acetate II of EVR (ACII)	8.6–23.4	1.41–3.46

TABLE 5. Coefficients for the principal-component analysis using terpene data

Terpene oils	Component		
	1	2	3
ISFR	0.309	0.129	-0.034
SFRL	0.265	-0.493	0.290
MEUG	0.289	-0.295	0.392
GRM3	-0.253	0.474	0.304
CLMN	-0.283	0.298	0.191
ELOL	0.279	0.130	-0.477
BR12	0.259	0.361	0.581
CBNL	0.306	0.124	0.092
GUDL	0.307	0.180	-0.088
BUDL	0.307	0.143	-0.076
IAC2	0.281	0.345	-0.206
ACII	0.315	0.049	0.044
Variance (eigenvalue)	9.564	0.858	0.504
Cumulative % total variance	79.70	86.85	91.08

4. Again, population numbers are arrayed on the ordinate, and the abscissa represents PC1 scores. Population 55 at the top of the figure represents "pure" *J. horizontalis* and population 49, third from the top, represents "pure" *J. virginiana*. As with the morphological analysis, marked separation along PC1 is apparent, and several populations near the Driftless boundary contain trees of intermediate terpene composition (particularly sites 4, Pine Bluff; 5, Black Earth; 6, Cross Plains 1; 7, Pine Hill Road Farm). Furthermore, the overall distribution of terpene PC1 scores is skewed

towards *J. virginiana*, which was also the case with the morphological scores. This is not surprising since the hybrid populations are in direct contact with pure *J. virginiana* populations but not with pure *J. horizontalis*. Perhaps the oddest plants, in terms of morphological and terpene phenotypes, are two specimens at Spring Green (site 11) which, based on terpene profiles, would be classified as *J. horizontalis* but morphologically are nearly perfectly intermediate. These plants have the *J. virginiana* growth habit, although they appear broadly conical with wide-spreading branches characteristic of the western and southern forms of *J. virginiana*.

Although samples were too limited to allow comparison of the variances for terpene PC1 scores in terms of hybrid versus parental, the ranges of PC1 scores of intermediate populations are considerably greater than those of the parentals, suggesting again that the peculiar populations along the Driftless boundary are of hybrid origin. This hypothesis can be tested in one additional way at this juncture; viz., there should be a correlation between morphological and terpene PC1 scores. That is, putative hybrids, which presumably are an admixture of both morphological and terpene determining genes, should have intermediate scores by both criteria. The correlation between morphological and terpene PC1 scores is ($r = -0.399$, $P = (0.01 \leq P \leq 0.05)$; $n = 61$), which is consistent with the prediction.

The terpene data were subjected to canonical variate analysis, and Wells distances (Wells 1980) were also calculated. These results were consistent with the PCA analysis, again strongly suggesting hybridization.

Electrophoretic results

Although genetic analysis of multiband peroxidase patterns can be complex (Van Huystee and Cairns 1981), we have found a peroxidase electromorph which, in our preliminary electrophoretic work, appears to be diagnostic for the two species. As shown in Fig. 5, eight peroxidase bands usually can be resolved in either species. Although some peroxidase electromorphs are polymorphic within both species, a single unique band consistently distinguishes the two species. With regard to this particular peroxidase, *Juniperus virginiana* is characterized by a fast mobility band, whereas that of *J. horizontalis* is relatively slow. Specimens from North Freedom (site 49) and Sheboygan, WI (site 55), were indistinguishable from Michigan *J. virginiana* (sites 1 and 2) and *J. horizontalis* (site 56), respectively (Fig. 5). Plants classified as hybrids on morphological and terpene criteria were electrophoresed along with pure *J. virginiana* and *J. horizontalis* extracts from Michigan. Most of the hybrids showed both bands and each band stained less intensely than in the parental species. We interpret this pattern to be that of a heterozygote as the additivity and apparent gene dosage effect certainly

suggest that explanation. Several hybrids were "homozygous" for one allele or the other. Thus, the electrophoretic results are consistent with a hypothesis of hybridization beyond the F_1 stage.

Discussion

Genetic implications

Multivariate analyses of both the morphological and terpene data from plants in the Driftless Area indicate that hybrids do indeed exist (Figs. 3 and 4). However, the intermediate morphological scores alone do not necessarily prove hybridization. As pointed out earlier, alleged introgressive hybridization between *Juniperus ashei* and *J. virginiana* (Hall 1952) was later refuted using terpene analysis (Von Rudloff et al. 1967; Adams and Turner 1970). In contrast with the former situation, earlier studies using morphological (Fassett 1945b), karyological (Ross and Duncan 1949), and our own morphological, terpene, and electrophoretic data strongly support the existence of hybridization between *J. virginiana* and *J. horizontalis*.

The terpene profiles of *J. virginiana* and *J. horizontalis* are quite distinct (refer to Table 4). The elemol-eudesmol types of sesquiterpenes and the aromatic ethers of the safrole isomers are particularly diagnostic, being present in large percentages in *J. virginiana* but only in trace amounts in *J. horizontalis*. The degree of hybridization was estimated by examining the terpene profile. Cases of both high intermediacy and combinations of various parental compounds were found, suggesting hybridization beyond the F_1 . This is consistent with the conclusions drawn from morphological and electrophoretic data.

The correlations among characters within either the morphological or terpene data sets are stronger than those between sets. Some of the correlations within the morphological data are probably due to pleiotropic effects as well as to linkage. In a similar fashion, no doubt some of the terpenes share common precursors and biosynthetic pathways. The elemol-eudesmol isomers may be derived from *trans*-farnesol via the same hypothetical intermediate (Von Rudloff 1975b). In a similar fashion, *p*-hydroxyphenylalanine is a common precursor to the phenylpropanoid ethers, methyl eugenol, safrole, eugenol, and estragole (Von Rudloff 1975a). While a significant correlation exists between principal-component scores derived from the morphological and terpene data, correlations of individual characters between the data sets are not particularly high, suggesting a large degree of independence.

Our electrophoretic data strongly support the existence of hybrids. In fact a plant that scored as a morphological parental in the Driftless Area may score as an electromorph heterozygote. Except for one *J. horizontalis* discussed below, all plants scoring as "*J.*

horizontalis" in the Driftless Area thus far examined are heterozygotes.

The biogeography of hybridization

The morphological, terpene, and electrophoretic analyses individually and in concert corroborate Fassett's (1945*b*) inference that the peculiar junipers occurring just inside the eastern boundary of the Driftless Area in south central Wisconsin are hybrids between *Juniperus virginiana* and the *J. horizontalis*. The distribution of hybridization could be described aptly as an "archipelago" of hybrid populations trailing along the Driftless boundary. Hybrid populations are small and localized but in most instances are in direct contact with massive *J. virginiana* populations to the west in the Driftless Area. In many cases the hybrid "islands" are surrounded by *J. virginiana* populations, but the latter show little sign of introgression. The southernmost hybrid "island" that we know of occurs at Blanchardville (31; Lafayette Co.) and the northernmost "island" occurs at Grand Marsh (39–44; Adams Co.). The most extensive hybrid populations occur approximately 7 mi (1 mi = 1.609 344 km) west of Madison in the vicinity of Pine Bluff, Cross Plains 1, Pine Hill Road Farm, Riley, and Cross Plains 2 (sites 4, 6, 7, 24, 25, respectively). There probably are undiscovered hybrid populations, but the collecting efforts of Fassett, other University of Wisconsin botanists, and ourselves make it doubtful that many undiscovered populations exist and, therefore, Fig. 2 is believed to be a nearly complete map of the hybrid zone.

Curtis (1959) noted that the cedar glades in Wisconsin are found on two very special sites in the prairie-forest province, steep hillsides of thin loess over either limestone or quartzite bedrock or glacial moraines. The hybrids seem to be most prevalent in very sandy soils on hilltops comprising friable sandstone. Cedar populations occur east of the Driftless Area, but this is not good habitat and populations are thin, small, and scattered. The several populations we sampled between the Driftless boundary and the Lake Michigan shoreline scored as pure *J. virginiana*.

Fassett (1945*b*) reported that pure *J. virginiana* and *J. horizontalis* were sympatric at Grand Marsh without hybrids and suggested that the two taxa were reproductively isolated there. However, our morphological analysis clearly indicates the existence of hybrids at what we believe to be exactly the same locale (see site 39, Fig. 2). In addition, electrophoretic analysis reveals that these plants are heterozygotes. In fact, with one possible exception, we found no populations where the two parental species are sympatric without hybridization. The possible exception is Black Hawk's Lookout (site 15) where a single, large *J. horizontalis* (based on morphological and electrophoretic criteria) male grows atop a high stone outcropping. The extensive surround-

ing population, however, appears to be pure *J. virginiana*. At this juncture, we do not know whether the surrounding *J. virginiana* are reproductively isolated or whether the male is infertile. This male was heavy with male cones in August 1980.

Another misconception regarding these hybrids is that the genetic differences between *J. virginiana* and *J. horizontalis* are slight. Curtis (1959) considered, as one hypothesis, the possibility that the creeping juniper was no more than a "cold-induced mutation" of the red cedar which affected only the growth habit. This obviously is not true since, in addition to the several morphological differences scored, the parental taxa are significantly different with regard to relative composition of 34 volatile foliage oils, at least one electromorph, and karyologically (Ross and Duncan 1949). Although the genetic distance between *J. virginiana* and *J. horizontalis* cannot be calculated at this juncture, it appears to be substantial with divergence perhaps dating from early Pleistocene or earlier.

The most striking feature of the hybrid zone is its one-sided nature; i.e., the hybrid populations are in direct contact with massive *J. virginiana* populations but are isolated by a distance of at least 160 km from the nearest *J. horizontalis* populations along the Lake Michigan dunes at Sheboygan, WI (site 55). This aspect of the hybrid zone has implications in terms of explaining its persistence. Plausible explanations of stable one-sided hybrid zones are limited to the possibilities that the hybrids are actually more fit than the parentals in the local environments where they occur or that the hybrids are maladapted everywhere, but *J. horizontalis* is more fit in the hybrid zone than *J. virginiana*. In the latter case continued gene flow from *J. virginiana* prevents fixation of the *J. horizontalis* genotypes (Nagylaki 1975). Since junipers are long-lived, it is also possible that the hybrid zone is not stable and that what we now see is a dilute residue of *J. horizontalis* germ plasm that will be eroded altogether as more generations accrue. However, post-Pleistocene vegetational history would seem to argue against this hypothesis.

Analyses of fossil pollens show that the Driftless Area was dominated by fir, spruce, and pine at the end of the Wisconsin glacial stage if not throughout that stage (Hansen 1939). Although *J. horizontalis* is very localized in distribution, being abundant on the sand dunes and bluffs of the northern Great Lakes, its range is generally within the northern forests characterized by these same species. Therefore, it is likely that *J. horizontalis* was at least locally abundant in suitable habitats in the Driftless Area during the Wisconsin stage, probably quite close to the glacial front as boreal forest occurred in proximity to the glaciers (West 1961). Extending this reasoning, it is probable that *J. horizontalis* was abundant on the high, friable sandstone bluffs

that now support hybrid populations. This would have been the circumstance some 14 000–15 000 years ago towards the end of the Cary advance. Retreat of the Wisconsin glaciers was apparently rather rapid as was vegetational succession in periglacial areas and areas vacated by the ice sheets (West 1961; Frye et al. 1965; Wright 1970). The recession of the Cary ice sheet was followed by two minor readvances, the Mankato and Valdres substages, but these did not approach the Driftless Area. Presumably *J. virginiana* rapidly colonized the Driftless Area from the south as the Cary ice sheet withdrew and, indeed, it is not certain that it was ever absent. In any case hybridization apparently ensued and the Driftless boundary populations took on the highly introgressed character they show today. With regard to *J. horizontalis* populations, since the region between the Driftless Area and Lake Michigan is not generally good juniper habitat, it is likely that this species rapidly retreated to the Lake Michigan shoreline where permanent populations were established. The important point is that the hybrid populations probably have not been in contact with pure *J. horizontalis* for 14 000 or more years and yet there is an abundant residue of *J. horizontalis* genes in these populations.

Although we have not made an extensive effort to determine the age structure of populations, we have cored a number of *J. virginiana* and hybrid specimens and taken cross sections from some few dead creeping specimens at Grand Marsh (site 39) and Riley site (24) (*J. horizontalis* is difficult to core). Large, presumably old, hybrids had from 31 to 47 growth rings, whereas a large main stem of a completely prostrate form at Grand Marsh had 73 growth rings, and a smaller prostrate main stem at Riley had 50 growth rings. The question here is it is possible that the hybrids are selected against, but some relict creeping junipers have sustained themselves clonally since the end of the Pleistocene, and these relicts are the continuing source of "pure" *J. horizontalis* germ plasm for renewed hybridization? The answer is probably no because even if the creeping forms reproduce asexually, clones would have to survive, conservatively, 140–187 generations of competition with *J. virginiana* and hybrids. (Assuming an average generation of 75–100 years over 14 000 years.) In fact, plants which scored morphologically as *J. horizontalis* are electrophoretically heterozygotes except for one case, that individual at Black Hawk's Lookout.

Taken in sum, then, the historical biogeography of hybridization between *J. virginiana* and *J. horizontalis* suggests that either *J. horizontalis* or the hybrids or both are favored by selection in certain locales in south central Wisconsin, although the particular ecological conditions that favor the latter are unclear. One possibility is that the high, somewhat exposed hills composed of friable sandstone with very sandy soils approximate the

dune habitats preferred by *J. horizontalis*, but the inland and somewhat southerly locale conjoin with other factors to make these environments intermediate between those favored by the parental taxa. Pleistocene glaciation, per chance, created the opportunity to meld the gene pools of ecologically distinct species, and the peculiar geological ecology of the Driftless boundary selected out the array of locally adapted genotypes seen today.

Acknowledgements

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- ADAMS, R. P. 1975. Gene flow versus selection pressure and ancestral differentiation in the composition of species; analysis of population variation of *Juniperus ashei* Buch. using terpenoid data. *J. Mol. Evol.* **5**: 177–185.
- 1977. Chemosystematics-analysis of populational differentiation and variability of ancestral and recent populations of *Juniperus ashei*. *Ann. Mo. Bot. Gard.* **64**: 184–209.
- 1983. Intraspecific terpenoid variation in *Juniperus scopulorum*: evidence for Pleistocene refugia and recolonization in western North America. *Taxon*, **32**: 30–46.
- ADAMS, R. P., M. GRANAT, L. HAGGE, and E. VON RUDLOFF. 1970. Identification of lower terpenoids from gas-chromatography—mass spectral data by on-line computer method. *J. Chromatogr. Sci.* **17**: 75–81.
- ADAMS, R. P., M. M. PALMA, and W. S. MOORE. 1981. Volatile oils of mature and juvenile leaves of *Juniperus horizontalis*: chemosystematic significance. *Phytochemistry*, **20**: 2501–2502.
- ADAMS, R. P., and B. L. TURNER. 1970. Chemosystematic and numerical studies of natural populations of *Juniperus ashei* Buch. *Taxon*, **19**: 728–751.
- BLACKRITH, R. E., and R. A. REYMENT. 1971. *Multivariable morphometrics*. Academic Press, New York.
- COMER, C. W., R. P. ADAMS, and D. F. VAN HAVERBEKE. 1982. Intra- and interspecific variation of *Juniperus virginiana* and *J. scopulorum* seedlings based on volatile oil composition. *Biochem. Syst. Ecol.* **10**: 297–306.
- COOLEY, W. W., and R. P. LOHNES. 1971. *Multivariable data analysis*. John Wiley and Sons, New York.
- CURTIS, J. 1959. The vegetation of Wisconsin. An ordination of plant communities. The University of Wisconsin Press, Madison, WI. p. 657.
- FASSETT, N. C. 1930. Preliminary reports on the flora of Wisconsin. V. Coniferales. *Trans. Wis. Acad. Sci. Arts Lett.* **25**: 177–182.
- 1944a. *Juniperus virginiana*, *J. horizontalis*, and *J.*

- scopulorum*. II. Hybrid swarms of *J. virginiana* and *J. scopulorum*. Bull. Torrey Bot. Club, **71**: 475-483.
- 1944b. *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum*. I. The specific characters. Bull. Torrey Bot. Club, **71**: 410-418.
- 1944c. *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum*. V. Taxonomic treatment. Bull. Torrey Bot. Club, **72**: 480-482.
- 1945a. *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum*. III. Possible hybridization of *J. horizontalis* and *J. scopulorum*. Bull. Torrey Bot. Club, **72**: 42-46.
- 1945b. *Juniperus virginiana*, *J. horizontalis*, and *J. scopulorum*. IV. Hybrid swarms of *J. virginiana* and *J. horizontalis*. Bull. Torrey Bot. Club, **72**: 379-384.
- FLAKE, R. H., L. URBATSCH, and B. L. TURNER. 1978. Chemical documentation of allopatric introgression in *Juniperus*. Syst. Bot. **3**: 129-144.
- FLAKE, R. H., E. VON RUDLOFF, and B. L. TURNER. 1973. Confirmation of clinal pattern of chemical differentiation in *Juniperus virginiana* from terpenoid data obtained in successive years. Terpenoids: structure, biogenesis, and distribution. Rec. Adv. Phytochem. **6**: 215-228.
- FRYE, J. C., H. B. WILLMAN, and R. F. BLACK. 1965. Outline of glacial geology of Illinois and Wisconsin. In *Quaternary of the United States*. Edited by H. E. Wright and D. G. Frye. Princeton University Press, Princeton, NJ.
- HALL, M. T. 1952. Variation and hybridization in *Juniperus*. Ann. Mo. Bot. Gard. **39**: 1-64.
- 1955. Comparison of juniper populations on an Ozark glade and old fields. Ann. Mo. Bot. Gard. **42**: 171-194.
- HALL, M. T., J. F. McCORMICK, and G. G. FOGG. 1962. Hybridization between *J. ashei* and *J. pinchotii* in south-west Texas. Butler Univ. Bot. Stud. **14**: 9-28.
- HANSEN, H. P. 1939. Post glacial vegetation of the Driftless Area of Wisconsin. Am. Midl. Nat. **21**: 752.
- KUPPERS, U., and M. WEIDNER. 1980. Seasonal variation of enzyme activities in *Laminaria hyperborea*. Planta, **148**: 222-230.
- NAGYLAKI, T. 1975. Conditions for the existence of clines. Genetics, **80**: 595-615.
- NIE, N. H., C. H. HULL, J. G. JENKINS, K. KISTEINBRENNER, and D. H. BENT. 1975. Statistical package for the social sciences. McGraw-Hill Publications, New York.
- PIMENTEL, R. A. 1979. Morphometrics. Kendall-Hunt, Dubuque, IA.
- ROSS, J. G., and R. E. DUNCAN. 1949. Cytological evidences of hybridization between *J. virginiana* and *J. horizontalis*. Bull. Torrey Bot. Club, **76**: 414-429.
- SCHURTZ, R. H. 1971. A taxonomic analysis of a triparental hybrid swarm in *Juniperus* L. Ph.D. thesis, University of Nebraska, Lincoln, NE.
- SYMEONIDIS, L., S. KARATAGLIS, and I. TSEKOS. 1979. Electrophoretic variation in esterases and peroxidases of Greek diploid *Aegilops* species (*Ae. caudata* and *Ae. comosa*, Poaceae). Plant Syst. Evol. **131**: 1-15.
- VAN HAVERBEKE, D. F. 1968. A population analysis of *Juniperus* in the Missouri River Basin. University of Nebraska Studies No. 38.
- VAN HUYSTEE, R. B., and W. L. CAIRNS. 1980. Appraisal of studies on induction of peroxidase and associated porphyrin metabolism. Bot. Rev. **46**: 429-446.
- VON RUDLOFF, E. 1975a. Volatile oil analysis in chemosystematic studies of North American conifers. Biochem. Syst. Ecol. **2**: 131-167.
- 1975b. Chemosystematic studies of the volatile oils of *Juniperus horizontalis*, *J. scopulorum*, and *J. virginiana*. Phytochemistry, **14**: 1319-1329.
- VON RUDLOFF, E., R. IRVING, and B. L. TURNER. 1967. Reevaluation of allopatric introgression between *Juniperus ashei* and *Juniperus virginiana* using gas chromatography. Am. J. Bot. **54**: 660.
- WELLS, H. 1980. A distance coefficient as a hybridization index: an example using *Mimulus longiflorus* and *Mimulus flemingii* (Scrophulariaceae) from Sta. Cruz Island, Calif. Taxon, **29**: 53-65.
- WEST, R. G. 1961. Late and post-glacial vegetational history in Wisconsin, particularly changes associated with the Valdres readvance. Am. J. Sci. **259**: 766-783.
- WRIGHT, H. E., JR. 1970. Vegetational history of the Central Plains. In *Pleistocene and recent environments of the Central Plains*. Edited by W. Dort and J. K. Jones. University of Kansas Press, Lawrence, KS.
- ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ.

Appendix: collecting sites

Collection site no.	Collection site name	County, state	Longitude × latitude, section and R and T nos.
1	Pinckney	Livingston, MI	83°56'39" × 42°27'36" Sect. 21, R4E T1N
2	Mattawan	Van Buren, MI	85°47'11" × 42°13'20" Sect. 12, R13W T35
4	Pine Bluff 2	Dane, WI	89°39'55" × 43°4'7" Sect. 21, R7E T7N
5	Black Earth	Dane, WI	89°45'29" × 43°8'24" Sect. 23, R6E T8N
6	Cross Plains I	Dane, WI	89°41'8" × 43°6'10.4" Sect. 5, R7E T7N
7	Pine Hill Road Farm	Dane, WI	89°38'27" × 43°4'37" Sect. 15, R7E T7N
8	Springfield Corner	Dane, WI	89°31'48" × 43°9'33.4" Sect. 15, R8E T8N

Appendix: (continued)

Collection site no.	Collection site name	County, state	Longitude × latitude, section and R and T nos.
9	Viaduct Road	Dane, WI	89°30' × 43°14'37" Sect. 14, R8E T9N
10	Lodi	Columbia, WI	89°30'47" × 43°18'29" Sect. 26, R8E T10N
11	Spring Green	Sauk, WI	90°2'36" × 43°12'55" Sect. 36, R4E T8N
12	Rockbridge	Richland, WI	90°21' × 43°27'4" Sect. 16, R1E T11N
13	Viola	Richland, WI	90°40'8" × 43°3'9" Sect. 18, R2W T12N
14	Prairie du Sac	Sauk, WI	89°49'29" × 43°17'35" Sect. 31, R6E T10N
15	Black Hawk's Lookout	Columbia, WI	89°42'16" × 43°17'5" Sect. 31, R7E T10N
16	Junction 60	Columbia, WI	89°41'44" × 43°15'59" Sect. 5, R7E T9N
17	Poynette	Columbia, WI	89°27'9.7" × 43°25'34" Sect. 20, R9E T11N
18	Lewiston	Columbia, WI	89°39'19" × 43°34'57" Sect. 27, R7E T13N
19	Baraboo	Sauk, WI	89°48'46" × 43°30'30" Sect. 20–20, Sect. 17–16, R6E T12N
20	R. Peetz Farm	Sauk, WI	89°47'7.4" × 43°21'22" Sect. 9, R6E T10N
21	Belleville	Dane, WI	89°36'39" × 42°52'45" Sect. 36, R7E T5N
22	New Glarus	Green, WI	89°37'21" × 42°49'54" Sect. 13–14, R7E T4N
23	County Road U	Dane, WI	89°39'11" × 42°54'19" Sect. 22, R7E T5N
24	Riley	Dane, WI	89°39'40" × 48°1'29" Sect. 3, R7E T6N
25	Cross Plains 2	Dane, WI	80°36'32" × 43°5'46" Sect. 12, R7E T7N
26	St. John	Dane, WI	89°40'53" × 43°8'54" Sect. 20, R7E T8N
27	Roxbury	Dane, WI	89°39'40" × 43°14'37" Sect. 16, R7E T9N
28	Mazomanie	Dane, WI	89°47'25" × 43°9'33.4" Sect. 10, R6E T8N
29	Daleyville	Dane, WI	89°46'19" × 42°52'45" Sect. 34, R6E T5N
30	Lee Valley	Dane, WI	89°47'10" × 42°52'44" Sect. 32, R6E T5N
31	Blanchardville	Lafayette, WI	89°51'10" × 42°47'1.3" Sect. 25–26, Sect. 35–36, R5E T4N
32	Monroe	Green, WI	89°32'3" × 42°38'31" Sect. 16–15, R8E T2N
33	Beloit	Rock, WI	89°10'4" × 42°33'35" Sect. 11, R11E T1N
34	Lima	Rock, WI	88°48'20" × 42°49'13" Sect. 11, R14E T4N
35	Mineral Point	Dane, WI	89°42'5.7" × 43°3'47" Sect. 19, R7E T7N
36	Mt. Horeb	Dane, WI	89°44'2" × 42°58'31" Sect. 25, R6E T6N
37	York Valley Road 1	Dane, WI	89°45'57" × 42°52'35" Sect. 34, R6E T5N
38	York Valley Road 2	Dane, WI	89°47'3" × 42°50'43" Sect. 4, R6E T4N
39	Grand Marsh 1	Adams, WI	89°42'16" × 43°49'51" Sect. 31, R7E T16N
40	Grand Marsh 2	Adams, WI	89°42'43" × 43°51'50" Sect. 6, R7E T16N
41	Grand Marsh 3	Adams, WI	89°42'43" × 43°50'1.6" Sect. 30, R7E T16N
42	Grand Marsh 4	Adams, WI	89°45' × 43°50'1.5" Sect. 26, R6E T16N
43	Grand Marsh 5	Adams, WI	89°41'48" × 43°49'32" Sect. 31–32, R7E T16N
44	Grand Marsh 6	Adams, WI	89°43'10" × 43°46'13" Sect. 24, R6E T15N
45	West Baraboo	Sauk, WI	89°46'53" × 43°29'13" Sect. 29, R6E T12N
46	Pine Hill Road Farm 2	Dane, WI	89°38'3" × 43°4'22" Sect. 23, R7E T7N

Appendix (concluded)

Collection site no.	Collection site name	County, state	Longitude × latitude, section and R and T nos.
47	Birch Trail	Dane, WI	89°37'30" × 43°4'56.6" Sect. 14, R7E T7N
48	Witwen	Sauk, WI	89°53'1.6" × 43°18'16" Sect. 34, R5E T10N
49	North Freedom	Sauk, WI	89°52'47" × 43°28'42" Sect. 35, R5E T12N
50	Kildare	Juneau, WI	89°52'50" × 43°45'39" Sect. 26, R5E T15N
51	Easton	Adams, WI	89°47'43" × 43°50'30" Sect. 30-29, R5E T15N
52	I90-I94	Juneau, WI	89°48'48" × 43°39'8" Sect. 31, R6E T14N
53	Mayville	Dodge, WI	88°32'12" × 43°26'34" Sect. 1, R16E T11N
54	Cedar Grove	Sheboygan, WI	87°51' × 43°33' Sect. 30, R23E T13N
55	Sheboygan	Sheboygan, WI	87°42'44" × 43°40'14" Sect. 23, R23E T14N
56	Mackinac	Emmet, MI	84°47'16" × 45°45'36" Sect. 24, R4W T39N