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Growth performance of hybrid families by crossing selfed lines of *Betula pendula* Roth

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Abstract Hybrid breeding is an effective approach in many agricultural crops. In allogamous tree species severe inbreeding depression and long reproductive cycles generally prohibit its use. However, three generations of selfing in silver birch (*Betula pendula* Roth) were obtained by forcing trees to flowering under greenhouse conditions. Hybrids were produced by crossing first-, second- and third-generation selfed lines. The effects of different levels of parental inbreeding on the growth performance of hybrid families were observed in a 9-year-old field progeny test. Also, provenance crosses were carried out between selfed lines from different parts of Finland and several other European countries. Observations of growth performance of the provenance hybrids were made in the same trial. The results indicated that the mean stem volumes were significantly different between classes of parental inbreeding coefficients (F_p) ($P<0.0001$), and were positively correlated with F_p ($r=0.9106$, $P<0.05$). Within-family variation of the hybrid families decreased with an increase of F_p. The performance of the provenance crosses between parents at a relatively close distance did not depart significantly from the standard controls. However, when the cross distance was extended far to the south, hybrids grew faster, indicating either higher heterozygosity or an extended growth period.

Key words *Betula pendula* · Selfing · Inbreeding · Provenance hybrid · Heterosis · Stem volume

Introduction

Hybridization between selfed lines to produce high-yielding varieties is a very useful approach in many crop plants, notably in maize. The possibility of using inbred material in forest-tree improvement has been suggested by some forest geneticists (Andersson et al. 1974; Lindgren 1975; Rudolph 1981). In allogamous tree species severe inbreeding depression and long reproductive cycles make it difficult to obtain selfed individuals. However, in silver birch (*Betula pendula* Roth) three generations of selfing were obtained by forcing trees to flowering under greenhouse conditions. This special technique has been developed at the Foundation for Forest Tree Breeding in Finland (Kärki 1977). Under optimal conditions the generation interval can be cut down to 8 months so that the "hybrid maize" breeding technique can be applied to trees. Hybrids were produced with selfed lines after one, two and three generations of selfing. This unique material is suitable for in-depth studies on the effect of parental inbreeding and heterosis in a cross-pollinating tree species. Thus, the efficiency of crossing selfed lines to obtain heterosis in forest-tree breeding programs can be evaluated. Provenance crosses were made with selfed lines originating in different parts of Finland and some other European countries to determine whether such crosses would further improve the performance of the offspring.

Inbreeding depression of selfs has been studied in several forest trees including, for example, *Pinus taeda* (Franklin 1968), *Pinus attenuata* (Conkle 1977), *Sequoia sempervirens* (Libby et al. 1981), *Pinus radiata* (Wilcox 1983) and *Eucalyptus regnans* (Griffin and Cotterill 1988). However, studies of hybridization between selfed or inbred lines are scarce. A 4-year-old progeny test of S_1 trees pollinated with a pollen mixture from S_1 individuals indicated a relief from inbreeding depression in *Pinus banksiana* Lamb. (Rudolph 1981). Comparisons in several traits of seeds and small seedlings were made between hybrid families by crossing first-generation selfs ($S_1 \times S_1$) and outcrosses of their S_0 parents in *P. taeda* (Sniezko

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1984). No previous experimental evidence has been available on the use of inbred materials in population improvement.

Provenance hybridization has been employed in forest genetics to capture heterosis, change growth rhythm and/or to combine desirable characteristics. However, mixed results have been obtained from past efforts. Height growth in interprovenance hybrids of Norway spruce (*Picea abies* Karst) and of Scots pine (*Pinus silvestris* L.) expressed slight positive heterosis (Nilsson 1973; Ekberg et al. 1982; Park and Gerhold 1986). Interprovenance hybrids of red pine (*Pinus resinosa* Arr.) (Holst and Fowler 1973) and Jack pine (*Pinus banksiana* Lamb.) (Magnussen and Yeatman 1988) were found to be intermediate to parental provenances. Wide crosses of loblolly pine (*P. taeda* L.) lacked hybrid superiority on a good site, but expressed significant heterosis on poor sites (Woessner 1972). Heterosis in the above examples was defined in the traditional sense of performance exceeding the mid-parent value. Attempts to combine parental characteristics have been more successful. Crosses have been made in Norway spruce to combine the vigor of a central European provenance with the winter-hardiness of a Swedish source (Nilsson 1973). Some hybrids produced by crossing Coastal Douglas-fir (*Pseudotsuga menziesii var. menziesii*) with the inland variety (*Pseudotsuga menziesii var. glauca*) have the vigor of the coastal variety combined with the winter-hardiness of the inland variety (Rehfeldt 1986). Such cases are not examples of heterosis, but rather of tailoring phenology for maximum yield.

Silver birch has a wide natural distribution and is thus a good candidate for provenance hybridization. However, long-distance hybrids between and within central and southern Finland do not seem to lead to hybrid vigor (Raulo and Koski 1977). In the present study the cross distance was extended to cover southern and northern Finland as well as other European origins from Estonia, Latvia, Germany and Austria (see Fig. 1).

Materials and methods

A 9-year-old progeny test of crosses between selfed individuals of silver birch, established by the Foundation for Forest Tree Breeding (experiment No. 1253), was the object of the present study. The trial was located in Loppi (latitude 60°42′, longitude 24°20′ and altitude 150 m) in southern Finland which was planted with 1-year-old seedlings in the late summer of 1987. It included 30 hybrid families produced by crossing inbreds and five controls of standard seed origins (Table 1). A complete randomized block design was used with 2×4 plants per plot (spacing at 2.5×2.5 m) and six blocks in the whole trial.

The 30 hybrid families comprised crosses between selfs with different origins (Table 1). The five controls contained four normal full-sib families and one stand seed origin from southern Finland. Southern central and northern Finland were defined here by the latitude about 62° and 64°, respectively, following county borders (Fig. 1).

The effect of parental inbreeding on yield (stem volume) was estimated on the basis of 12 hybrid families (S×S). Their performance was compared to that of the S-stand and the four S-FS full-sib families. The parental inbreeding coefficient (F_p) was given as the mean of the two parents as they often came from different selfing generations. We assumed that the inbreeding coefficient (F) of the non-selfed parent is 0, then $F_1=0.5$ for the first-generation selfs (S_1), $F_2=0.75$ for the second-generation selfs (S_2), and $F_3=0.875$ for the third-generation selfs (S_3). Five classes of F_p between 0 and 0.75 were obtained in the observed material (see Table 2). Inbreeding depression was very strong after selfing as can be expected in the case of an obligate outbreeder like *B. pendula*.

The analysis of the effect of provenance hybrids on growth performance was based on all such entries, grouped as close-distance north-cross, close-distance south-cross, long-distance north-cross and long-distance south-cross. South- and north-ward crosses were seperated in order to distinguish true heterosis from the effects of a changed growth period. The stand seed origins, and the S-FS and S×S families were used as controls (Table 1).

Results

Effect of parental inbreeding (F_p) on stem volume

The mean stem volumes differed significantly among different classes of F_p ($P<0.0001$). The stand seed origin and

Table 1 Combinations of provenance crosses and the number of crosses in each combination

Category	Cross	No. of crosses	Combination of the provenance cross	Group
Controls	S-stand	1	Standard stand origin from southern Finland	Local stand origin
	S-FS	4	Non-inbred full-sib families southern Finland	Non-inbred local cross
Hybrids	S × S	12	Southern Finland × Southern Finland	Local cross
	S × C	5	Southern Finland × Central Finland	Close-distance north-cross
	C × S	5	Central Finland × Southern Finland	
	S × Estonia	1	Southern Finland × Estonia	Close-distance south-cross
	Estonia × S	1	Estonia × Southern Finland	
	S × N	1	Southern Finland × Northern Finland	Long-distance north-cross
	N × S	1	Northern Finland × Southern Finland	
	C × Estonia	1	Central Finland × Estonia	Long-distance south-cross
	N × Latvia	1	Northern Finland × Latvia	
	Germany × S	1	Germany × Southern Finland	
	C × Austria	1	Central Finland × Austria	

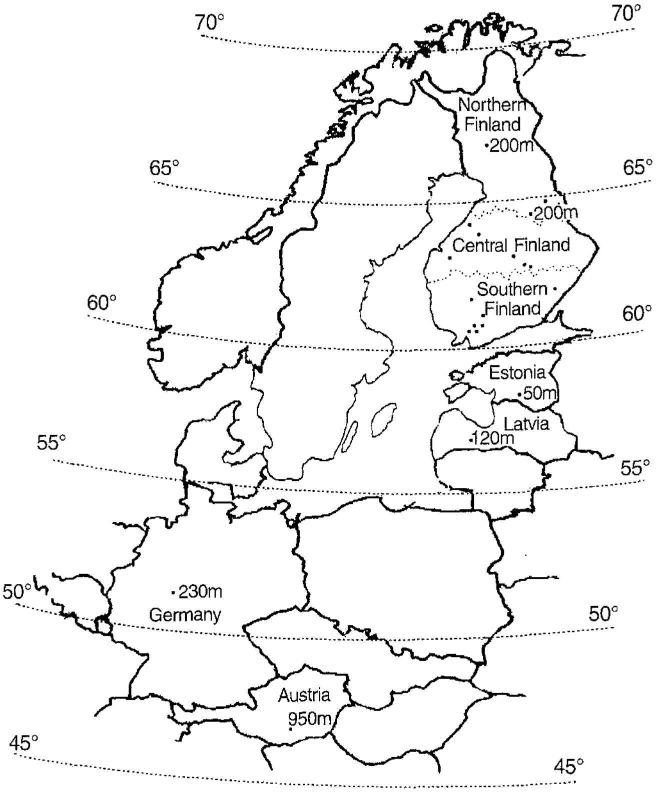

Fig. 1 Geographical origins of the selfed lines and controls. ● indicates the location of the origin, while the *number* beside indicates the altitude of the origin. Origins without indicated altitude are between 40–150 m

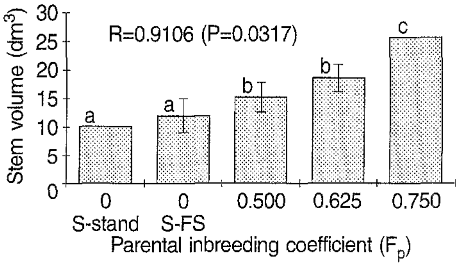

Fig. 2 Mean stem volumes of different classes of parental inbreeding coefficient (F_p). *Error bars* indicate the standard deviation. Data without error bars have only one cross. Means with the *same letter* at the top of the data bars are not significantly different at 0.05 in Tukey's Studentized Range (HSD) Test

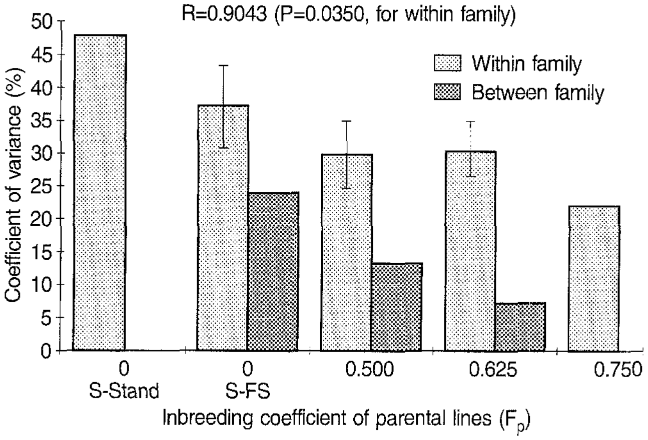

Fig. 3 Within-family variation in different classes of parental inbreeding coefficient (F_p). *Error bars* indicate the standard deviation. Data without error bars have only one cross. Means with the *same letter* at the top of the data bars are not significantly different at 0.05 in Tukey's Studentized Range (HSD) Test

Table 2 Classes of parental inbreeding coefficients (F_p) and the number of families in each class

Class of F_p	No. of families	Category
0	1	S-stand
0	4	S-FS
0.5	5	Hybrids
0.625	6	
0.75	1	

the normal full-sib families ($F_p=0$) were the poorest ones (Fig. 2). The cross between the second-generation selfed parents ($F_p=0.75$) was the best and was significantly better than all other classes. There was a steady drop in performance with decreasing mean parental inbreeding. The correlation between stem volume and F_p was positively significant (r=0.9106, P<0.05), However, the differences in stem volume among the families within the classes were also significant (P<0.0001).

Within- and between-family variation of the hybrid families

Within-family variation or the uniformity of hybrid families was described with the coefficient of variation (CV) excluding family and block effects. We assumed that the interaction among individuals in each plot was small because of the unclosed canopy and thus insignificant competition. The mean CV of families in different F_p classes ranged from 21.84 to 47.75% (Fig. 3). The stand origin and the normal full-sib family classes (F_p=0) were the highest ones and the cross between the second-generation selfed parents (F_p=0.75) was the lowest. The correlation between the coefficients of variation and F_p was negatively significant (r=0.9043, P<0.05). Thus, as was expected, the uniformity of the family increased with an increase of the parental inbreeding coefficient F_p.

Between-family variation within each level of F_p could only be observed at the F_p levels of 0 (normal full-sib families), 0.5 and 0.625, which contained several families within each of these levels (Table 2). Coefficients of variance at the three F_p levels were 23.73, 13.35 and 7.00% respectively (shown in Fig. 3), decreasing with increasing F_p.

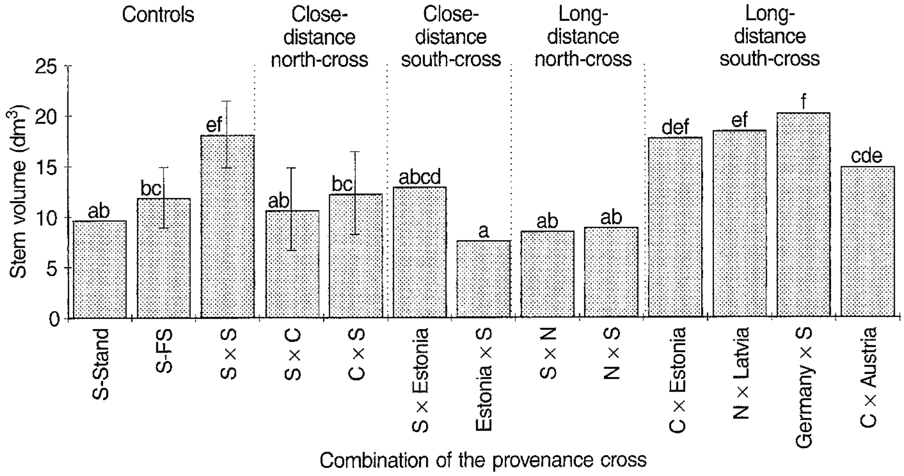

Fig. 4 Mean stem volumes of different provenance cross combinations. *Error bars* indicate the standard deviation. Data without error bars have only one cross. Means with the *same letter* at the top of the data bars are not significantly different at 0.05 in Tukey's Studentized Range (HSD) Test

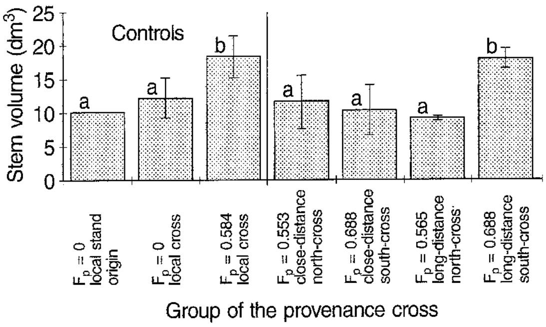

Fig. 5 Mean stem volumes of grouped combinations of provenance crosses. Means with the *same letter* at the top of data bars are not significantly different at 0.05 in Tukey's Studentized Range (HSD) Test. F_p values are averages over the parents involved in a respective class

Effect of provenance hybrids on stem volume

The effect of the parental inbreeding coefficient was ignored in provenance hybrids because the correlation between stem volume and F_p was not significant (r=0.1954, P=0.5031). The mean stem volumes of cross combinations between different origins are shown in Fig. 4.

The performance of the hybrids in the groups of close-distance north-cross, close-distance south-cross, and long-distance north-cross was not significantly different from S-stand and S-FS, but was significantly poorer than the inbred local hybrids (S×S). These particular crosses were made between carefully selected elite trees and thus outperformed all other groups, except the long-distance south-crosses. However, the hybrids in the long-distance south-cross group generally performed better than others. The cross of Germany×S was the best of all provenance combinations, followed by N×Latvia and C×Estonia, which were significantly better than S-stand, S-FS, and all crosses among other origins except S×S and S×Estonia (Fig. 4). However, there was only a single cross for each of these combinations, and the family mean of stem volume in Germany×S was the forth best in the whole experiment, whereas the first three best families were present in S×S. Differences between S×C and C×S, S×N and N×S, or S×E and E×S were not significant (Fig. 4). Thus, maternal effects could not be found.

In summary, if the maternal effect is ignored and the reciprocal crosses are grouped together, then the performance of provenance hybrids can be presented according to the group of their origins (Fig. 5). Growth performance of the long-distance south-cross group significantly outperformed all other groups, but did not differ from the inbred local cross. All other provenance cross groups were not significantly different from the local stand origin and the local non-inbred crosses.

Discussion

This is the first field experiment of crossing different generations of inbred lines in forest-tree breeding that we know of. Results show a clear positive correlation between the degree of inbreeding (F_p) and heterosis in the F_1, indicating the possibility of using inbred material to improve forest trees. This also demonstrates that inbreeding and selection need not be limited to one or two generations of selfing, but could be continued for additional generations to further increase homozygosity and improve the potential hybrid vigor in crosses among the advanced generation inbreds as suggested by others (Lindgren 1975; Rudolph 1981). However, Sniezko (1984) found that percent filled seed, germination rate, percent mortality, hypocotyl length, number of cotyledons, and root collar diameter of S_0 single crosses were greater than that of S_1 single crosses in *P. taeda*. No comparison can be made between these two studies since we did not make the same observations at these stages.

Principally, if a large number of lines derived from a base population, inbred without selection so that the mean gene frequencies are not changed, are crossed at random, then the (average) inbreeding coefficient in the crossbred progeny reverts to that of the base population. Thus the mean value of any character in the crossbred progeny is expected to be the same as the population mean of the base population (Falconer 1989). The positive correlation between the parental inbreeding coefficient and stem volume observed in this study must be attributed to the effect of selection, natural and/or artificial, performed on each generation of selfing. The percentages of filled seed gave the impression of being very low (no data available). In some other species, the percent filled seed generally shows the greatest inbreeding depression compared to other traits (Franklin 1970; Sniezko 1984). It has been suggested that the genetic cause of the embryo collapse for producing an empty seed is due to the action of homozygous lethal genes (Sarvas 1962). Thus, this is an intensive procedure for eliminating lethal alleles by inbreeding. The average rate of germination of selfed seeds of *B. pendula* is 1.6%, whereas that of the normal crossed seed is from 15 to 50% (Hagqvist, unpublished data). Therefore, the more generations of selfing, the stronger the early selection and the better the growth rate of the F_1. However, attention must be paid to the fact that the selfed lines were not derived from one population only and a higher level of heterozygosity and more heterosis can be expected than if parentals came from the same population (Falconer 1989). Thus, the effect of selfed parents may be confounded by the heterosis produced by the cross between different populations. No heterosis has been found in crosses between populations in silver birch in an earlier study (Raulo and Koski 1977). Thus the better growth performance of the hybrid families compared with that of the normal full-sib families must still be attributed to the effects of inbreeding, selection, and heterosis.

The total genetic variance after crossing inbred lines is the same as that in the base population from which the inbred lines were derived, provided there is no selection or else that selection has not changed gene frequencies. The more inbred the parents, the smaller the F_1 within-family variation, and the larger the F_1 between-family variation. If the parental lines are fully inbred, all members of the same F_1 have identical genotypes and the variance between families is equal to the total variance (Falconer 1989). In this study, within-family variation decreased with an increase of parental inbreeding level; in other words, the uniformity of the individuals in each family was enhanced by crossing highly inbred parental lines, all in accordance with theory. However, between-family variation did not increase correspondingly to the decrease of within-family variation. This is probably due to an insufficient number of families at each level of F_p as well as to selection. As pointed out earlier, the selfed lines were not derived from one base population and this may have distorted the level of variation between F_1 families.

The results suggest that selfing and crossing selfed lines may also be very effective in forest trees provided the long reproductive cycle and severe inbreeding depression can be overcome. Inbreeding depression is also severe in the first several generations in allogamous crops, e.g. in maize (Allard 1960), but levels out in later generations. Fortunately, as mentioned above, the long reproductive cycle can be overcome in silver birch. Inbreeding depression differs among individuals, e.g. the percent filled seed of selfed individuals varied between 2 and 45% among 21 clones in a *Pinus tabulaeformis* seed orchard in China (Wang et al., unpublished data), and a similar result was noted in *P. taeda* (Franklin 1968). Heavy inbreeding does not invariably mean decreased vigor because the decrease is caused by the accumulation of deleterious recessive genes rather than by selfing itself (Wright 1976). Thus, inbreeding depression allows an effective elimination of deleterious recessive genes and results in some gene complexes which could not be developed by means of normal selection. These complexes, when combined with others, may produce an exceptionally well-adapted type of tree (Wright 1976). Wright's definition of adaptation in this case means better growth. This does not conform with the original meaning of adaptation, which is based on fitness and which we use in our deliberation. Inbreeding and crossing inbred lines may be most effective when plants carry many deleterious recessive genes, as in many forest trees. Furthermore, linkage would reduce the expected symmetrical distribution because unfavorable recessive genes could be linked to favorable dominant genes. This would result in a reduced frequency of multiple dominant alleles on a given chromosome (Stoskopf 1993). However, linkage disequilibrium seems to be very rare and new recombinants are frequent in every generation in forest trees (Muona 1990).

In this study provenance hybrids between selfed lines with a relatively close cross-distance, including south-cross and north-cross, as well as involving origins from southern, central and northern Finland and Estonia, did not show better performance than the local stand seed origins and normal full-sib families, but showed a significantly poorer performance than the hybrids produced by selfed lines of the southern Finnish origin. According to our study and a previous one in silver birch by Raulo and Koski (1977), in which no heterosis was found in the hybrids obtained by crossing plus trees from different populations at least 100 km apart in southern and central Finland, the good growth performance of the hybrids by crossing individuals from different populations in southern Finland is probably attributable to carefully selected inbred parents and not to heterosis in population hybrids. When the cross distance was extended from the previously mentioned crosses to long-distance crosses, the long-distance north-cross, the cross between southern and northern Finnish origins, gave the same result as the above mentioned crosses. However, the growth performance of the long-distance south-cross, including central Finland×Estonia, central Finland×Austria, northern Finland×Latvia, and southern Finland×Germany, was significantly better than the close-distance crosses among the origins within Finland, stand seed origins and normal full-sib families. Nevertheless, these extended long-distance crosses did not significantly outperform the crosses between selfed elite lines originating from the local populations.

Among other things, the amount of heterosis depends principally on the differences of gene frequency between the two populations crossed. This difference should increase with geographic distance. However, the cross between southern and northern Finnish origins had a longer cross distance than the cross between southern and central Finnish origins, and had a similar cross distance to that between the origins from central Finland and Latvia, but gave the poorest growth performance. The cross between the origins from central Finland and Austria had the longest latitudinal cross distance. However, its intermediate growth performance must depend on the high altitude (950 m) of the father, which curtails the growth period. The results indicated that, when the long-distance crosses were between local and northern origins, the growth performance of the hybrids was poor, whereas when the crosses were between local and southern origins, a good growth performance of the hybrids could be expected. Populations that are widely differentiated through adaptations to local environments may fail to show heterosis (Wallace and Vetukhiv 1955; Falconer 1989) due to genetic breakdown affecting adaptation. This may be the explanation in our experiment as well. However, the better performance of the local×south crosses may also be due to the extended growth period of such offspring.

The good performance of the birch hybrids obtained by crossing Finnish and central European origins may have an entirely different genetic background from the S×S elite families. Both heterosis (gene frequency differences) and a manipulated growth rhythm may be involved. Further improvement may be possible by careful selection of elite parents in populations located far apart.

We conclude that genetic yield improvement in silver birch is most likely to succeed with selection and the breeding of local well-adapted material within well-defined breeding zones. It is also possible to produce equally fast-growing hybrids by wide crosses. Ultimately these two breeding strategies could be combined for maximum yield. However, when determining the final importance of wide crosses and crossing inbred lines for practical breeding work, stem and branch quality should also be taken into account to ensure not only maximum fiber production but also a high quality timber.

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