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Kazuhito Kita · Takaaki Fujimoto · Kazuko Uchiyama
Makoto Kuromaru · Hisashi Akutsu

Estimated amount of carbon accumulation of hybrid larch in three 31-year-old progeny test plantations

Abstract Hybrids generated by crossing Kuril larch (*Larix gmelinii* var. *japonica*) and Japanese larch (*L. kaempferi*) are expected to have high carbon accumulation ability because of fast growth and high wood density in Hokkaido, Japan. We estimated the amount of carbon accumulation of the hybrid larch in three progeny test plantations consisting of 21 full-sib families and compared the results to the carbon accumulation of open-pollinated progenies of Japanese larch plus-trees (improved Japanese larch). Gene–environment interactions were not observed for tree height and diameter at breast height but were seen for wood density (area-weighted density, *AWD*). The amount of carbon accumulated per unit area (C_{stand}) positively correlated with the stand volume. The AWD did not correlate with the C_{stand}; therefore, families with high wood density can be selected independent of the C_{stand}. The C_{stand} of the best full-sib family, female parent half-sib family, and male parent half-sib family at three sites were 106.1, 84.6, and 93.2 Cton·ha^{-1}, respectively. All these values exceed the mean for the improved Japanese larch, which has a C_{stand} value of 82.5 Cton·ha^{-1}.

Key words Hybrid larch · Stand volume · Wood density · Carbon accumulation · Family selection

K. Kita (✉) · K. Uchiyama
Hokkaido Forestry Research Institute, Koshunai, Bibai 079-0198, Japan
Tel. +81-126-63-4164; Fax +81-126-63-4166
e-mail: kita@hfri.pref.hokkaido.jp

T. Fujimoto · H. Akutsu
Hokkaido Forest Products Research Institute, Asahikawa 071-0198, Japan

M. Kuromaru
Dohoku Branch of Hokkaido Forestry Research Institute, Nakagawa 098-2805, Japan

Introduction

By the end of the twenty-first century, the global mean temperature is projected to increase from the 1980–1990 level by 1.1°–6.4°C because of the increasing concentration of greenhouse gases in the atmosphere.[1] The Kyoto Protocol, enforced in February 2005, required a reduction in Japan's greenhouse gas emission during the first commitment period, 2008–2012, by 6.0% of the greenhouse gases in 1990.[2] The Japanese Government planned to reduce 3.8% (13.0 Mt-C · year^{-1}) of the greenhouse gas emissions by human-induced activities such as forest management, afforestation, and reforestation.[3]

Larix kaempferi (Japanese larch) is one of the most important afforestation species in the boreal temperate zone of Japan because of its ability to grow rapidly. However, this species has some disadvantages for afforestation in Hokkaido Island, North Japan, including its sensitivity to the gnawing of a vole (*Clethrionomys rufocamus bedfordiae*) and crooked stems. Therefore, various interspecific crosses of this genus, especially those distributed in East Asia, have been tested in Hokkaido.[4–6] These studies have shown that the F$_1$ hybrid of *Larix gmelinii* var. *japonica* (Kuril larch) and *L. kaempferi* (Japanese larch) exhibits the most desirable traits: rapid juvenile growth, straight stems, and resistance to vole gnawing. About 700 000 seedlings of this hybrid are planted annually in Hokkaido.

The amount of carbon accumulated in tree stems is estimated by using two parameters: basic density of wood and stem volume.[7] The basic density is higher in *L. kaempferi* than in *Abies sachalinensis* (Todo fir) and *Picea glehnii* (Sakhalin spruce), which are important afforestation conifers native to Hokkaido.[8] Furthermore, *L. gmelinii* var. *japonica* has higher basic density than *L. kaempferi,* and the basic density of their hybrid is intermediate to that of the parent species.[5] The diameter growth of the hybrid larch is the same as that of *L. kaempferi*, but the height increase of the former is greater than that of the latter at the juvenile stage.[5] Therefore, the hybrid larch is expected to have high potential for carbon accumulation.

To increase the carbon-accumulating potential of the hybrid larch further, we investigated hybrid families to identify superior hybrids with high carbon accumulation. Another advantage of hybrid selection is that families with desirable traits such as straight stems[5] and strong wood[9] can be selected. In the long term, effective use of the produced wood is more important than carbon storage in forests for preventing increase in the atmospheric CO_2 content, because the growth rate of trees usually decreases and the stand volume becomes saturated with increasing tree age.

This study was undertaken to achieve the following objectives: (1) to reveal gene–environment interactions and heritability of tree height, diameter, and wood density; and (2) to estimate the amount of carbon accumulated in full-sib families of 31-year-old hybrid larch for selecting families with high carbon-accumulating ability.

Materials and methods

Progeny test stands and wood samplings

We investigated three progeny test stands for estimating hybrid larch growth. The progeny test consisted of an incomplete factorial mating design involving four female and six male parent trees (Table 1). The number of full-sib families in Kunneppu (43°46′ N, 143°41′ E; elevation = 230 m), Niikappu (42°26′ N, 142°26′ E; elevation = 120 m), and Bibai (43°16′ N, 141°51′E; elevation = 250 m) were 21, 21, and 19, respectively (Table 1). The test stands were established in 1974 with 2-year-old seedlings according to a randomized complete block experimental design with two replicates. Each full-sib family was planted in a plot of three rows with seven trees in each row at 2 × 2 m intervals in Kunneppu and Niikappu. In Bibai, however, the planting interval was 2 m within a plot and 4 m between plots. As a result, the overall planting densities at the Kunneppu, Niikappu, and Bibai sites were 2500, 2500, and 1640 trees·ha⁻¹, respectively. In Kunneppu, third-row thinning (remove every third row) was carried out at the age of 15 years, and selective thinnings (aimed primarily at the elimination of poorly formed dominants and release of intermediate trees of better form) were carried out with 25% and 15% stand density at the ages of 20 and 24 years, respectively. In Niikappu, third-row thinning was carried out at the age of 9, and selective thinnings were carried out with 20% stand density at the ages of 20 and 28 years. In Bibai, selective thinnings were carried out with 30%, 17%, and 28% stand density at the ages of 11, 24, and 29 years, respectively.

Open-pollinated half-sib families of *L. kaempferi* plus-trees were planted next to the hybrid larch plots within each replication at every site. Their female parents were L1, L2, L3, L5, and L6 in Kunneppu and Niikappu, and L2, L5, and L6 in Bibai, all of which were common to the clones used as male parents for the hybrid larch. Plus-trees were selected according to the phenotypes for growth, trunk straightness, and/or crown shape. Therefore, we expected the selected traits to be improved in the progeny of the plus-trees. In this study, we called the half-sib families of *L. kaempferi* plus-trees as "improved Japanese larch," which was used as a control for the hybrid larch.

The diameters at breast height (DBH) and tree heights of all the trees were measured at 31 years of age with calipers and Vertex II (Haglöf Sweden), respectively.

Wood samples were collected from the trees at 20 years of age from Kunneppu and at 29 years from Bibai. We harvested 5–8 (mean, 6.1) sample trees in Kunneppu and 1–6 (mean, 5.0) sample trees in Bibai from each of the full-sib families while adding up two replications. In total, 128 and 95 trees were sampled from Kunneppu and Bibai, respectively. A 40-mm-thick diametric flitch was obtained from each tree at a height of approximately 2 m above ground level. Wood samples were not collected from Niikappu.

Wood density measurements

A 2-mm-thick (longitudinal) section including the pith, free from decay and knots, was cut from the top end of each flitch. Resin was then extracted from these sections with a solution of benzene–ethanol (2:1) for a week. Thereafter, the sections were dried to an equilibrium moisture content of about 12%. Wood density information was obtained by using X-ray densitometry.[10,11] The methods for measuring wood density have been described previously by Fujimoto et al.[12] For each of the annual rings in the samples, early-wood density (ED), latewood density (LD), average ring density (RD), earlywood width (EW), latewood width (LW), and ring width (RW) were obtained based on the densitometric profiles. Demarcation between earlywood and latewood in each ring was 0.55 g·cm⁻³.[12]

The number of growth rings was 13–16 in the sample sections obtained from Kunneppu and 24–27 in those from Bibai, because the trees reached the sampling height at different ages. To avoid confusion owing to the differences in tree age, the annual rings were identified and numbered starting from the bark and moving toward the pith.[13] Therefore, we analyzed growth rings formed at 7–19 years of stand age in Kunneppu and 7–28 years of stand age in Bibai.

Area-weighted averages from the pith to the bark were calculated for individual wood density by using the following formula:[14]

$$AWD = \sum a_i.d_i \Big/ \sum a_i \tag{1}$$

Table 1. Diagram of the mating design in three progeny test sites

Females (*L. gmelinii* var. *japonica*)	Males (*Larix kaempferi*)					
	L1	L2	L3	L4	L5	L6
G1		○	○ᵃ	○	○	○ᵃ
G2	○	○	○	○	○	○
G3	○	○	○	○	○	○
G4		○	○	○	○	

ᵃ Missing crosses in the Bibai test site

where AWD is the area-weighted density (g·cm^{-3}), a_i is the cross-sectional area of the ith growth ring while assuming that each ring is perfectly circular, and d_i is the wood density of the ith growth ring.

Statistical analysis

Analyses of variance for the observed values of the DBH and tree height were performed according to the model presented in Eq. 2.[15]

$$Y_{ijkln} = \mu + S_i + R_{j(i)} + F_k + M_l + FM_{kl} + SF_{ik} + SM_{il} + SFM_{ikl} + RF_{jk} + RM_{jl} + RFM_{jkl} + \varepsilon_{ijkln} \tag{2}$$

where Y_{ijkln} is the observed value of the nth tree of the kth female parent and lth male parent in the jth replicate within the ith site, μ is the general mean, S_i is the fixed effect of site i, $R_{j(i)}$ is the random effect of replicate j within site i with variance $\sigma_{r(s)}^2$, F_k is the random effect of the kth female with variance σ_f^2, M_l is the random effect of the lth male with variance σ_m^2, FM_{kl} is the random interaction effect between the kth female and lth male with variance σ_{fm}^2, SF_{ik} is the random interaction effect between the ith site and kth female with variance σ_{sf}^2, SM_{il} is the random interaction effect between the ith site and lth male with variance σ_{sm}^2, SFM_{ikl} is the random interaction effect between the ith site, kth female, and lth male with variance σ_{sfm}^2, RF_{jk} is the random interaction effect between the jth replicate and kth female with variance σ_{rf}^2, RM_{jl} is the random interaction effect between the jth replicate and lth male with variance σ_{rm}^2, RFM_{ikl} is the random interaction effect between the jth replicate, kth female, and lth male with variance σ_{rfm}^2, and ε_{ijkln} is the within-plot random-effect error with variance σ_e^2.

Because the number of thinning trees was limited and we could not harvest a sufficient number of wood samples for each replicate, analyses of variance for the observed values of AWD were performed without regard for replicates according to the model provided in Eq. 3.

$$Y_{ikln} = \mu + S_i + F_k + M_l + FM_{kl} + SF_{ik} + SM_{il} + SFM_{ikl} + \varepsilon_{ikln} \tag{3}$$

where each variable is the same as that used in Eq. 2. Variance components for each trait were estimated by using the SAS mixed procedure (version 9.1).

The factorial mating design allowed estimation of the genetic variance components: additive variance and dominance variance. Additive variance (σ_A^2) could be derived directly from female and male variance components, assuming that epistatic and maternal effects were negligible and that the coefficient of inbreeding among the parents was zero.[16]

$$\sigma_A^2 = 2(\sigma_f^2 + \sigma_m^2) \tag{4}$$

Similarly, dominance variance (σ_D^2) was estimated from the following equation:

$$\sigma_D^2 = 4\sigma_{fm}^2 \tag{5}$$

Narrow-sense heritability is given by the following formula on an individual tree basis:[15]

$$h^2 = 2(\sigma_f^2 + \sigma_m^2) / (\sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 + \sigma_{sf}^2 + \sigma_{sm}^2 + \sigma_{sfm}^2 + \sigma_{rfm}^2 + \sigma_e^2) \tag{6}$$

Standard errors of narrow-sense heritability were calculated as described by Becker.[17]

Because dominance variance (σ_D^2) was estimated in the factorial mating design, broad-sense heritability was given by the ratio of all the genetic variation to phenotypic variation,[18] calculated with the following formula:

$$H^2 = (2(\sigma_f^2 + \sigma_m^2) + 4\sigma_{fm}^2) / (\sigma_f^2 + \sigma_m^2 + \sigma_{fm}^2 + \sigma_{sf}^2 + \sigma_{sm}^2 + \sigma_{sfm}^2 + \sigma_{rfm}^2 + \sigma_e^2) \tag{7}$$

Estimation of carbon accumulation in tree stems

The amount of carbon accumulated per unit area of tree stem was estimated by using the following formula:

$$C_{\text{stand}} = V_{\text{stand}} \times bd / 1000 \times CW \tag{8}$$

where C_{stand} is the amount of carbon in stems per unit area (Cton·ha^{-1}), V_{stand} is the stand volume (m^3·ha^{-1}), bd is the basic density (kg·m^{-3}), and CW is the carbon content in wood substance. Single stem volume was estimated according to the equation for $L.\ kaempferi$[19] from observed values of the DBH and tree height, and V_{stand} was calculated by multiplying the single stem volume with the stand density in each plot. CW was set at 0.5 as the constant in this study because CW has a smaller range of variation than V_{stand} and bd, and does not affect variation of carbon accumulation in stems, although it varies among species and within the stem.[20]

AWD, the density of wood containing about 12% moisture determined by X-ray densitometry, was transformed into bd, defined as a fully dried wood weight per unit green wood volume, to estimate the C_{stand}. The bd were measured from wood sampled in Bibai by using the water displacement method.[21] Sample blocks including two growth rings were successively cut from pith to bark of the radial strip. The data from each radial direction were averaged to mean value. The relationship between the AWD and bd are as shown in Eq. 9:

$$bd / 1000 = 0.438\,AWD + 0.231\,(n = 95,\ R^2 = 0.6026,\ P < 0.001) \tag{9}$$

We calculated the bd by using Eq. 9 and estimated the C_{stand} by substituting the bd for Eq. 8.

To select families with superior carbon accumulation ability, analyses of variance for plot mean values of the C_{stand} were performed according to Eqs. 9 and 10 by using the SAS GLM procedure:

$$Y_{ijkl} = \mu + S_i + R_{j(i)} + F_k + M_l + FM_{kl} + \varepsilon_{ijkl} \text{ (for multiple sites)} \tag{10}$$

$$Y_{jkl} = \mu + R_j + F_k + M_l + FM_{kl} + \varepsilon_{jkl} \quad \text{(for a single site)} \tag{11}$$

where Y_{ijkl} and Y_{jkl} are the plot mean values of the kth female parent and lth male parent in the jth replicate of the ith site and μ is the general mean. S_i, $R_{j(i)}$ and R_j, F_k, and M_l are the fixed effects of the ith site, replicate j within the ith

site, the kth female parent, and the lth male parent, respectively. FM_{kl} is the fixed interaction effect between the kth female parent and lth male parent, and ε_{ijkl} and ε_{jkl} are the within-replicate errors. The least square means (LS means) of the female parent, male parent, and full-sib family were estimated by using the LSMEANS statement in the GLM procedure because there were missing crosses. Multiple comparisons of the female parent, male parent, and full-sib family means were also performed by using the PDIFF option of the LSMEANS statement. Wood samples were not harvested in Niikappu. We calculated the C_{stand} value in Niikappu by using the LS means of AWD in Bibai and Kunneppu.

Results

Genetic variations of tree height, DBH, and AWD

The stand densities at 31 years of age in Kunneppu, Niikappu, and Bibai were 896, 706, and 460 trees·ha^{-1}, respectively (Table 2). The stand density was consistently lower in Bibai than in the other two sites since the test sites were established. Consequently, the mean DBH of the 31-year-old trees was the largest in Bibai (27.4 cm), followed by 23.9 cm in Niikappu and 21.4 cm in Kunneppu (Table 2). The largest full-sib family mean of the DBH was 11.5%, 18.2%, and 12.9% higher than the site mean in Kunneppu, Niikappu, and Bibai, respectively. The mean tree height of the 31-year-old trees was the highest at 22.4 m in Niikappu, followed by 21.6 m in Kunneppu and 20.8 m in Bibai (Table 2). The largest full-sib family mean of the tree height was 9.3%, 7.1%, and 11.1% higher than the site mean in Kunneppu, Niikappu, and Bibai, respectively. Earlywood density slightly decreased with increasing stand age, whereas latewood density was almost constant at around 0.8 g·cm^{-3} in Kunneppu and Bibai (Fig. 1a). These densities were not different between the two sites at the same stand age. On the other hand, earlywood and latewood width decreased with increasing stand age, and earlywood width was greater in Bibai than in Kunneppu at the same stand age (Fig. 1b). As a result, the average density of each growth ring (RD) was higher in Kunneppu than in Bibai (Fig 1c). The RD increased with increasing stand age until about 17 years and fluctuated in Bibai. The site mean of AWD was also higher in Kunneppu (0.565 g·cm^{-3}) than in Bibai (0.516 g·cm^{-3}) because of the difference in earlywood width between the two sites. The full-sib family mean of AWD ranged from 0.523 to 0.637 g·cm^{-3} and from 0.439 to 0.570 g·cm^{-3} in Kunneppu and Bibai, respectively (Table 2).

The site × female, site × male, and site × female × male interactions of the DBH and tree height were zero, whereas the site × female and site × male interactions with respect to AWD were similar in amount to the main effects of the female and male parents (Table 3). Narrow-sense heritability, representing additive genetic variance, was 0.16 for the DBH, 0.20 for tree height, and 0.28 for the AWD (Table 3). Additive genetic variances derived from the male parents for the DBH and tree height were larger than those derived from the female parents, but additive variances for the AWD were similar between the male and female parents. Broad-sense heritability, representing the sum of additive and dominance genetic effects, was 0.26 for the DBH, 0.40 for tree height, and 0.41 for the AWD.

Estimated carbon accumulation in tree stems

The site mean and range of full-sib family means for the C_{stand} in Kunneppu were 85.2 Cton·ha^{-1} and 47.2–116.9 Cton·ha^{-1}, respectively (Table 4); in Bibai, these values

Table 2. Site mean and maximal and minimal full-sib family mean of tree height, DBH, stem volume, stand density, stand volume, and AWD in three sites

Site	Trait	Age	Mean (±SD)	Full-sib family mean	
				Max.	Min.
Kunneppu	Tree height (m)	31	21.6 ± 2.5	23.6	18.9
	DBH[d] (cm)	31	21.4 ± 4.4	23.8	18.0
	Stem volume (m³·tree^{-1})	31	0.393 ± 0.176	0.485	0.259
	Stand density (tree·ha^{-1})	31	896	1190	595
	Stand volume (m³·ha^{-1})	31	352	548	194
	AWD (g·cm^{-3})	19	0.565 ± 0.054	0.637	0.523
Niikappu	Tree height (m)	31	22.2 ± 2.1	23.8	18.9
	DBH[d] (cm)	31	23.9 ± 4.6	28.2	20.4
	Stem volume (m³·tree^{-1})	31	0.490 ± 0.199	0.678	0.324
	Stand density (tree·ha^{-1})	31	706	1012	298
	Stand volume (m³·ha^{-1})	31	353	526	231
Bibai	Tree height (m)	31	20.8 ± 1.6	23.1	19.7
	DBH (cm)	31	27.4 ± 3.9	30.6	24.0
	Stem volume (m³·tree^{-1})	31	0.587 ± 0.179	0.811	0.452
	Stand density (tree·ha^{-1})	31	460	547	156
	Stand volume (m³·ha^{-1})	31	276	443	207
	AWD (g·cm^{-3})	28	0.516 ± 0.061	0.570	0.439

DBH, diameter at breast height; AWD, area-weighted average wood density; SD, standard deviation; Max., maximal full-sib family mean of each trait; Min., minimal full-sib family mean of each trait

Fig. 1. Time trends of (a) earlywood density (*ED*) and latewood density (*LD*), (b) earlywood width (*EW*) and latewood width (*LW*), (c) average ring density (*RD*), and (d) ring width (*RW*) of the hybrid larch. The *filled and open symbols* show the values obtained from Kunneppu and Bibai, respectively
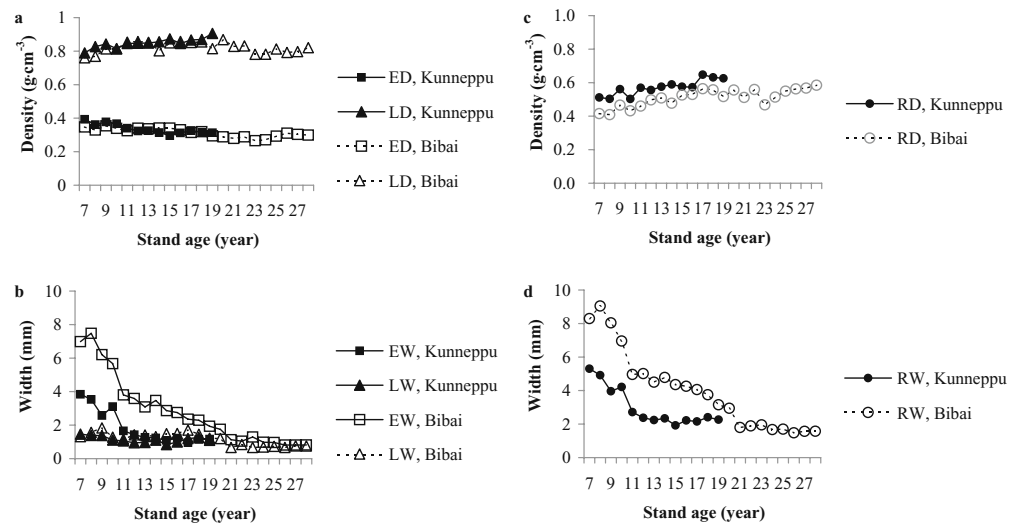

Table 3. Variance component and heritability of the DBH and tree height estimated by using the pooled data from Kunneppu, Niikappu, and Bibai, and *AWD* estimated with pooled data in Kunneppu and Bibai

Source	DBH		Tree height		AWD^c	
	Variance component	Standard error	Variance component	Standard error	Variance component	Standard error
Replication (site)	0.18	0.40	0.10	0.23		
Female	0.57	0.70	0.08	0.17	0.00020	0.00030
Male	0.95	0.77	0.34	0.30	0.00017	0.00024
Female × male	0.44	0.42	0.20	0.15	0.00009	0.00011
Site × female	0.00	–	0.00	–	0.00018	0.00020
Site × male	0.00	–	0.00	–	0.00019	0.00018
Site × female × male	0.00	–	0.00	–	0.00000	–
Replication × female (site)	0.57	0.48	0.36	0.22	–	–
Replication × male (site)	0.00	–	0.21	0.14	–	–
Replication × female × male (site)	0.74	0.51	0.44	0.17	–	–
Residual	15.83	0.86	3.05	0.17	0.00183	0.00019
Narrow-sense heritability	0.16	0.26	0.20	0.27	0.28	0.43
(Female)[a]	0.06		0.04		0.15	
(Male)[b]	0.10		0.17		0.13	
Broad-sense heritability	0.26		0.40		0.41	

[a] Narrow-sense heritability derived from the female parents
[b] Narrow-sense heritability derived from the male parents
[c] Area-weighted average wood density

were 65.7 Cton·ha^{-1} and 45.3–97.0 Cton·ha^{-1}. The lower C_{stand} values in Bibai could be the result of the lower stand density and stand volume than those in Kunneppu; however, the single stem volume was higher in Bibai than in Kunneppu (see Table 2). The site mean for the C_{stand} in Niikappu, estimated by using the least square means of the *AWD* in Kunneppu and Bibai because wood samples were not harvested in Niikappu, was 84.0 Cton·ha^{-1}. The maximal and minimal full-sib family means of the C_{stand} in Niikappu were 121.4 Cton·ha^{-1} and 52.5 Cton·ha^{-1}, respectively. The overall C_{stand} mean and range of full-sib family LS means of the three sites were 76.7 Cton·ha^{-1} and 50.4–106.1 Cton·ha^{-1}, respectively. The best full-sib family had a C_{stand} value 38% higher than the overall mean of the three sites. The C_{stand} value significantly differed between the female and male parents in Bibai and in the three sites as well as between the male parents in Kunneppu (Table 5). No interaction

effects were observed between the female and male parents at any of the sites. With regard to the overall value of the three sites, G3 and L2 showed the highest LS means of the C_{stand} in the female and male parent family, respectively. The C_{stand} value of G3 significantly differed from that of G1, and the C_{stand} value of L2 significantly differed from those of L3, L4, and L5 (Table 4; pairwise comparisons of the LS means, $P < 0.05$). The G3 family and L2 family had 10% and 22% higher C_{stand} values, respectively, than the overall mean of the three sites.

As shown in Eq. 8, the C_{stand} is determined by two factors: stand volume and basic density of wood. The full-sib family mean C_{stand} of the three sites correlated with stand volume (Fig. 2a; $r = 0.993$, $P < 0.001$) but not with *AWD* (Fig. 1b; $r = 0.055$, $P = 0.811$). Growth of individual trees is prohibited by the competitive effect as the stand volume increases. However, the single stem volume positively correlated with

Table 4. Estimated amount of accumulated carbon in stems per unit area (C_{stand}) of the 31-year-old hybrid larch at Kunneppu, Bibai, and three sites

Site	Females (*L. gmelinii* var. *japonica*)	Males (*L. kaempferi*)						
		L1	L2	L3	L4	L5	L6	LS mean
Kunneppu	G1		97.1	63.1	80.4	47.2	85.9	75.7[a]
	G2	109.8	116.9	90.2	59.7	50.5	109.6	89.5[a]
	G3	80.4	101.0	74.0	130.2	71.3	89.6	91.1[a]
	G4		99.5	79.5	94.5	50.7		84.6[a]
	LS mean	90.0[ab]	103.6[a]	76.7[bc]	91.2[a]	54.9[c]	94.8[ab]	85.2
Bibai	G1		66.9		61.2	57.5		59.2[ab]
	G2	48.8	61.1	53.3	47.7	53.2	55.2	53.2[b]
	G3	56.1	97.0	45.3	61.5	67.8	87.8	70.8[ab]
	G4		73.4	58.5	73.8	57.3		65.7[a]
	LS mean	52.6[c]	74.6[a]	54.5[bc]	61.0[bc]	59.0[bc]	71.8[ab]	62.2
Three sites[e]	G1		72.4	50.4	65.1	55.6	86.9	66.0[b]
	G2	87.0	96.2	76.3	60.2	56.5	77.7	75.6[ab]
	G3	79.9	106.1	57.1	95.7	74.1	92.3	84.6[a]
	G4		98.1	66.9	81.7	63.9		80.7[a]
	LS mean	80.1[ab]	93.2[a]	63.4[cd]	75.7[bc]	62.3[d]	85.6[ab]	76.7

LS mean, least squares mean
[abcd]The same letters indicate no significant difference between the parents ($P > 0.05$)
[e]Three sites include Kunneppu, Niikappu, and Bibai. C_{stand} values of Niikappu used for the three site means were estimated by using the LS means of the *AWD* in Kunneppu and Bibai sites because wood samples were not harvested in Niikappu

Table 5. Analyses of variance of accumulated carbon in stems per unit area (C_{stand}) in Kunneppu, Bibai, and three sites

Site	Source	DF	SS	MS	*F* value	*P*
Kunneppu	Replication	1	558	558	1.43	0.245
	Female	3	1439	480	1.23	0.324
	Male	5	11462	2292	5.89	0.002
	Female × male	12	7353	613	1.57	0.179
	Error	20	7784	389		
Bibai	Replication	1	29	29	0.18	0.679
	Female	3	1687	562	3.49	0.039
	Male	5	2513	503	3.12	0.035
	Female × male	10	1689	169	1.05	0.448
	Error	17	2739	161		
Three sites[a]	Site	2	12243	6121	13.09	<0.0001
	Replication (site)	3	915	305	0.65	0.584
	Female	3	4669	1556	3.33	0.023
	Male	5	16741	3348	7.16	<0.0001
	Female × male	12	7183	599	1.28	0.243
	Error	94	43949	468		

[a]Three sites include Kunneppu, Niikappu, and Bibai: C_{stand} values of Niikappu used for the three site means were estimated by using the LS means of the *AWD* in Kunneppu and Bibai sites because wood samples were not harvested in Niikappu

the stand volume on the basis of the full-sib family mean (Fig. 2c; $r = 0.643$, $P = 0.02$). This result indicated that the competitive effect was not a factor determining the C_{stand} value of the 31-year-old hybrid larches. The stand volume was smaller in the G1 family than in the other families showing similar single stem volume, which was the result of the lower survival rate of the G1 family. The *AWD* did not correlate with the stand volume (Fig. 2d; $r = -0.056$, $P = 0.809$), showing that it was possible to select families with higher growth and carbon accumulation without decreasing the *AWD*.

The DBH and tree height of open-pollinated and improved *L. kaempferi* planted at the same site tended to be higher than the corresponding values of the hybrid larches at any of the sites. However, the *AWD* of *L. kaempferi* was lower than that of the hybrid larches (see Tables 2, 6). The C_{stand} of *L. kaempferi* was higher in Kunneppu and in the three sites, but this value was lower than that of the hybrid larches in Bibai. The low C_{stand} value of the improved *L. kaempferi* in Bibai compared with that of the hybrid larches was caused by its lower *AWD* and survival rate (28.1% for hybrid larch, 24.6% for improved Japanese larch). The best female parent family, male parent family, and full-sib family of the hybrid larch had C_{stand} values 3%, 13.0%, and 29% higher than that of the improved *L. kaempferi*.
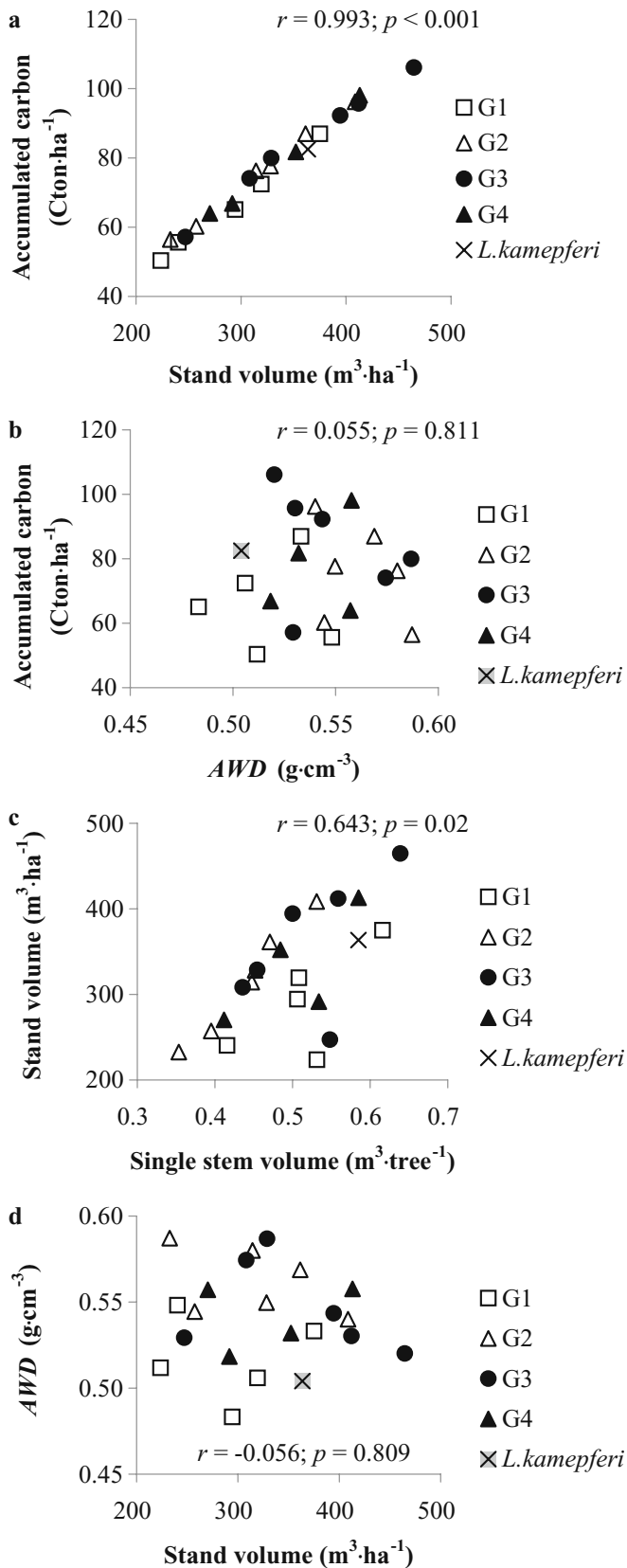
Fig. 2. Relationship between (a) stand volume and accumulated carbon per unit area, (b) area-weighted average wood density (*AWD*) and accumulated carbon per unit area, (c) single stem volume and stand volume, and (d) stand volume and *AWD* based on the least square full-sib family mean values of the 31-year-old hybrid larches at the three sites, but only two sites (Kunneppu and Bibai) for the *AWD*

Discussion

We analyzed the genetic structure of growth and wood density, and estimated the amount of carbon accumulation of 31-year-old hybrid larches for selecting families with high carbon storage ability.

When a gene–environment interaction is observed in a given trait, the estimation of good and bad families depends on the study site. However, there were no gene–environment interactions for tree height and DBH. These results are in agreement with the studies at other progeny test sites for *L. kaempferi* in Hokkaido.[22] However, *L. kaempferi* showed gene–environment interaction for tree height in progeny test plantations established within the natural distributed range.[23] One of the reasons for the gene–environment interaction for tree growth in Hokkaido is the genetic differentiation along the snow gradient.[24] *L. kaempferi* was originally distributed in central Japan. It was artificially introduced in Hokkaido in the late 1800s[25] and has experienced selective pressure of the snow gradient in Hokkaido only for a few generations. *L. gmelinii* var. *japonica* was distributed in Hokkaido in the last glacial age but disappeared from Hokkaido with the end of the glacial age.[26] This species is distributed in Sakhalin and the southern part of the Kuril Islands at present and was artificially introduced in Hokkaido in the late 1800s.[25] The short period from introduction of the parent species in Hokkaido might be related to the lack of gene–environment interactions for tree height and diameter growth of the hybrid larch, but further studies are needed.

On the other hand, we observed a gene–environment interaction for the *AWD*. According to Eqs. 7 and 9, the *AWD* is a determined factor of the C_{stand}. If the *AWD* strongly affects the C_{stand} and exhibits gene–environment interaction, estimation of the families for the C_{stand} depends on the study site. However, the C_{stand} correlated with the stand volume and not the *AWD* (Fig. 2), because the family mean range of the *AWD* was smaller than that of the stand volume (Table 2). These results suggest that we can estimate good and bad families for the C_{stand} without considering the gene–environment interaction for the *AWD*.

The narrow-sense heritability, which includes components of additive genetic variance, was 0.16 for the DBH, 0.20 for tree height, and 0.28 for the *AWD*. Previous reports have shown that the heritability estimates of wood density are higher than those of growth traits.[27,28] The narrow-sense heritability of wood density in this study was higher than those of the growth traits; however, it was lower than the estimates for wood density reported in other conifers,[29–31] probably because of the gene–environment interactions observed in this study.

Table 6. Mean values in each site and least squares means in three sites of open-pollinated improved *Larix kaempferi*

Trait	Kunneppu	Niikappu	Bibai	Three sites[b]
DBH (cm)	21.4	25.2	29.4	24.5
Tree height (m)	22.7	25.2	21.0	23.4
Stand volume (m^3)	453	365	273	364
AWD[a] (g·cm^{-3})	0.542		0.484	0.504
Accumulated carbon in stems per unit area (C_{stand}) (Cton·ha^{-1})	105.7		60.3	82.5

[a] Area-weighted average wood density
[b] Three sites include Kunneppu, Niikappu, and Bibai: C_{stand} values of Niikappu used for the three site means were estimated by using the LS means of the AWD in Kunneppu and Bibai sites because wood samples were not harvested in Niikappu

Revealing the inheritance mode is important for deciding breeding strategies. The additive genetic variance components associated with the male parents (*L. kaempferi*) were larger than those associated with the female parents (*L. gmelinii* var. *japonica*). Another study of hybrid larch progeny tests also reported that the additive genetic components associated with *L. kaempferi* were larger in the DBH of the *L. gmelinii* var. *japonica* × *L. kaempferi* hybrids and in the DBH and tree height of the *L. kaempferi* × *L. gmelinii* var. *japonica* hybrids compared with those associated with *L. gmelinii* var. *japonica*.[32] *L. kaempferi* tended to have stronger effects on the growth of the hybrid progeny than *L. gmelinii* var. *japonica*. These results suggest that male family selection is more efficient for improving growth traits of the hybrid larch than female family selection. On the other hand, as the additive genetic components of the AWD were similar between the female and male parents, each of the female and male family selections is expected to have a similar effect for improving the AWD. However, a mating design with few full-sib families for every half-sib family, as used in this study, is not adequate for estimating variance components. Further, the standard errors of the heritability estimates were large due to the small sample size, especially for evaluating the AWD. Therefore, further studies are required to understand the genetic structure of growth and wood density traits of the hybrid larch better.

We used the AWD data obtained from tree samples harvested at 19 years of age in Kunneppu for estimating the amount of carbon accumulation of the 31-year-old stand. Wood density of the hybrid larch increases steadily with age and reaches a plateau at approximately 18 years of age,[12] consistent with the age of transition from juvenile to mature wood based on tracheid length.[33] The age-related genetic and phenotypic correlations for the AWD between 19 and 28 years are greater than 0.9.[12] Therefore, precise estimation of carbon accumulation is possible; however, there is a time lag between the AWD measurement and estimation of carbon accumulation.

The overall mean C_{stand} value of the 31-year-old hybrid larch was 76.7 Cton·ha^{-1}, which is 2.47 Cton·ha^{-1}·year^{-1} in terms of the annual accumulated carbon (C_{stand} per year). The C_{stand} per year of the typical forests in Hokkaido has been calculated by using the published data for stem volume and basic density and reported as follows: 0.85 Cton·ha^{-1}·year^{-1} for a boreal mixed forest, 1.14–1.69 Cton·ha^{-1}·year^{-1} for a 60-year-old *Abies sachalinensis* plantation, 0.80 Cton·ha^{-1}·year^{-1} for a 60-year-old *Picea glehnii* plantation, 1.69–2.25 Cton·ha^{-1}·year^{-1} for a 40-year-old *L. kaempferi* plantation, 0.41 Cton·ha^{-1}·year^{-1} for a 100-year-old secondary forest of *Betula maximowicziana*, and 1.59 Cton·ha^{-1}·year^{-1} for a 32-year-old willow-dominated riparian secondary forest.[34] Therefore, the hybrid larch shows superior carbon accumulation ability compared with the typical forests in Hokkaido.

Two perspectives are suggested with regard to wood use for preventing global warming: high-level utilization of wood as a sustainable material for buildings and furniture, and utilization of wood biomass as a next-generation fuel.[35] It is important to clarify which perspective is the objective for breeding the studied species. The family mean C_{stand} of the hybrid larch was 84.6 Cton·ha^{-1} for the best female parent, 93.2 Cton·ha^{-1} for the best male parent, and 106.1 Cton·ha^{-1} for the best full-sib family, and their corresponding annual values were 2.72, 3.01, and 3.42 Cton·ha^{-1}·year^{-1}. These values were 3%, 13.0%, and 29% higher than that of the improved *L. kaempferi*, respectively. In Hokkaido, higher biomass production than that from the hybrid larch has been reported from selected clones of *Salix* and *Populus* species with short rotation practice for pulp wood production. The annual biomass production is 15 t·ha^{-1}·year^{-1} (7.5 Cton·ha^{-1}·year^{-1}) for *Populus maximowiczii*[36] and 17.5 t·ha^{-1}·year^{-1} (9.75 Cton·ha^{-1}·year^{-1}) for *Salix pet-susu*.[37] Therefore, the *Populus* and *Salix* species have a considerable advantage in terms of the second perspective: utilization of wood biomass as a next-generation fuel. On the other hand, the hybrid larch has an advantage in terms of the first perspective, high-level utilization of wood as a sustainable material for buildings, because the hybrid larch inherently has strong wood.[9] The C_{stand} value of the hybrid larch was determined by the stand volume, and the family mean value did not reveal any relationship between the C_{stand} value and AWD. This result suggests that families with high AWD can be selected independent of their carbon accumulation ability. In other words, it is possible to improve the carbon accumulation ability and efficiency of wood utilization of the hybrid larch simultaneously.

If the stem volume of individual trees negatively correlates with the stand volume because of the competitive effect, the single stem volume will decrease because of the

selection of families with high stand volume; this reduces the utilization rate of the harvested wood and is inconsistent with the second perspective. In the case of the 31-year-old hybrid larch, however, the single stem volume positively correlated with the stand volume, and no competitive effect was observed (Fig. 2). Therefore, selection of families with high carbon accumulation ability per unit area helps in increasing the single stem volume and wood utilization efficiency of the hybrid larch.

We selected the best female parent (G3) and plan to produce half-sib hybrid larches of G3 by rooted cuttings at the juvenile seedling stage.[38] We selected a half-sib family instead of a full-sib family to enable seed production by open pollination and decrease the cost of seed production, which is higher in the case of controlled pollination. We have already established a seed orchard consisting of a single G3 clone and a multiclone of *L. kaempferi*.[39] *Larix* species have a long life and require a long period from the time of planting to flowering. After the trees flower, the initial seed production is low. Propagation of rooted cuttings encourages the spread of selected hybrid larches despite low seed production.

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