ORIGINAL ARTICLE

The timing of latewood formation determines the genetic variation of wood density in *Larix kaempferi*

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Abstract

Key message **The seasonal change of the wood formation stage, especially the timing of the cessation of cell division, are key to understanding the genetic difference of wood density.**

Abstract Wood density is important in terms of various wood properties and forest carbon sequestration properties. Wood density is genetically variable, although the mechanism that causes this variability is not well clarified. We hypothesized that wood formation dynamics differ genetically and that this affects wood density. We analyzed the dynamics of wood formation, including the timing of earlywood-latewood transition and the cessation of cell division, and their relationship to clonal differences in wood density using nine clones of *Larix kaempferi*, through microscopy observations and X-ray densitometry. Wood density showed a strong correlation with the proportion of latewood. The clonal variation of the timing of the earlywood-latewood transition was small. The timing of the cessation of cell division differed clonally by a maximum of 42.7 days and was correlated with the duration of latewood formation. The duration of latewood formation showed a correlation coefficient of 0.925 with the proportion of latewood. We described a hierarchical relationship that explained the genetic difference in wood density in mature *L. kaempferi* wood in the following sequence; the timing of cessation of latewood formation, the duration of latewood formation, the proportion of latewood, and wood density.

Keywords Japanese larch · Proportion of latewood · Wood formation dynamics · Timing of cessation of cell division · Timing of transition from earlywood to latewood

Introduction

Wood density, the amount of cell wall substance in a unit volume of wood, is an important wood property for lumber that affects wood stiffness and wood strength, and is related to biomass production and carbon sequestration properties

of trees and forests. In conifers, variations in wood density are mainly determined by differences in the cell size and cell wall thickness of tracheids. Recently, in a wider eco-physiological perspective, inter-specific, inter-annual, and intra-annual variations in wood density have been studied in the connection to the response of forest trees to environmental change, including climate change and water availability (Roderick and Berry 2001; Rossi et al. 2011; Cuny et al. 2014; Battipaglia et al. 2016; Björklund et al. 2017).

As wood density is also known vary genetically (Zobel and Van Buijtenen 1989), it is one of the objective traits to be improved in many tree breeding programs (e.g., Louzada and Fonseca 2002; Bouffier et al. 2009; Fries 2012). There are many reports on the moderate to high genetic inheritance of wood density in various species (e.g., Zobel and Talbert 1984; Zobel and Van Buijtenen 1989; Zobel and Jett 1995; Fries 2012; Park et al. 2012; Chen et al. 2014), although the mechanism responsible for these genetic differences has not been well clarified. Recently, molecular genetics tried to understand the mechanism of wood formation and wood

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density variation by genome-wide association studies and gene expression analyses (e.g., Gonzalez-Martinez et al. 2007; Mishima et al. 2014; Jokipii-Lukkari et al. 2017). Nonetheless, phenotypic studies are essential to understand the mechanism of wood density inheritance, including the dissection of heritable objective traits into sub-component traits and the evaluation of the degree of the impact of sub-component traits on upper-level traits. Such a hierarchical procedure will provide a deeper insight into wood formation.

Wood density can be divided into sub-parts, namely earlywood and latewood, based on differences in the cell size and cell wall thickness of tracheids in coniferous species. The density of earlywood and latewood in addition to the proportion of latewood, which is defined as the ratio of latewood width to the width of a growth ring, are major sub-component traits of wood density (Zobel and Van Buijtenen 1989). Therefore, the inheritance of wood density can be understood by considering the inheritance of these sub-components, their developing process, and their contribution to wood density. These sub-component traits differ in their magnitude of genetic control, variation, and influence on wood density, which is dependent on the species and the age of the wood formation (Zobel and Van Buijtenen 1989). Among them, the proportion of latewood has been reported to have a high correlation with wood density (Zobel and Jett 1995). Three causal factors for the variation in the proportion of latewood can be proposed, especially for examining genetic variations; the duration of latewood formation, the production rate of latewood cells during the latewood formation period, and the relationship between earlywood and latewood in the cell production period and in the cell production rate. Such seasonal patterns of wood formation in the cambium zone, in other words, "the dynamics of wood formation," vary between genotypes and thus determine the variation in the proportion of latewood. There are a few reports on the genetic variation of wood formation dynamics. Vargas-Hernandez and Adams (1994) reported that, in *Pseudotsuga menziesii* (Douglas fir), wood density was negatively correlated with the transition date from earlywood to latewood. Jayawickrama et al. (1997) reported a provenance difference in the date of latewood transition in *Pinus taeda* (loblolly pine). Perrin et al. (2017) reported provenance differences in the reactivation of xylem differentiation in *Picea mariana* (black spruce). Genetic variations in the dynamics of wood formation and their effect on wood density must be studied further to derive a general understanding on the mechanism responsible for genetic variations in wood density.

We investigated *Larix kaempferi* (Japanese larch) in this study. *L. kaempferi* is one of major plantation species in Japan and it is also used in plantations in China (Lai et al. 2014). In addition, this species is used as a breeding material for inter-specific hybridization with other *Larix* species

in Europe (Pâques 2004), North America (Li and Wyckoff 1994), and Hokkaido Island in Japan (Fujimoto et al. 2006; Kita et al. 2009). *Larix kaempferi* is naturally concentrated in the central mountainous area of Japan (latitude range from 35 to 37° north). There are several reports on its genetic variation in wood density and sub-components traits for wood density (Koizumi et al. 1990; Takata et al. 1992; Fujimoto et al. 2006; Fukatsu et al. 2013) that describe a relatively high heritability for wood density and the proportion of latewood. We believe that *L. kaempferi* is a suitable research material given its specific characteristics as a deciduous conifer, it has a limited natural distribution range compared to other well-studied conifers, and it is a globally used species.

In this study, we investigated the clonal variation in wood density and its sub-components using nine clones of *L. kaempferi* and analyzed the relationship of the sub-components to wood density. We also investigated the dynamics of wood formation, including earlywood-latewood transition timing and the cessation of cell division in the cambium, under the hypothesis that the timing of latewood formation affects wood density.

Materials and methods

Plant materials

This study used grafted clones of *L. kaempferi* plus trees grown at a clonal archive of breeding materials located in the Nagano campus (36°21′N 138°28′E, a.s.l. 1030 m, see Figure S1 in Online Resource) of the Forest Tree Breeding Center, Forestry and Forest Products Research Institute, Japan. The archive was established in 1960 on a relatively flat and uniform forest area by a row plot design with 10–20 ramets for each clone. The number of trees of each clone in 2007 was reduced to an average of 35% of the initial number because several thinnings had been performed, although accurate records were missing. We selected nine clones from the archive as research materials in this study (see Figure S1 and Table S1 in Online Resource). The number of ramets for each clone ranged from 3 to 6 (4.0 in average) for the wood density measurement and 2–3 for the analysis of wood formation dynamics (see Table S1 in Online Resource).

Wood density measurement

We collected increment cores of 5.15 mm diameter from each of the sample trees (see Table S1 in Online Resource). Four cores were collected from each tree, one for each cardinal point. Sampled cores were cut into 2.2 mm thick transverse sections using a Dendrocut table saw (Walesch ElectronicGmbH, Zurich, Switzerland). The sections were

incubated in acetone (45 °C) and then water (60 °C) over a week for each solvent to remove extractives. Acetone and water were replaced several times until the change in solvent color become subtle. Growth ring components, including ring width (RW), average wood density (RD), proportion of latewood (PLW), average earlywood density (ED), and average latewood density (LD) (hereafter, those parameters are termed "ring structure components") were calculated for each ring by X-ray densitometry using the procedure described in Fukatsu et al. (2013). Briefly, core sections and a stepped internal standard block were irradiated by soft X-ray on an X-ray sensitive film. The film was then processed and digitized with a scanner, and the radial variation in the image intensity of the core section on the film was converted into radial variation of wood density with a calibration curve constructed from the image of the stepped internal standard block. The boundary between earlywood and latewood was determined to be 0.55 g/cm^3 density (Ohta 1970). Growth rings with inclined ring borders against the X-ray irradiation direction and those with blurred borders between the latewood and the next year's earlywood in the X-ray films were excluded from PLW, ED, and LD analyses. Finally, 94% of the total number of rings was used in the analysis of the three traits.

Ring structure component data obtained from the four cores from each single tree were averaged for each year and used in the following analyses. The average number of obtained rings for each individual was 44.2 ± 2.3 standard deviation (SD). We could collect ring structure component data from 85% of all investigated trees at stand age 6 (i.e., from the growth ring produced in 1965), and from 100% of trees at stand age 8. In this paper, we use stand age rather than cambial age to represent the age of each ring. This is because the climate condition affects the ring structure components of a ring, and it is preferable to use the rings created at the same stand age, namely under the same climatic condition, so that year-to-year variations do not affect the clonal comparison of ring structure components.

The ramets used in this study were planted in one row, so the replication of ramets for each clone was not spatially randomized with ramets of other clones, but the ramets in each clone were grown under a similar micro-environment. The replication of ramets in this study could be considered a pseudo-replication. To verify that the materials used in this study can represent RD genetic characteristics for each clone, we compared RD with the breeding values of ring density estimated from a full-diallel mating design of *L. kaempferi* (Fukatsu et al. 2015). Six out of the nine clones used in this study were common to the parents used in Fukatsu et al. (2015), which reported estimated breeding values of wood density from stand ages 27–31 from a test site with five replications.

Dynamics of wood formation

For the microscopic observation of the cambial activity stage, we randomly sampled 2–4 microcores (2 mm in diameter) from the inner bark to the outer xylem of sample trees around the stems 1.3 m above the ground using the Trephor sampling tool (Rossi et al. 2006). Samples were collected from all nine clones in 2009 and for five of the nine clones over 2009 and 2010. Sampling was conducted 14 times from June 4 to September 28 in 2009 and 13 times from May 25 to September 25 in 2010. The sampling interval in 2009 and 2010 was 5–14 days (average of 8.9 d) and 5–17 days (average of 10.3 days), respectively. The obtained microcores were fixed in FAA (formalin:acetic acid:ethanol:water at a ratio of 1:1:9:9). Sections 9 μm thick were prepared using Kawamoto's film method (Kawamoto and Shimizu 2000; Cryofilm transfer kit, Leica Microsystems GmbH, Wetzlar, Germany). Several microcores were embedded in LR-White Resin (London Resin Co., Basingstoke, UK), they were then cut into sections 9 μm thick and stained with safranin and Alcian blue for detailed observation. The sections from microcores were observed using a microscope (Leica DMR; Leica Microsystems GmbH, Wetzlar, Germany) with conventional optical microscopy and polarizing microscopy.

The stage of wood formation for each section was classified as either the "earlywood stage," "latewood stage," or "cessation stage," which was determined by the radial diameter of the latest tracheids that were starting the deposition of a secondary wall. Deposition of a secondary wall was determined by the presence of birefringence in the transvers sections under polarizing microscopy (Fig. 1). Sections in which the latest tracheids depositing a secondary wall had a similar radial diameter to earlywood tracheids in the previous year were classified as the earlywood stage (Fig. 1a, b). Sections taken in May or early June that had enlarging tracheids but deposition of a secondary wall had not yet started were also classified as the earlywood stage. Sections in which the latest tracheids depositing a secondary wall had an apparently smaller radial diameter compared to their previous tracheids in the same radial file were classified as latewood stage (Fig. 1c–f, h, j). This visual criterion is different from Mork's definition (Denne 1989), because Mork's definition is difficult to apply directly on cells which is not finished their secondary cell wall thickening in the sampled microcores. *L. kaempferi* is classified as 'abrupt' (Itoh et al. 2006) concerning the transition from earlywood to latewood. In mature *L. kaempferi* wood, both radial diameter and cell wall thickness show abrupt change at the earlywood / latewood transition (Fig. 1g–j). As the results, the positions of earlywood / latewood in one growth ring differ little between our cell diameter method and Mork's definition (Fig. S2 in Online Resource). Sections in which the latest tracheids depositing secondary cell wall had radial
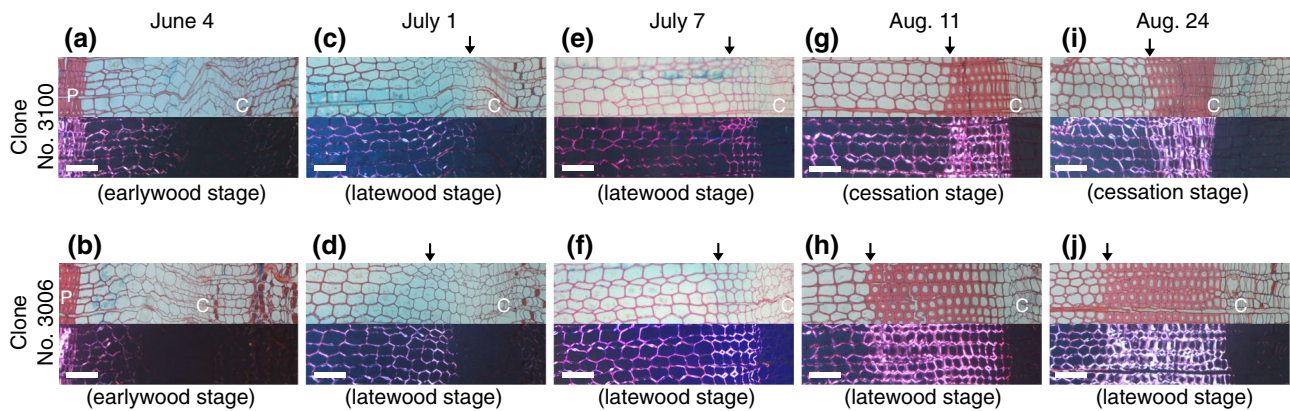
Fig. 1 Microscopy results for seasonal variation of differentiating xylem in mature *Larix kaempferi* wood. Two contrasting clones are shown. For each clone, the top image shows the optical microscopic image and the image below shows the corresponding polarizing microscopic image. C is the cambium zone, and arrow heads show the transition point from earlywood to latewood. Scale bars = 100 μm

diameters as small as the outermost tracheids in the previous year's latewood were determined to have ceased cell division (Fig. S3 in Online Resource) and classified as cessation stage (Fig. 1g, i). In cessation stage, the cell wall thickening and lignification continue but cell number and cell size have been determined. We used this classification to associate wood formation dynamics with latewood width or the proportion of latewood in this study. The timing of the transition from earlywood to latewood was determined as the first microcore sampling date in which the latewood stage was confirmed, and the timing of cessation of latewood formation was determined as the first microcore sampling date in which the cessation stage was first confirmed for each individual tree. The duration of latewood formation was calculated from the timing of transition from earlywood to latewood and the timing of the cessation stage for each individual tree.

Ring widths and latewood widths of the outermost five growth rings were measured using sections obtained from microcores sampled in 2009, which were used for the measurement of the timing of wood formation using a profile projector (PROFILE PROJECTOR PJ500, Mitutoyo, Kawasaki, Japan). Borders between earlywood and latewood were defined as the point where an apparent decrease in radial diameter of the tracheid and apparent increase in cell wall thickness of the tracheid were observed in a radial profile (Fig. 1j–j). We collected microcores 14 times in 2009. The average RWs and average latewood width (LW_s) were calculated for each tree each year (2005–2008) from width data obtained from 14 microcores per tree. Because several microcores did not contain some rings due to the limitation of the Trephor sampling tool length or where several rings had been damaged by sectioning, the number of measured sections per ring and per tree was reduced from 14 to an average of 11.9 (range 1–14). RW_s and LW_s in 2009 were

measured on a few microcores (average of 3.3, range 3–7) because the latewood width could only be measured after the cessation stage. The proportion of latewood from sections by the profile projector (PLW_s) was calculated from RW_s and LW_s as LW_s/RW_s per tree per year. Finally, LW_{s5} and PLW_{s5} were calculated as a five-year average (2005–2009) of LW_s and PLW_s for each tree, respectively. The latewood production rate (μm/day) was calculated as the ratio of LW_{s5} to the duration of latewood formation in 2009.

Statistical analyses

The magnitude of the clonal effect on the variation of ring structure components was evaluated using clonal repeatability. Repeatability (R^2) was calculated for each year and each ring structure component, using the following formula:

$$R^2 = \sigma_c^2 / (\sigma_c^2 + \sigma_e^2), \tag{1}$$

where, σ_c^2 is the clonal variance and σ_e^2 is the residual variance. Variances in (1) were obtained based on the following model:

$$y_{ij} = \mu + C_i + e_{ij}, \tag{2}$$

where, y_{ij} is the phenotypic value of a ring structure component for ramet j in clone i, μ is the ground mean, C_i is the random effect of the clone i, and e_{ij} is the residual for ramet j in clone i. The clonal value (genotypic value) for each clone for each ring structure component was predicted for each year as the best linear unbiased predictor (Henderson et al. 1959) of C_i. Variances and clonal values were estimated using the lmer package (Bates et al. 2015) in R (R Core Team 2017).

The clonal differences of traits relating the dynamics of wood formation, including the timing of transition from earlywood to latewood, the timing of cessation of cell division,

the duration of latewood formation, and the latewood production rate was tested by the Kruskal–Wallis rank sum test using the kruskal test function in *R*. The correlation coefficients of traits relating the dynamics of wood formation against PLW_{s5} were calculated.

To depict a seasonal pattern in wood formation and its clonal variation, the relationship between day of year and ring width developed up to that day was modeled using the Gompertz function in each clone for 2009. The ring width varied even between microcores obtained from the same tree because of the directional heterogeneity of ring growth in trees. Therefore, a developing ring width in a section from a microcore in 2009 was normalized as a ratio to the ring width in 2008 in that section ($RW_{normalized}$). After that, $RW_{normalized}$ modeling was executed using the following formula:

$$RW_{normalized} = A \exp(-B \cdot C^t), \tag{3}$$

where, *t* is the day number in the year when a microcore was collected, $RW_{normalized}$ is developed ring width up to *t* normalized from 0 to 1, and *A, B,* and *C* are parameters for the Gompertz function. We assumed that $RW_{normalized}$ on May 1 was zero for all trees. The SSgompertz function in R was used for fitting, and after that, the developed ring width for each clone was estimated using the following formula:

$$RW_{estimated} = RW_{normalized} \cdot RW_{s2008} \tag{4}$$

where, $RW_{estimated}$ is the estimated developed ring width up to *t* and RW_{s2008} is the average RW_s in 2008 calculated for each clone. $RW_{estimated}$ was plotted as an estimated developed ring width against *t* to show the ring growth curve for each clone.

Results

Inheritance of wood density and its sub-component traits

RW showed a trend of having a peak around stand age 10 after which RW decreased gradually with stand age (Fig. 2a), and clonal differences in RW genotypic value became unclear at higher stand ages. RD and PLW showed gradually increasing trends with stand age which leveled off after around age 25 (Fig. 2b, c). The genotypic values of RD and PLW showed similar trends against stand age, and the clonal differences of genotypic values were greater with stand age (Fig. 2b, c). The clonal differences were clear, especially in stand ages > 25. These results were compared with those of Fukatsu et al. (2015) who investigated progeny (i.e., offspring made by control crossing between the parent clones that were investigated in this study). The correlation coefficient between the clonal RD values from six clones investigated in this study and RD breeding values from the six common parents in the study by Fukatsu et al. (2015) was 0.918 for the mean values in stand ages 27–31 corresponded to the age of the maximum common outermost five rings in both studies.

Different ring structure components exhibited different age trends in clonal repeatability (Fig. 3). The repeatabilities in RD and PLW increased with stand age from only 0.2 up to 0.8. The repeatabilities in RW, ED, and LD tended to decrease with age and fluctuated more than that in RD and PLW. In particular, RW showed a severe
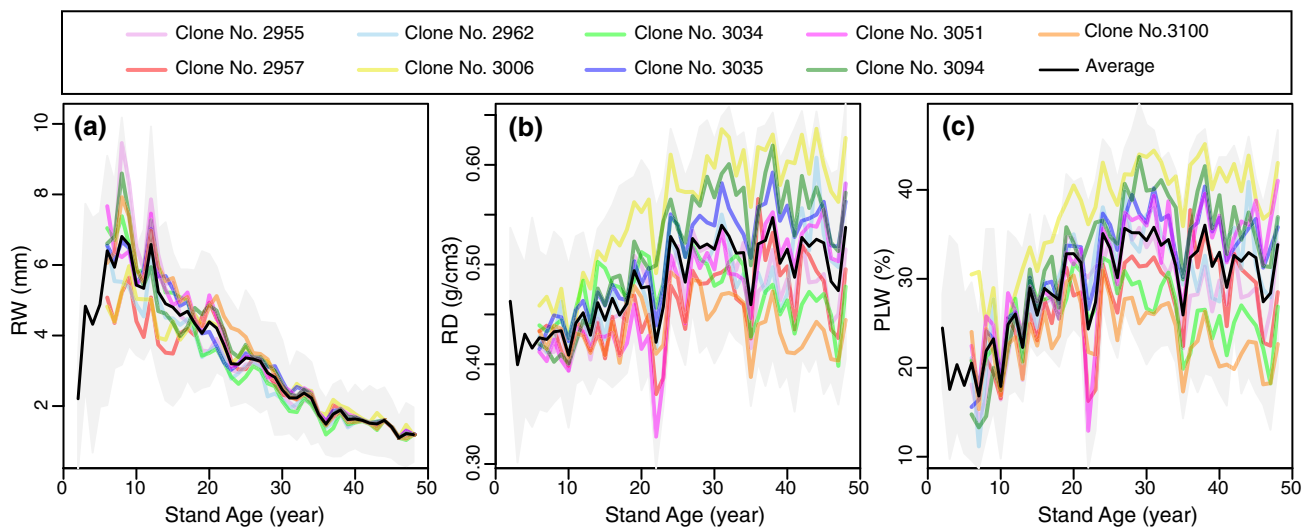

Fig. 2 *Larix kaempferi* time trends for **a** ring width (RW), **b** ring density (RD), and **c** the proportion of latewood (PLW) measured by X-ray densitometry. The range of individual values (gray area), the average value for all individuals (bold black line), and genotypic values for each clone are shown. Stand ages 6–48 are plotted
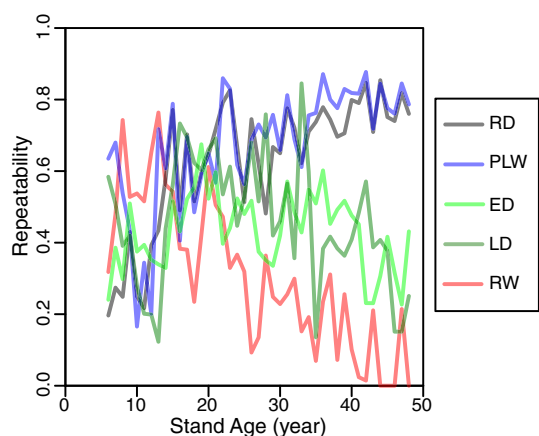
Fig. 3 Time trend of clonal repeatabilities of ring structure components of mature *Larix kaempferi* wood. Ring density (RD), proportion of latewood (PLW), earlywood density (ED), latewood density (LD), and ring width (RW) are plotted for stand ages 6–48
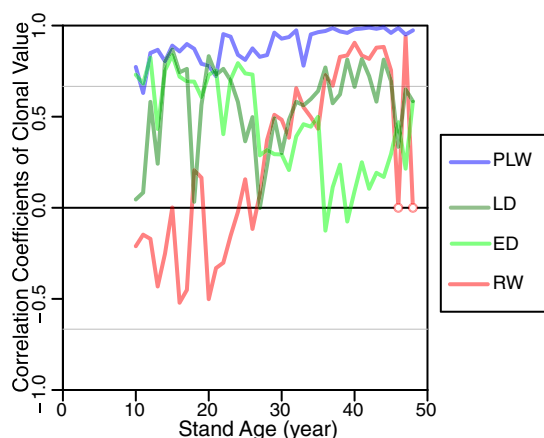

Fig. 4 Time trends of correlation coefficients of clonal values of ring density against the clonal value of other ring structure components in mature *Larix kaempferi* wood. The proportion of latewood (PLW), earlywood density (ED), latewood density (LD), and ring width (RW) are plotted for stand ages 6–48. The points where repeatability is zero and, therefore, the clonal value was estimated as zero are depicted by an open circle. $n = 9$ for each point. The gray line shows the 5% t distribution for correlation coefficients that were not significantly different from zero

decrease in repeatability from 0.7 at around age 10–0.0 at ages 44 and 45 (see Fig. 3).

When clonal RD values were compared with the clonal values in the other ring structure components, the age trend of the correlation coefficient differed between the ring structure components (Fig. 4). PLW showed a constant high positive correlation with RD and the correlation coefficients were always significant ($p < 0.05$). The correlation coefficients between RD and RW tended to increase with age from negative (but not significant, $p > 0.05$) to high positive (significant in ages > 35, $p < 0.05$). ED showed several

significant correlations before stand age 25. LD showed significant correlations after stand age 29.

The relationship between RW and PLW for each clone after age 25, which was the age that the time trends in RD variation became relatively stable (Fig. 2), varied depending on the clones (Fig. 5). Seven clones showed a significant positive correlation with each or several individuals, although other clones showed a non-significant correlation.

Clonal variation in the dynamics of wood formation

The variation in the timing of transition from earlywood to latewood was small between individuals and between clones (Fig. 6a; Table 1). Transition to latewood was observed in sections obtained from July 1 to 7 and from July 2 to 9 in 2009 and 2010, respectively, with the exception of an individual tree in which the transition was observed on July 20, 2010. Clonal differences in the timing of transition was not significant in 2009 and 2010, and the timing range was approximately 6 days in clonal mean for both years (Table 1).

The timing of cessation of latewood cell division was observed from July 21 to September 7 in 2009, and from August 2 to September 25 in 2010 (Fig. 6a; Table 1). The clonal mean difference in timing was large, with a range of approximately 40 days. The clonal difference in the timing of cessation of latewood cell division was significant in both years.

The average duration of latewood formation was 50.3 days in 2009 and 58.5 days in 2010 in the clonal mean base (Table 1). The clonal difference was significant in 2009 ($p < 0.01$) but was not significant in 2010 ($p = 0.061$), and the range between clones was 40.7 days in 2009 and 45.0 days in 2010.

The duration of latewood formation was significantly correlated with the timing of cessation of cell division in 2009 and 2010; individual Kendall's rank correlation coefficients were 0.989 ($p < 0.001$) in 2009 and 0.880 ($p < 0.001$) in 2010, and the clonal mean was 0.990 ($p < 0.001$) in 2009 and 1.00 ($p < 0.05$) in 2010. The correlation of the duration of latewood formation with the timing of latewood transition was not significant for either year.

Relationship between the dynamics of wood formation and the width and proportion of latewood

A positive correlation was observed between the duration of latewood formation and LW_{s5} and Pearson's correlation coefficients were 0.529 ($p < 0.01$) for individual base and 0.781 ($p < 0.05$) for the clonal mean base. Higher positive correlation was observed between the duration of latewood formation and PLW_{s5} (Fig. 7). Pearson's correlation
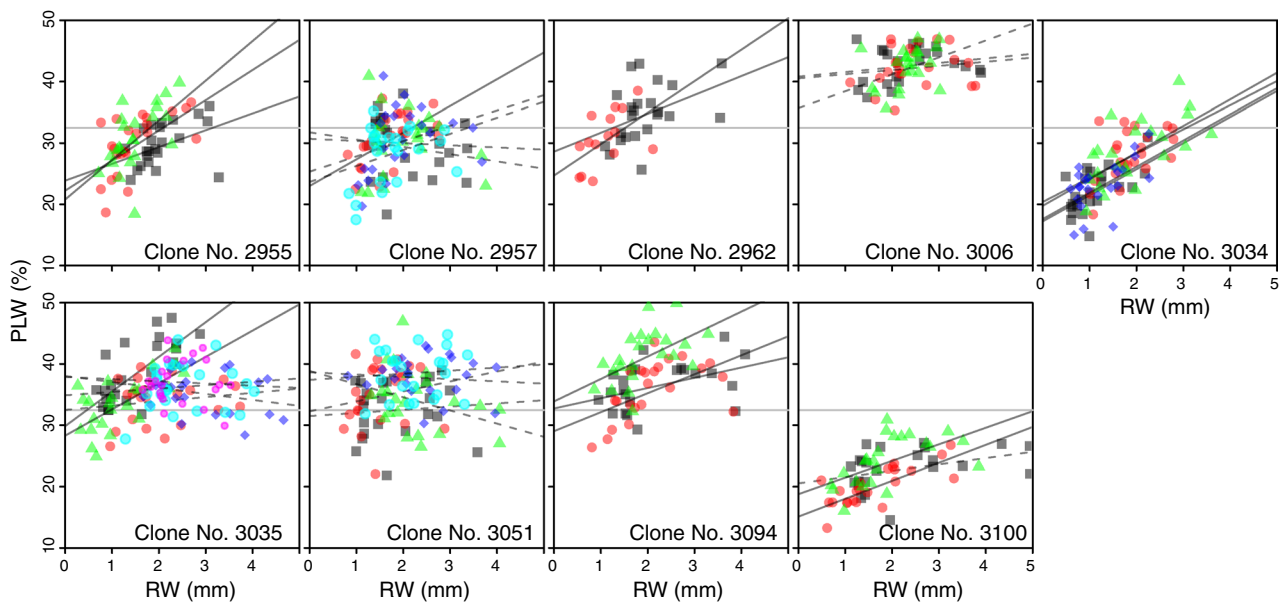
Fig. 5 Intra-tree relationship between ring width (RW) and the proportion of latewood (PLW) in mature *Larix kaempferi* wood. Data from stand ages > 25, which is the ages where increasing trend in RD and PLW against stand age are leveled off, were plotted. Each panel shows several individuals of one clone. The different symbols in each panel represent different individuals. Black solid and dotted lines are the regression line for each individual; a solid line represents a correlation coefficient that is significantly different from zero ($p < 0.05$), and a dotted line represents a correlation coefficient that is not significantly different from zero ($p > 0.05$). A gray horizontal line represents the ground mean for individual PLW

coefficients were 0.829 ($p < 0.001$) for the individual base and 0.925 ($p < 0.001$) for the clonal mean base. A weak correlation was observed between the latewood production rate and PLW_{s5}: correlation coefficients were 0.405 ($p < 0.05$) for the individual base and 0.497 ($p = 0.173$) for the clonal mean base. The clonal difference in the latewood production rate was not significant ($p = 0.297$).

The wood formation pattern for each clone represented by $RW_{estimate}$ and meteorological data are depicted in Fig. 8. Patterns of wood formation were different between clones. There were several crossings among the curves, indicating that some clones that grew faster in the earlywood stage might stop growing earlier in the latewood stage (such as clone No. 2955), and that clones that grew moderately during earlywood formation continued growing at a relatively high rate during latewood formation (clone No. 3094).

Discussion

The clonal value of ring density obtained in this study had a high correlation with breeding values obtained from the progenies in a previous study (Fukatsu et al. 2015), irrespective of the difference in propagation methods and growing environment. Based on this result, we concluded that the inheritance of wood density presented in this study can be included in general discussions on the inheritance of wood density in *L. kaempferi* and that the causal factors of wood density variation are worth discussing. We dissected wood density into sub-component traits and further investigated the dynamics of wood formation. Hereafter in this paper, we discuss the mechanism that causes a genetic variation in wood density based on a stepwise and hierarchical approach from the smaller scale to the larger scale.

Transition from earlywood to latewood

This study revealed a very small variation in the timing of transition from earlywood to latewood. This means that the timing of transition is not a causal factor in the variation in the proportion of latewood and variation in wood density between clones.

The transition from earlywood to latewood occurred at the beginning of July for all sampled trees except one tree in this study. Imagawa and Ishida (1970) reported that the transition date from earlywood to latewood was July 4 for *L. kaempferi* growing on Hokkaido Island, Japan. Rossi et al. (2009) reported that the transition date was the end of June for *Larix decidua* (European larch) in Italy, similar to our results. Rossi et al. (2009) reported a transition date of mid-August for *Pinus cembra* (Swiss pine) and *Picea abies* (Norway spruce), and Grotta and Gartner (2005) reported a transition date from the end of June to the middle of July for *P. menziesii*. Funada et al. (1987) and Funada et al. (1990)
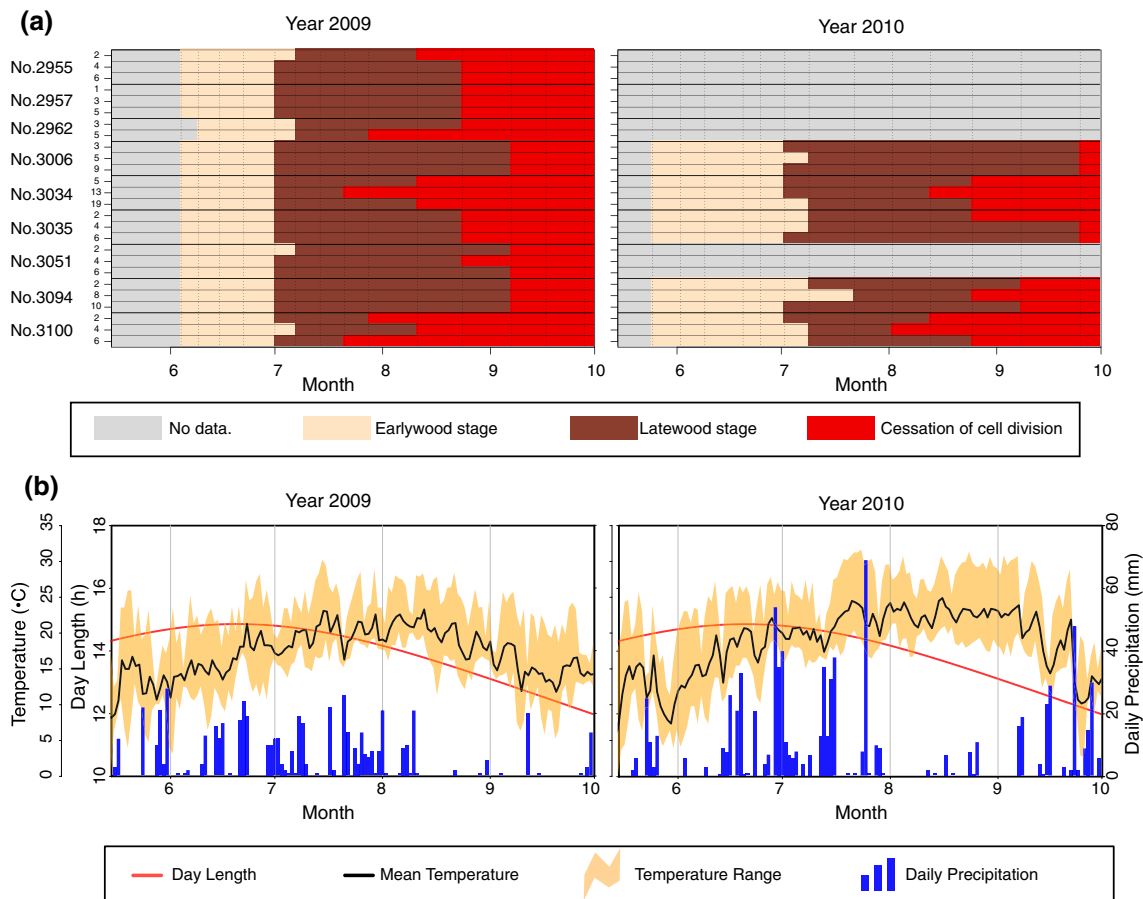
(a)

(b)

Fig. 6 Seasonal pattern of wood formation in mature *Larix kaempferi* wood and meteorological data. In **a** seasonal pattern of wood formation are depicted for 2009 and 2010. No. represents the clone number. The small numbers after a clone No. represents the individual numbers for each clone. One row shows one individual. Two to three individuals for each clone were measured. In **b** meteorological data, day length, daily mean temperature, temperature range between a daily maximum and minimum temperature, and daily precipitation are shown for 2009 and 2010. Temperature data were obtained from weather station No. 48,331 (Karuizawa) of the automated meteorological data acquisition system that was set by the Japan Meteorological Agency. The station is about 8 km in distance and less than 30 m in altitude from the test site. Day length were calculated from latitude, longitude, and altitude of the test site using the method presented in Nagasawa (1999)

Table 1 Summarized results of the dynamics of wood formation in mature *Larix kaempferi* wood

Trait	Year	Individual value				Clonal mean				Clonal difference *p* value
		n	Average	SD (days)	Range (days)	*n*	Average	SD (days)	Range (days)	
Date of latewood transition	2009	26	July 2	2.4	6.0	9	July 2	2.0	6.0	0.125 ns
	2010	15	July 6	5.5	20.0	5	July 6	2.7	6.7	0.743 ns
Date of cessation of cell division	2009	26	Aug. 21	15.3	48.0	9	Aug. 21	14.2	38.7	0.005**
	2010	15	Sep. 3	18.5	54.0	5	Sep. 3	17.4	42.7	0.030*
Duration of the latewood formation	2009	26	51.6 d	15.0	48.0	9	50.3 d	14.0	40.7	0.005**
	2010	15	58.5 d	20.2	61.0	5	58.5 d	17.9	45.0	0.061 ns

Range is the difference between the minimum and maximum value

The clonal difference p value was obtained by Kruskal–Wallis rank sum test

sd is standard deviation, *ns* non-significant
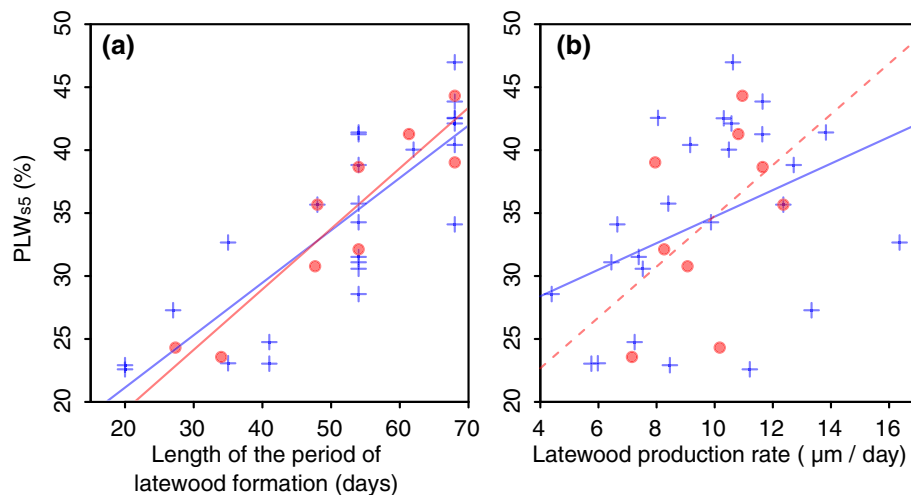
*$p < 0.05$

**p value < 0.01

Fig. 7 Relationship between the averaged proportion of latewood in the latest 5-year ring (PLW$_{s5}$) against **a** the period of latewood formation and **b** the latewood production rate in mature *Larix kaempferi* wood. PLW$_{s5}$ was measured from microcores. The duration of latewood formation and the latewood production rate was measured in 2009. The cross symbol represents an individual value and a filled circle the clonal mean. A black line represents the regression line for individual values and a gray line represents the regression line for the clonal mean. The coefficient of determination (R^2) is 0.688 and 0.856 for individual values and clonal means, respectively, in (**a**), and 0.164 and 0.247 for individual values and clonal means, respectively, in (**b**). A solid line shows that the correlation coefficient is significantly different from zero ($p < 0.05$) and the dotted line shows that the correlation coefficient is not significantly different from zero ($p > 0.05$)

Fig. 8 Estimated growth curves in mature wood of nine *Larix kaempferi* clones. The estimated growth curve for estimated ring width developed up to a specific date (RW$_{estimated}$) are plotted against date for each clone for 2009. A thin line and bold line represents the earlywood stage and latewood stage, respectively
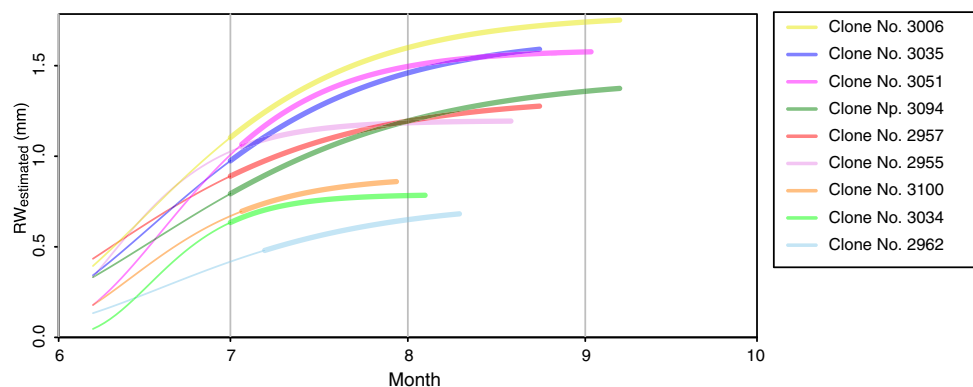
reported that the transition from earlywood to latewood in *Cryptomeria japonica* (Japanese cedar) and *Pinus densiflora* varied from early May to mid-August and late May to mid-September, depending on the crown proportion of sampled tree and vertical sampling position in the stem. Nagao et al. (1985) also investigated *C. japonica* and reported that the transition occurred from early June to August depending on the clones. The transition from earlywood to latewood depends on the species and on individual trees or genotypes within a species, whereas a similar transition timing has been reported for different *Larix* species and individual trees. Concerning the cause of transition from earlywood to latewood, Antonova and Stasova (1997) stated that the formation of latewood is initiated by water stress in *Larix sibirica*. Begum et al. (2016) summarized that rapid decrease in temperature might be the critical factor in the control of

latewood formation. In our research, the meteorological condition around the timing of the transition from earlywood to latewood was, no consistent decrease of temperature or shortage of precipitation was observed in 2 measured year (Fig. 6b). Some authors thought that the transition from earlywood to latewood was associated with shoot growth in coniferous species (Larson 1964; Jayawickrama et al. 1997; Devine and Harrington 2009), although Renninger et al. (2006) argued that the two events occurred at the same time without any causal relationship. *Larix* species are characterized as having a deciduous habit and show clear differences in their shoots (long or short) and differences in growth pattern morphology and phenology (Owens and Molder 1979). In addition, *Larix* species was reported to cease shoot elongation around the end of June (Rossi et al. 2009; Swidrak 2013). This suggests a possible sensitivity of larch to the

summer solstice compared to evergreen conifers. The above argument could explain the similarity in transition timing in *Larix* species from earlywood to latewood in individual trees, genotypes, and species, irrespective of the variable environments.

The small genetic variation in the timing of transition from earlywood to latewood in this study can also be explained by differences in the natural distribution range among species. In previous reports on genetic variations in the timing of transition from earlywood to latewood, the variations were 17 and 47 days among *P. menziesii* families (Vargas-Hernandez and Adams 1994) and *P. taeda* families (Jayawickrama et al. 1997), respectively. In this study, the variation was smaller than those in the above reports, at only 6 days between clones. The natural distribution of *L. kaempferi* is limited to a small latitudinal range; from 35°N to 37°N (Hayashi 1951; see Figure S1 in Online Resource) and from 35°N to 38°N when a northern small relic population is considered (Yano 1994). This is in contrast to species that have a much broader natural distribution area, such as 19°N–55°N in *P. menziesii* (St Clair et al. 2005) and from around 30°N to 40°N in *P. taeda* (Little 1971). The large natural distribution of these species causes genetic differences in the timing of transition from earlywood to latewood through the adaptation to various local environments.

Cessation of cell division

A large clonal variation in the timing of cessation of cell division was observed in this study (Fig. 6a; Table 1). The possible over- or under-estimation of clonal variation would exist in this research caused by not a large (three or two) number of ramet for each clone, whereas the difference was statistically significant in 2009. This study also showed that the cessation timing determined the duration of latewood formation due to the very small variation in the timing of transition from earlywood to latewood. This is in contrast to *P. menziesii* results, in which the timing of transition determined the duration of latewood formation (Vargas-Hernandez and Adams 1994).

The triggers for the cessation of latewood cell division are not fully understood. The cessation of cambial activity was not associated with the amount of indole-3-acetic acid (IAA) in the cambial region (Funada et al. 2001; Uggla et al. 2001). Funada et al. (2001) mentioned that the seasonal change in cambial responsiveness to IAA was caused by the temperature and/or the photoperiod. Begum et al. (2016) reported that cambium dormancy was induced by local cooling of the trunk in *C. japonica*. By contrast, at the time when most clones went into the cessation of cell division stage (early to late August in 2009), the temperature range was higher compared to the temperature during earlywood formation in this study (as shown in Fig. 6b). On the other hand, comparing between the two investigated years, cell division continued longer term in 2010 than in 2009 (Fig. 6a), and days with high mean temperature continued until early September in 2010 (Fig. 6b). Therefore, temperature would affect the timing of cessation of cell division but cannot solely explain the timing of cessation of cell division and its genetic variance in *L. kaempferi*. Photoperiod and temperature are the main factors that mediate growth cessation in plant (Maurya and Bhalerao 2017). In addition, the cessation timing of tracheid production varies depending of the vertical position in a stem (Sundberg et al. 1987; Funada et al. 1990). The effect of a combination of extrinsic factors, including the level and changes in photoperiod and temperature, consequent internal factors including the amount and change of phytohormones and carbohydrates, some of which are regulated in the tree crown, and clonal differences in responsiveness to those factors should be considered in future research.

Causal factors of wood density variation

The proportion of latewood was highly correlated with wood density at the clonal level (Fig. 4). This result is consistent with research on *L. kaempferi* using clones (Nakada et al. 2005) and families in progeny tests (Fujimoto et al. 2006; Fukatsu et al. 2013). Clonal repeatability stabilized at high values after age 25. This result is consistent with the results of Fukatsu et al. (2013).

The proportion of latewood was strongly correlated with the duration of latewood formation in this study. On the other hand, the latewood production rate was not correlated with the proportion of latewood in clonal means. This indicates that the duration of latewood formation is much more important than the latewood production rate in terms of the mechanism of genetic difference in the proportion of latewood.

Our results revealed a hierarchical relationship in the variation in wood density between individual trees and between *L. kaempferi* clones. First, the timing of the cessation of cell division determined the duration of latewood formation. Second, the duration of latewood formation controlled the variation in the proportion of latewood. Finally, the genetic variation in the proportion of latewood caused the genetic variation in wood density. Our hypothesis that the timing of latewood formation affects the variation in wood density was confirmed. Here the timing of the cessation of cell division was determined by the latewood formation and the timing of transition from earlywood to latewood had little effect. Our results also confirmed that the dissection of wood density into sub-component traits was an effective method to understand wood density variation between trees.

The variation in the proportion of latewood can be explained by the duration of latewood formation, which was as much as 85.6 and 68.8% in the clonal mean and individuals, respectively (Fig. 7a), indicating that the formation of earlywood has a small effect in terms of understanding the genetic variation of wood density. Vargas-Hernandez and Adams (1994) showed a moderate negative genetic correlation between the date of initiation of earlywood formation and wood density, but there are no other reports on the genetic variation of earlywood formation dynamics and its relationship with wood density. Further research is necessary from this perspective.

Effects of growth on wood density variation

The relationship between wood density and growth traits has been mentioned in many reports. Zobel and Van Buijtenen (1989) summarized species-dependent relationships and mentioned the difficulty of generalizing this relationship. Bouffier et al. (2009) reported a low correlation between wood density and growth in *Pinus pinaster* (maritime pine) at the phenotype level and a negative or positive correlation at the genetic level. Fukatsu et al. (2015) reported a positive genetic correlation between growth and wood density but no residual correlation between them in *L. kaempferi*.

It is meaningful to consider those relationships from the perspective of wood formation dynamics. As shown in Fig. 5, the intra-tree correlations between ring width and the proportion of latewood was generally positive but it seemed to depend on clones. If the latewood proportion of mature wood was mainly determined by the duration of latewood formation, our results (Fig. 5) imply that some clones ceased latewood formation earlier with a decrease in ring width, whereas some clones maintained their latewood formation duration irrespective of the decrease in ring width. This clone/individual dependent relationship between the proportion of latewood and ring width would cause a change in the clonal rank of the proportion of latewood depending on the age or climate when the rings were formed. Nonetheless, there was a large difference in the proportion of latewood between clones when the same ring width was compared (Fig. 5). Therefore, the clonal difference of the proportion of latewood and the difference in the duration of latewood formation would need to be dissected into growth-dependent part and growth-independent part.

Wood formation is mainly affected by environmental change indirectly via dynamic changes in several factors, including the amount of photosynthate or phytohormones, which is caused by the direct environmental effect on vegetative growth in a tree crown (Larson 1964), except for factors such as direct sensing of temperature in the tree trunk (Begum et al. 2016). It remains unclear where these genetic differences exist in whole-tree processes that concern the dynamics of wood formation. While our results have provided evidence for the understanding of genetic differences of wood density, more intensive researches concerning the dynamics of wood formation, including the genetic difference of whole-tree processes, are required.

Conclusion

The present study demonstrates that wood density is highly correlated with the proportion of latewood at the clonal level, and the proportion of latewood was associated with the genetic difference in wood formation dynamics. Our results describe the hierarchical relationship of the genetic difference of causal components that explain clonal differences in wood density in mature *L. kaempferi* wood in the following sequence from smaller scale to larger scale; the timing of cessation of latewood formation, the duration of latewood formation, the proportion of latewood, and wood density. We found that the timing of transition from earlywood to latewood were similar among genotypes, and species dependency on wood formation dynamics were discussed. The mechanism causing the base difference, i.e., the genetic difference of the timing of cessation of latewood formation, is still unclear and requires further study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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