Variation in the amount of pollen per male flower on *Abies sachalinensis*

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Abstract

It is important to evenly increase the amount of scattered pollen per clone for maintaining high genetic variation in clonal seed orchards. It is thus imperative to determine not only the number of male flowers per tree, but also the amount of pollen per male flower in each clone. In this study, the existence of annual variation on the fresh weight of male flowers (FW) and the clonal and annual variation, and ramets' variation with the same or different tree ages on the weight of pollen per male flower (WP) were investigated using 21 *Abies sachalinensis* clones for 3 years. The results indicated that there were significant differences in FW and WP among clones each year and the relationships between FW and WP were linearly significant for every year. WP also showed significant annual variation, while there was also significant variation in ramets. The clonal repeatability regarding WP was 0.37—0.47 for data obtained for 2 or 3 years and the generalized linear mixed models with the random effects of clone, year, and ramet indicated that the effect of clone more strongly affected WP than other effects. These results suggested that WP on *A. sachalinensis* is a trait that is considerably influenced by clonal characteristics; there is thus a need to characterize this trait in each clone when using seed orchards.

Keywords: *amount of pollen per male flower, clonal variation, annual variation, ramet's variation, Sakhalin fir*

Introduction

Clonal seed orchards are expected to provide a large amount of seed with high genetic variation. To maintain high genetic variation, it is important to evenly increase the amount of scattered pollen per clone. When the same number of trees are planted for each clone in a seed orchard, amount of scattered pollen per clone is determined by multiplying the number of male flowers by the amount of pollen per male flower (Schoen and Stewart, 1986). This suggests the need to consider not only the number of male flowers but also the amount of pollen per male flower in order to determine the amount of scattered pollen per clone.

In a seed orchard, it has been reported that there are major variations in the number of male flowers among clones, while this variable also exhibits annual variation in each clone, for tree species such as Norwegian spruce, Scots pine and red pine (Nikkanen and Ruotsalainen 2000, Sivacioğlu 2010, Kang and Lindgren 1998). Burczyk and Chalupka (1997) also reported that the 25 % of clones with the best male flowering produced 45 % of total pollen in a Scots pine clonal seed orchard. These findings suggest that, when the same number of trees are planted in each clone in a seed orchard, the amount of scattered pollen per clone is heterogeneous each year. Similarly, some reports have been published about the variation in the amount of pollen per male flower. For example, it has been reported in *Cryptomeria japonica* that there is marked variation among clones in terms of the amount of pollen per male flower and that the pollen production of individual trees is not high if the amount of pollen per male flower is small (Kondo and Hakamada, 2007). Similarly, in *Betula pendula*, Ranpal et al. (2022) reported major annual and ramets' variation in the pollen production per catkin in each clone. These findings suggest that the amount of scattered pollen per male flower is also heterogeneous among clones each year. Therefore, to

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investigate the amount of scattered pollen in a clonal seed orchard, the clonal, annual and ramets' variation of not only the number of male flowers but also the amount of pollen per male flower must be considered.

Abies sachalinensis is a coniferous tree species distributed on Hokkaido, southern Kuril Islands and Sakhalin, and afforested actively in Japan. As a tree breeding project, more than 700 plus trees of *A. sachalinensis* have also been selected on Hokkaido and 19 seed orchards are now established using these plus trees. In the seed orchards of *A. sachalinensis*, the numbers of male and female flowers have been investigated in each tree over many years, with the results showing large variations in the numbers of male and female flowers among clones and also an annual variation in these numbers in each clone (Sugata, 1984). However, although major variations among clones have also been reported regarding the size of cones and the number of seeds produced per clone of *A. sachalinensis* (Itahana et al. 1995), the clonal variations, annual variations, and the ramets' variations in trees with the same or different ages regarding the amount of pollen per male flower have not been determined. Most of *A. sachalinensis* clonal seed orchards on Hokkaido are now too old to produce many seeds, so they need to be reconstructed by using new clonal seedlings. Therefore, determination of the variation in these variables of *A. sachalinensis* should enable utilization of the number and arrangement of each clone for the reconstruction of each clonal seed orchard with maintaining high genetic variation.

In this study, male flowers just before flowering were collected from 21 plus tree *A. sachalinensis* clones in 3 consecutive years. After that, the relationship between the number of male flowers and the amount of pollen per male flower was analyzed to determine whether it was significant. Clonal variation, annual variation, and ramets' variation with the same or different ages were also determined regarding the amount of pollen per male flower. Furthermore, the relationship between the number of male flowers and the amount of pollen per male flower in each tree was investigated to determine whether the former affects the latter. From the obtained results, the trait of the amount of pollen per male flower was characterized and a method for managing clonal seed orchards was also proposed.

Materials and methods

Study site and sample trees

This study was conducted at the Forest Tree Breeding Center, Hokkaido Breeding Office located in Ebetu City, Hokkaido, Japan, from May 2020 to June 2022. Twenty-one clones were used for the study and all of the sample trees except one (a seedling) were grafted. In each year from early to mid-May, 30-70 male flowers were randomly collected just before flowering from each *A. sachalinensis* plus tree. The collect rate was more than half for trees with less than 11 years old and less than 10 % for trees with 50 years old. The numbers of clones and ramets used, and the number of male flowers collected and measured in each year were determined, as shown in Table 1. Diameter at breast height was around 30 cm for trees at 50 years old, 21.5 cm for a tree at 33 years old, around 3 cm for trees at 10 years old, and 0.5cm for a tree at 4 years old.

Male flowers

Male strobilus are egg-shaped and the length of their long diameter is around 1cm. They are borne on branches from the previous year at the position of the leaf axil. They consist of approximately 100 pollen sacs.

Measurements

After collecting male strobilus, their fresh weight (FW) was immediately measured in 2020 and 2021. Furthermore, in all years, collected male flowers were placed on medicine wrapping paper indoors until June. Subsequently, the weight of pollen per male flower (WP) and the weight of pollen sacs per male flower (WS) were measured, except for strobilus, containing at least one unpunctured pollen sac, as determined using a stereoscopic microscope.

In 2022, to determine the relationship between the number of male flowers and the amount of pollen per male flower in each tree, the number of male flowers per tree was investigated for the sample trees at 50 years old. The number of male flowers per tree, i.e., male flowering index, was categorized into five classes as follows:

1: trees with no male flowers;
2: trees with male flowers covering less than one-quarter of the crown;
3: trees with male flowers covering from one-quarter to one-half of the crown;
4: trees with male flower covering from one-half to three-quarters of the crown;
5: trees with male flowers covering more than three-quarters of the crown

Statistical analysis

For statistical analysis, R Ver3.4.2 or Statistica 03J was used. The following traits were analyzed. In each year, overall relationships between FW and WP, and WS and WP were plotted graphically to determine whether they were significant. The significance of the intercept and the slope of the regression line between or among years was also tested by ANCOVA. Since the overall data of WP were not normally distributed for all years (Kolmogorov-Smirnov test, $P < 0.01$), one-way ANOVA or t-test for clonal variations, annual variations, and ramets' variations with the same or different tree ages on WP was conducted after logarithmic conversion (Kolmogorov-Smirnov test, $P > 0.05$). Clones used in each item are shown in Table 2. Clonal repeatability (the fraction of the variance that is genetic among clones, H^2) was estimated employing clones used for annual variations as follows:

$$H^2 = \sigma_c^2 / (\sigma_c^2 + \sigma_e^2)$$

where σ_c^2 is the variance among clones and σ_e^2 is the variance within a clone. The relationship between the number of male flowers and the weight of pollen in each tree was also plotted graphically to determine whether it was significant.

Table1
Tree age of sample trees and number of ramets used, male flowers sampled, and male flowers measured in each year

Clone	Tree age in 2020	Year					
		2020		2021		2022	
		Ramets used	Male flowers measured/ sampled	Ramets used	Male flowers measured/ sampled	Ramets used	Male flowers measured/ sampled
1	10	1	27/30	1	19/41	1	12/40
2	10	1	21/30				
3	10	1	27/35				
4	10	1	7/35				
5	10	1	5/30				
6	10	1	3/40				
7	50	1	24/35	1	37/40	3	93/110
8	50	1	19/30	1	25/32	4	114/130
9	50	1	23/35	1	20/33	4	125/148
10	50			1	17/32	1	33/34
	10	1	15/35	1	15/43	1	24/42
11	50			1	13/30	1	73/114
	10	1	6/35	1	17/38	1	42/51
12	33			1	36/44	1	33/37
	4			1	26/32	1	31/32
13	10			1	13/15	1	37/41
14	50			1	16/36	3	82/102
15	50			1	18/31	3	92/96
16	50			1	9/41	3	92/103
17	50			1	32/41	2	65/67
18	50			1	23/31	3	111/112
19	50			1	33/35	3	81/100
20	50			1	19/33	3	106/119
21	50			1	29/30	3	92/98
Sum.		11	177/370	19	417/658	42	1338/1576

Table 2
List of clones used for each analysis

Items	Year	Tree age	Clones used
Clonal variation	2020	10	1, 2 , 3, 4, 5, 6, 10, 11
		50	7, 8, 9
	2021	10	1, 10, 11, 13
		50	7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 19, 20, 21
	2022	10	1,10,11,13
Annual variation	2020-2022	10	1, 11
		50	7, 8, 9
	2021-2022	4	12
		10	13
		33	12
		50	10, 11, 14, 15, 16, 17, 18, 19, 20, 21
Variation among ramets	2022	50	7, 8, 9, 11, 14, 15,
Variation among tree age	2021 and 2022	10 vs. 50	10, 111
		4 vs. 33	12

1 = Same ramets of 50 years old tree was used on both years

Table 3
Equation of model and number of clones, years and ramets in each analysis

Formula	Equation of model	No. of clones	No. of years	No. of ramets
1	WP~WS+(1|clone)+(1|year)	5	3	
2	WP~WS+(1|clone)+(1|year)	13	2	
3	WP~WS+(1|clone)+(1|ramet)	13		4
4	WP~WS+(1|clone)+(1|year)+(1|ramet)	3	2	4

Goal of each formula is as follows: formula 1 is the comparison with clone and year that affects the WP more, formula 2 is the comparison with clone and year that affects the WP more , formula 3 is the comparison with clone and ramet that affects the WP more, and formula 4 is the comparison among clone, year and ramet that affects the WP most.

Table 4
Results of variance from random effects for each formula

Formula	Groups	Variance (Std. Dev.)
1	Clone	7.91 (2.81)
	Year	0
	Residual	6.15 (2.48)
2	Clone	7.28 (2.70)
	Year	4.15 (2.04)
	Residual	3.94 (1.99)
3	Clone	12.29 (3.51)
	Ramet	1.61 (1.27)
	Residual	3.32 (1.82)
4	Clone	3.19 (1.79)
	Year	1.12 (1.06)
	Age	0.65 (0.81)
	Residual	3.47 (1.86)

Four generalized linear mixed models were used to evaluate the factors that may contribute to WP, indicating that the variance of each random factor is an indicator that shows the influence of WP. The first formula was as follows: the model included WS as a fixed effect and the intercepts of the differences between each clone and each year as random effects using five clones with the data for 3 years. The second formula was as follows: the model included WS as a fixed effect and the intercepts of the differences between each clone and each year as random effects using 13 clones with the data for 2 years. The third formula was as follows: the model included WS as a fixed effect and the intercepts of the differences between each clone and each ramet as random effects using 13 clones with data for multiple ramets of the same age. The fourth formula was as follows: the model included WS as a fixed effect and the intercepts of the differences among each clone, each ramet, and each year as random effects using three clones with data for trees of multiple ages. Equations of the models for each formula are shown in Table 3.

Results

Figure 1 shows histograms of overall fresh weight (FW), weight of pollen per male flower (WP) and pollen sacs per male flower (WS) in each year. The ranges of FW were 30—314 mg and 37—253 mg in 2020 and 2021, those of WP were 3.2—24.7 mg, 2.8—22.1 mg, and 3.7—29 mg in 2020—2022, and those of WS were 3.3—26.6 mg, 3.1—18.9 mg, and 2.3—22.9 mg in 2020—2022, respectively. The ranges for FW, WP and WS were not markedly different between or among years. None of the distributions of each graph was consistent with a Gaussian distribution ($P < 0.01$).

Figures 2 and 3 show the overall relationships between overall WP and FW, and WP and WS in each year. The relationships between WP and FW were significant in both years ($P < 0.01$) and the values of the intercept and the slope were almost the same for all years (ANCOVA, $P > 0.05$). Moreover, the relationships between WP and WS were linearly significant for 3 years ($P < 0.01$) and the value of the slope in 2022 was significantly different from those in 2020 and 2021 (ANCOVA, $P < 0.01$).

Figure 4 shows the clonal variations in WP in each year at each tree age. Significant variations were detected for all years and tree ages ($P < 0.01$) and the differences between the minimum and maximum values of the mean WP in each clone were 1.96-, 1.89-, 2.71-, 2.54- and 1.43-fold, respectively.

Figure 5 shows the annual variations in WP for 2 or 3 years in each clone. F or t values obtained by ANOVA for each clone are also shown in the graph. Significant variations were detected for three of the five clones investigated for 3 years and 12 of the 14 clones investigated for 2 years ($P < 0.01$). There were more than 2-fold differences in the mean WP in each year in clones 11 and 18. The mean WPs in 2022 were larger than those in 2021 for 14 of the 18 cases, suggesting that WP tended to increase with conjunction with each clone. Clonal repeatabilities for WP were 0.47 and 0.37 for clones investigated for 3 and 2 years, respectively.

There were no significant differences in WP for 4 out of 12 clones (ANOVA, $P > 0.05$, Figure 6). In clone 18, there was more than 2-fold difference among ramets for WP, but the differences were less than 1.5-fold among ramets on the other 7 clones. In clones 10 and 11 (Figure 7), there were significant differences in WP between the ramets in 2022 and in both years, respectively (*t*-test, $P < 0.01$), whereas no significant differences were found in clone 12 in both years (*t*-test, $P > 0.05$). There was a greater than 2-fold difference in mean WP for clone 10 in 2022, but the ranges were less than 1.5-fold for clone 11 in both years. In clones 11 and 12, the mean WP was higher in ramets with a younger age. The WP decreased slightly with the number of male flowers, with a slope value of —1.18 (Figure 8). The regression line was of marginal significance, with the *P* value of 0.054. Upon applying formula 1, the variance was 0 in the group of year, whereas it was 7.91 in the group of clone (Table 4). Similarly, upon applying formula 2, the variance was 4.15 in the group of year, whereas it was 7.28 in the group of clone. Using formula 3, the variance of clone was much higher than that of ramet. Finally, with formula 4, the variance of clone was higher than those of year and ramet.

Discussion

This study revealed that the WP of *A. sachalinensis* differed among clones, among years, and among ramets with the same and different ages. It also decreased slightly with the number of male flowers per tree. Therefore, the WP varies under the influence of multiple factors.

The FW and WP did not show a Gaussian distribution (Figure 1) with the distribution showing a bias to the right. This suggests that in a *A. sachalinensis* tree, the mean FW and WP is larger than the mode of them due to the production of some extremely large male flowers. Since the WP increased linearly with increasing FW and WS (Figs 2 and 3), WP is easily predicted by FW or WS. Klinkhamer and Catharina (1999) also suggested that the corolla length is highly correlated to the number of pollen grains. In this study, however, significant annual variation was detected in the slope of the relationship between WP and WS (Fig.3). Moe (1998) suggested that, in *Alnus incana*, the high temperatures during June-October affected the length of the growing season and also pollen development. Hicks et al. (1994) also reported that, in *Betula*, the abundance of flowering was significantly correlated with the thermal sum of the previous year. Meanwhile, in *A. sachalinensis*, Seki (2016) reported that the difference between the mean temperature of the previous year in June and that of the present year in June was significantly positively related to the number of female flowers produced. This suggests that the difference of the mean temperature also influences the production of male flowers. Indeed, the differences among 2019—2020, 2020—2021, and 2021—2022 in Ebetu City were 0.54, —0.19 and —1.18, respectively, suggesting that the largest difference for 2021—2022 may have affected pollen production.

Significant annual variations were shown in 14 out of 18 clones in this study (Fig. 4). Damialis et al. (2011) reported that, in anemophilous woody taxa, environmental factors affected pollen production. Meanwhile, Ranpal et al. (2022) also reported that the pollen production per catkin on *Betula pendula* varied annually. They suggested that the degree of influence of environmental factors differed among clones. In this study, although the pollen production in 2022 increased dramatically compared with that in 2021 for many clones, some clones instead showed a decrease, suggesting that environmental factors affect the annual variation and also that the degree of this influence affects the difference of response of each clone.

Similarly, significant variations among ramets with the same and different ages were also detected for some clones regarding WP (Figs. 6 and 7). Furthermore, the WP with an older age was not always higher than that with a younger age, suggesting that the WP was not dependent on the tree size. Ranpal et al. (2022) also reported large variations among ramets with the same age and suggested that these variations might be caused by resource imbalance of individual trees. Therefore,

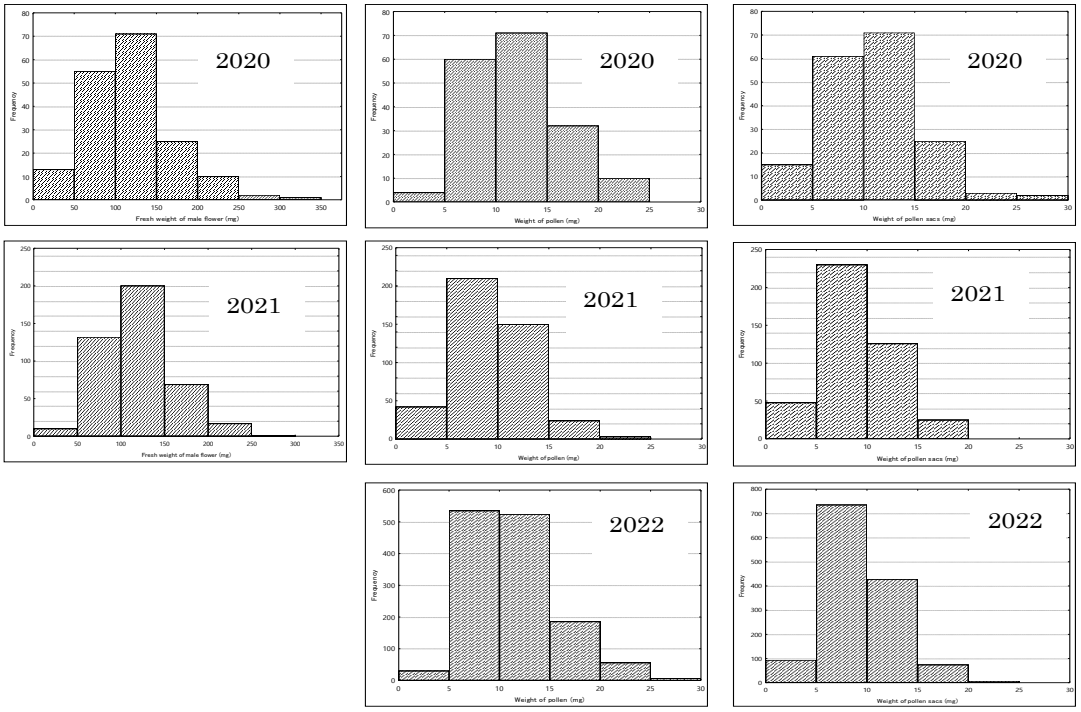

Figure 1
Frequency distribution of FW, WP and WS in each year. Fresh weight of male flowers is shown as 50 mg intervals, whereas weight of pollen and weight of pollen sacs are shown as 5 mg intervals.

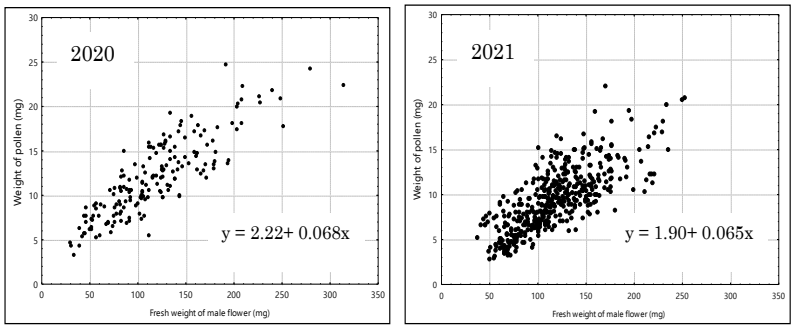

Figure 2
Relationship between FW and WP in each year. Regression formulas are also shown in the graphs.

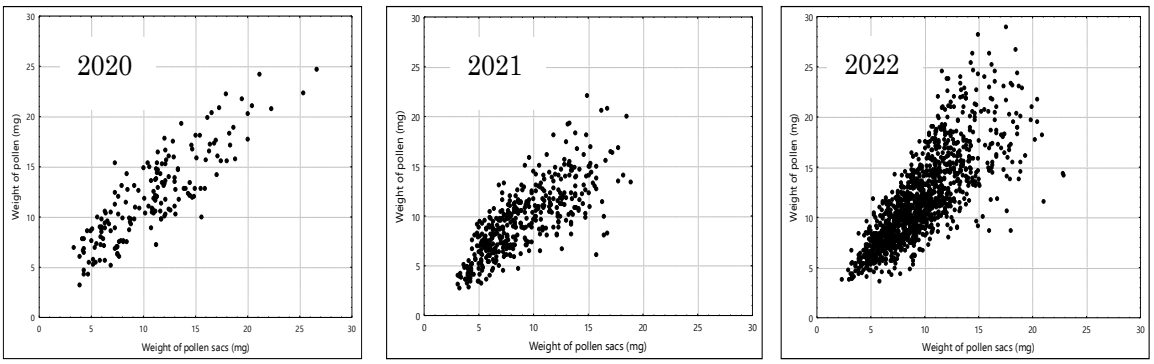

Figure 3
Relationship between WP and WS in each year. Regression formulas are also shown in the graphs.

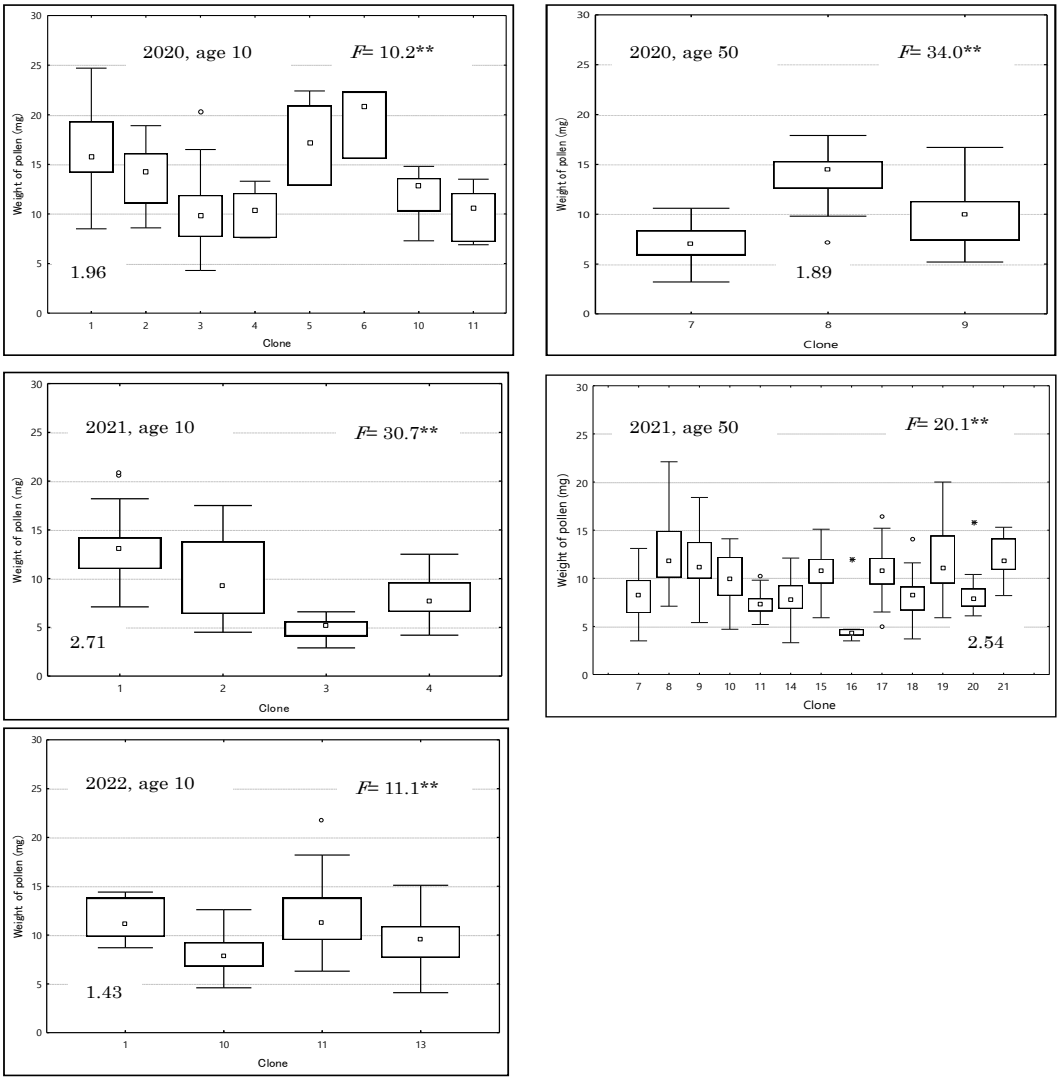

Figure 4
Clonal variations in WP in each year in each tree age. Box, interquartile range (the range of 50 % of the data); whiskers, the lower and upper hinges (1.5 times of the interquartile range); and circle, outlier according to the weight of pollen. F values obtained by ANOVA are also shown in the graphs. The ranges of difference in mean WP between the minimum and the maximum values in each clone are also shown in the graphs. **: Significant at the 1 % level.

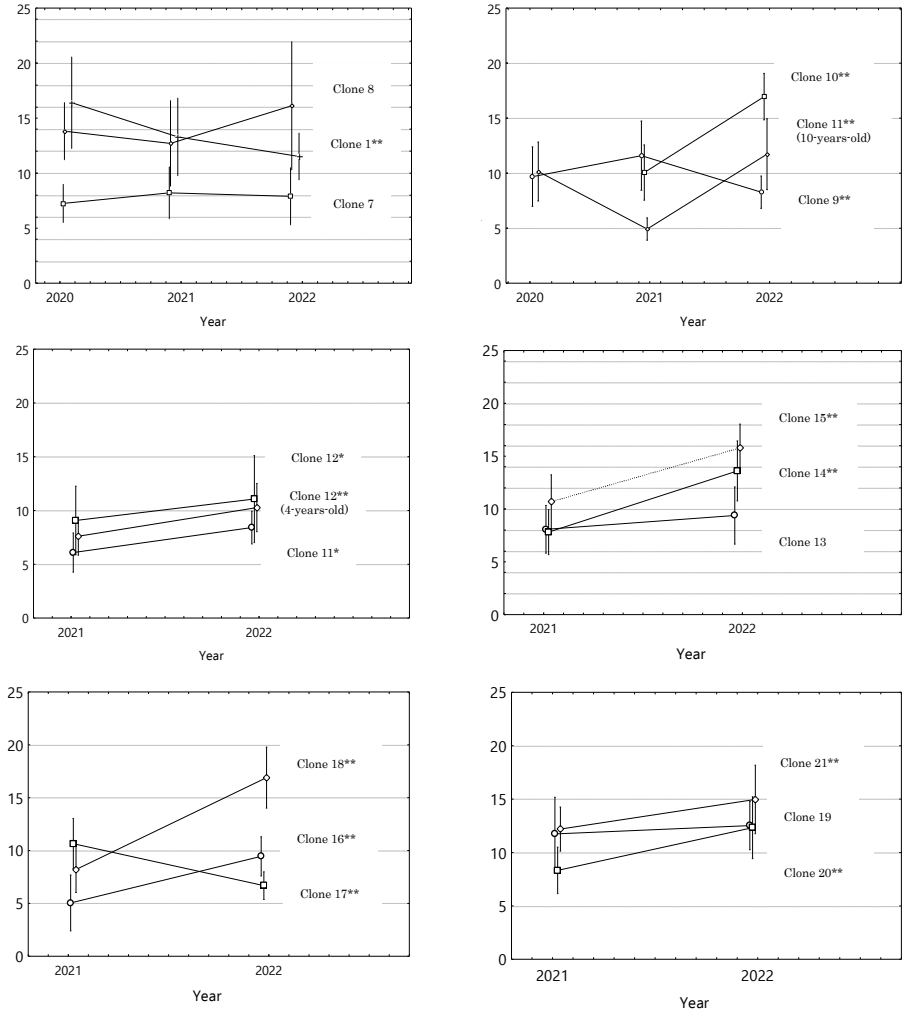

Figure 5
Annual variations in WP for 2 or 3 years in each clone. Bars indicate standard deviations. Clones shown in the figure are in the same order with values from the upper part in 2022. *: Significant at the 5 % level, **: Significant at the 1 % level.

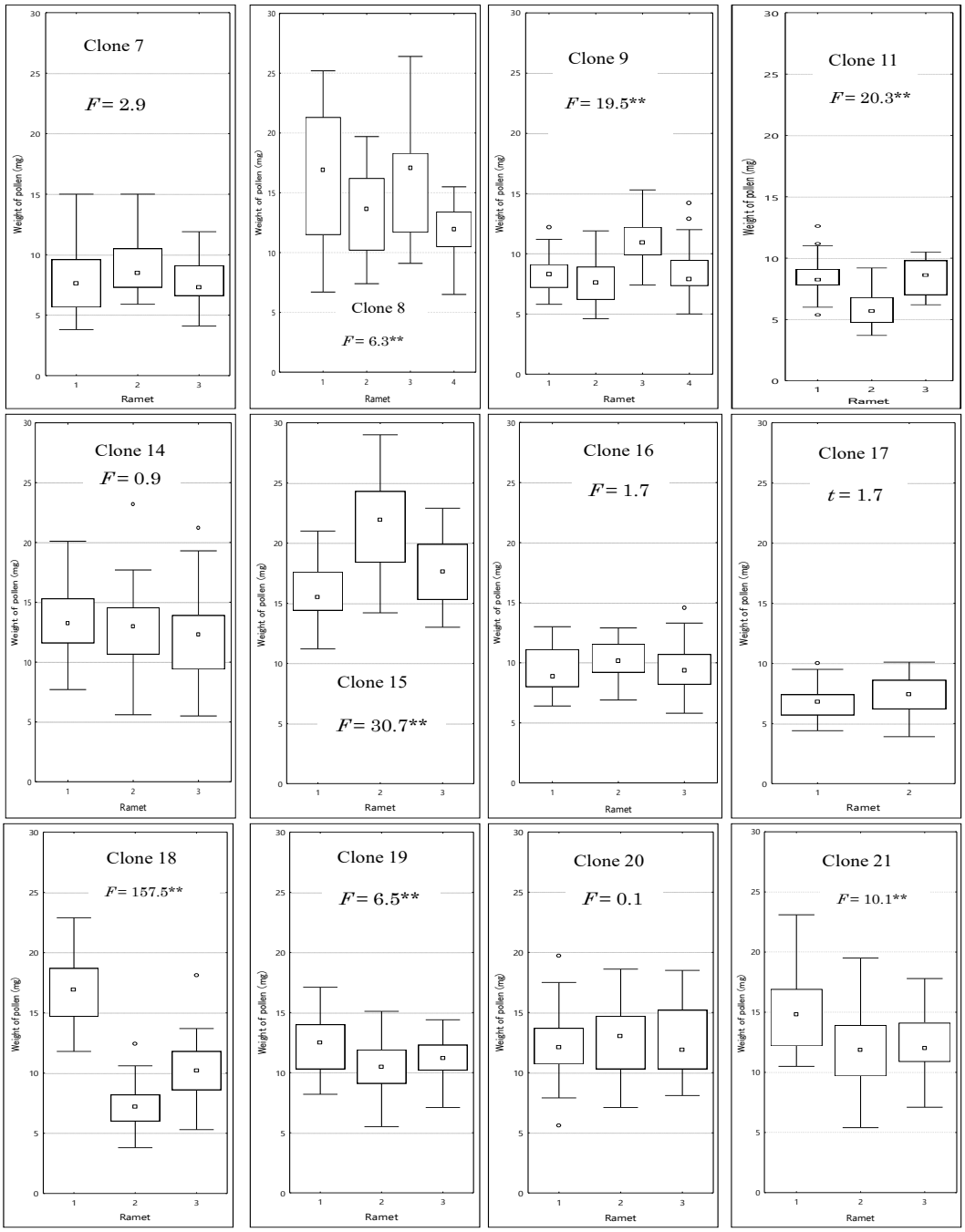

Figure 6
Ramets' variations in WP with the same age in each clone. Box, interquartile range (the range of 50 % of the data); whiskers, the lower and upper hinges (1.5 times of the interquartile range); and the circle, outlier according to the weight of pollen in each ramet for 11 clones. **: Significant at the 1 % level.

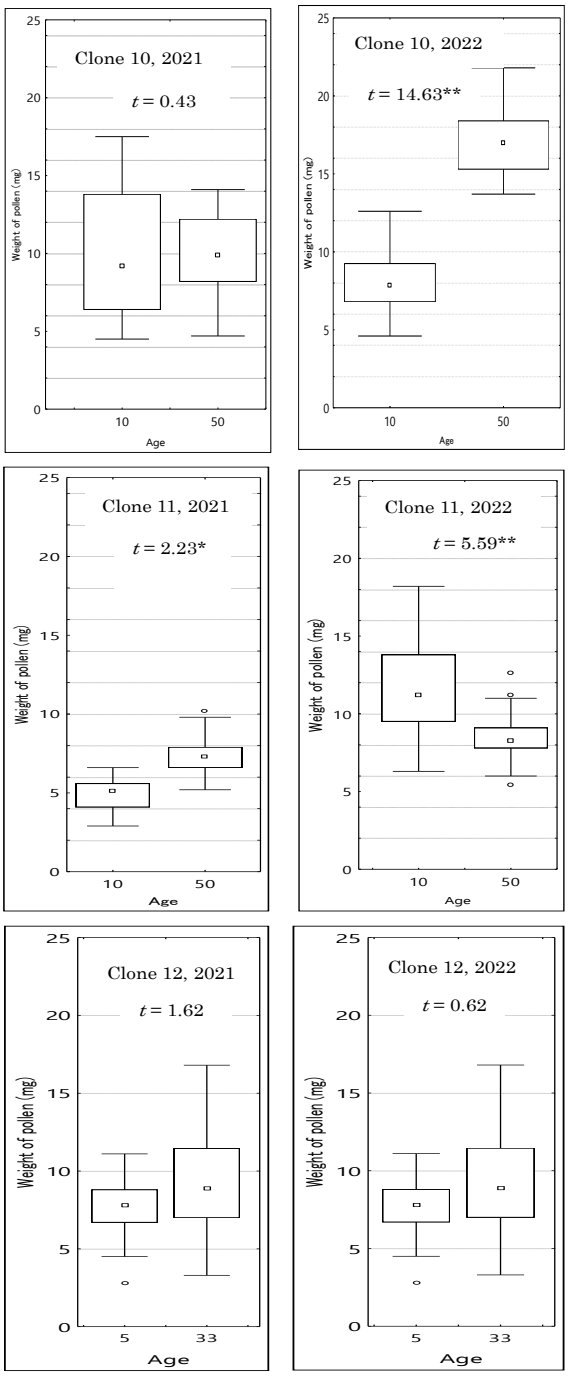

Figure 7
Ramets' variations on WP with different ages in each clone in each year. Box, interquartile range (the range of 50 % of the data); whiskers, the lower and upper hinges (1.5 times the interquartile range); and the circle, outlier according to the weight of pollen in each age in each year for three clones. *: Significant at the 5 % level, **: Significant at the 1 % level.

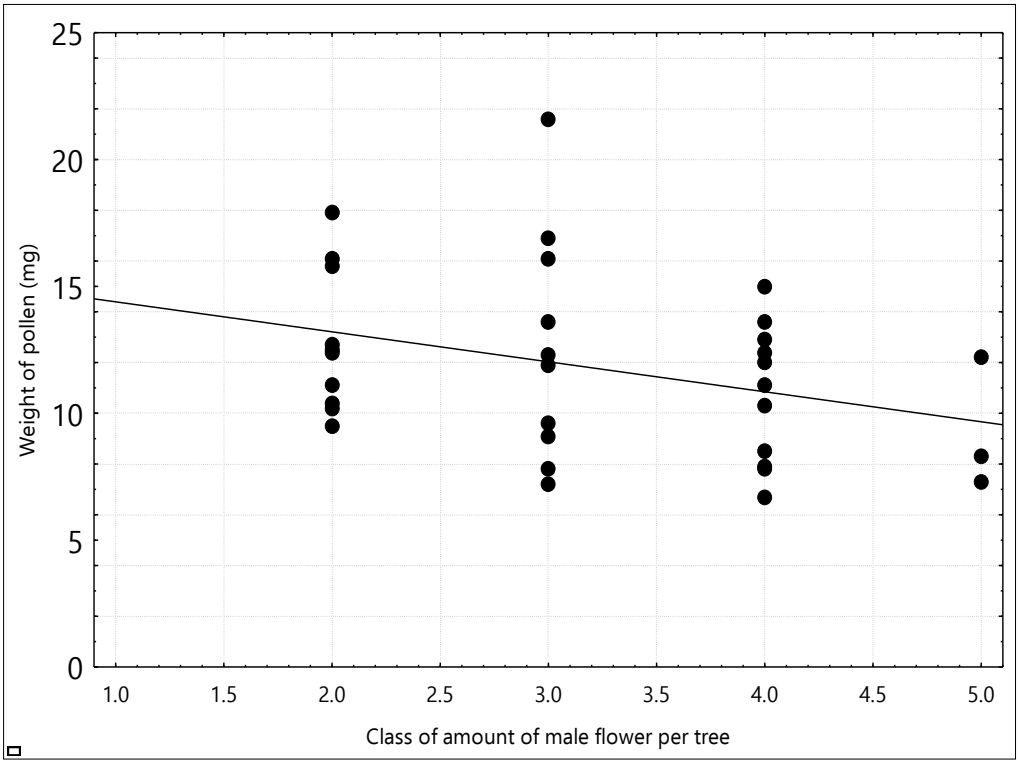

Figure 8
Relationship between mean WP and the number of male flowers per tree. Regression formula is also shown in the graph.

resource imbalance may also affect the difference among ramets for *A. sachalinensis*.

As with the detection of annual and ramets' variations in WP, significant clonal variation in WP was also detected in this study (Fig. 5). There was an almost threefold difference among the clones in 2 of the 3 investigated years. Kondo and Hakamada (2007) also reported that there was almost 3-fold difference in the weight of pollen per male flower for *C. japonica* using 51 clones. Similarly, Ranpal et al. (2022) reported that there was a 1.7-fold difference in pollen production per catkin for *B. pendula* using five clones. Therefore, clonal variation in relation to pollen production per male flower is generally, a common phenomenon in tree species. In this study, clonal repeatability in WP was 0.37 and 0.47 for data obtained in 2 or 3 years. Akutsu et al. (2008) also reported that the clonal repeatability of average ring width, ring density, modulus of elasticity, and bending strength on *A. sachalinensis* was 0.36, 0.46, 0.64 and 0.68, respectively. Conversely, Sato (1994) reported that the heritability of height on *A. sachalinensis* for four sites was 0.04, indicating that clonal repeatability on WP is higher than the growth trait. Moreover, Nikkanen and Ruotsalainen (2000) reported that the average clonal repeatability for male flowering abundance was 0.38 for Norway spruce. Furthermore, Klinkhamer and van der Veen-van Wijk (1999) also reported that the variation of clonal families in pollen production was extremely high and the clonal repeatability of the number of pollen grains was 0.39 for *Echium vulgare*. Furthermore, Hannerz et al. (2001) reported that clonal repeatability on male strobilus production was 0.64 and 0.39 in *Pinus contorta* for different years. These findings suggest that clonal repeatability in the trait of male flowers is around 0.5 for each tree species, as with the results in this study. The results of generalized linear mixed models suggest that the variance of clones was clearly higher than those of year or ramet (Table 4), suggesting that WP is quite a heritable trait.

Alternatively, WP slightly decreased with the number of male flowers per tree (Fig. 8), with the slope showing a value of 0.92 with the class of the amount. In *C. japonica*, there was no decrease in the pollen production per male flower with the number of male flowers per tree (Kondo and Hakamada, 2007). This suggests that the relationship may differ among tree species. Besides, in *C. japonica*, the number of male flowers per tree was reported to increase 2.6-fold with the increase of each male flowering index (Kato et al., 2020). If the relationship between the number of male flowers per tree and the class of the amount on *A. sachalinensis* is also the same in *C. japonica*, the pollen production per tree may increase 2.5-fold with the increase of each male flowering index (0.92×2.6). This suggests that, even if there was a difference of male flowering index regarding the number of male flowers per tree between trees, the amount of pollen per tree may be equal between

trees since there was more than 2.5-fold difference in WP (Fig. 5).

Thus, although the amount of pollen per male flower varied with clones, years and ramets, clonal repeatability was comparatively high level. Similarly, the results of generalized linear mixed models suggested that, as a random effect, the variance value of clones was high compared with those of years and ramets (Table 4). Therefore, to evenly increase the amount of scattered pollen per clone for newly established seed orchards, it is better to determine not only the number of male flowers per tree but also the amount of pollen per male flower in each clone, thereby regulating the number of planted trees in each clone.

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References

Akutsu H, Kita K, Uchiyama K, Kuromaru M (2008) Genetic variation and genotype-environment interaction in annual ring structure in progeny test of Sakhalin fir (Abies sachalinensis). Japanese Journal of Forest Society 90: 137-144 (in Japanese with English summary). https://doi.org/10.4005/jjfs.90.137

Burczyk J, Chalupka W (1997) Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. Annales des Sciences Forestières 54:129-144. https://doi.org/10.1051/forest:19970201

Damialis A, Fotiou C, Halley JM, Vokou D (2011) Effects of environmental factors on pollen production in anemophilous woody species. Trees 25:253-264. https://doi.org/10.1007/s00468-010-0502-1

Hannerz M, Aitken SN, Ericsson T, Ying CC (2001) Inheritance of strobili production and genetic correlation with growth in lodgepole pine. Forest Genetics 8:323-329

Hicks S, Helander M, Heino S (1994) Birch pollen production, transport and deposition for the period 1984-1993 at Kevo, northernmost Finland. Aerobiologia 10: 183-191. https://doi.org/10.1007/bf02459234

Itahana N, Kubota M, Iizuka K, Ubukata M (1995) Cone morphology and amount of seed production per cone for Abies sachalinensis plus trees. Annual report of the Forest Tree Breeding Center, Hokkaido Breeding Office 16:88-94 (in Japanese)

Kang KS, Lindgren D (1998) Fertility variation and its effect on the relatedness of seeds in Pinus densiflora, Pinus thunbergii and Pinus koraiensis clonal seed orchard. Silvae Genetica 47: 196-201

Kato K, Yamanobe T, Ohira M, Tsubomura M, Takashima Y (2020) Relationship between the total value of male flower estimated by visual observation and the actual volume of male flower. Boreal Forest Research 68:47-48 (in Japanese)

Klinkhamer PGL, van der Veen-van Wijk CAM (1999) Genetic variation in floral traits of Echium vulgare. Oikos 85: 515-522. https://doi.org/10.2307/3546700

Kondo A, Hakamada T (2007) Study on the evaluation of clonal trait about sugi (Cryptomeria japonica) plus tree selected at Shizuoka Prefecture -genetic variation on the weight of male flower objecting the selection of sugi tree producing few pollen. Bulletin of the Shizuoka Prefecture Forestry Technology Center 35:7-12 (in Japanese)

Moe D (1998) Pollen production of Alnus incana at its south Norwegian altitudinal ecotone. Grana 37: 35-39. https://doi.org/10.1080/00173139809362637

Nikkanen T, Ruotsalainen S (2000) Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. Silva Fennica 34: 205–222. https://doi.org/10.14214/sf.626

Ranpal S, Sieverts M, Wörl V, Kahlenberg G, Gilles S, Landgraf M, Köpke K, Kolek F, Luschkova D, Heckmann T, Traidl-Hoffmann CT, Büttner C, Damialis A, Jochner-Oette S (2022) Is pollen production of birch controlled by genetics and local conditions? International Journal of Environmental Research and Public Health 19: 1-18. https://doi.org/10.3390/ijerph19138160

Sato T (1994) Time trends for genetic parameters in progeny tests of Abies sachalinensis (Fr. Schm.) Mast. Silvae Genetica 43: 304-307

Schoen DJ, Stewart SC (1986) Variation in male reproductive investment and male reproductive success in white spruce. Evolution; International Journal of Organic Evolution 40:1109-1120. https://doi.org/10.1111/j.1558-5646.1986.tb05737.x

Seki T (2016) Evaluation of thermal conditions for the explanation of annual variation in the seed-cone bud production of Abies sachalinensis. The 15th international conference on ecology and silviculture of fir.

Sivacioğlu A (2010) Genetic variation in seed and cone characteristics in a clonal seed orchard of Scots pine (Pinus sylvestis L.) grown in Kastamonu-Turkey. Romanian Biotechnological Letters 15: 5695-5701

Sugata S (1984) Flowering condition at the seed orchards of Abies sachalinensis. Bulletin of Experimental Forest of Hokkaido University 1: 81-84 (in Japanese with English summary)