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Original Research

Frond size, shape and fertility of *Thelypteris confluens* (Thunb.) C. V. Morton in wetlands disturbed by human activities in Hokkaido, northern Japan

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ABSTRACT

Thelypteris confluens (Thunb.) C. V. Morton exhibits dimorphism between sterile and fertile fronds when fully matured. To assess the frond fertility patterns in this species, the frond size and the shape and degree of fertile fronds were measured in individuals growing in disturbed wetlands in Hokkaido, northern Japan, between late summer and early autumn in 2017 and 2018. This species often establishes on human-disturbed wetlands in boreal regions. The measured parameters were frond fertility, number of veins (NV), dry weight (DW), frond length (FL), blade length (BL), blade width (BW), frond area and blade perimeter. The Dissection Index, aspect ratio and specific frond area were calculated. The data were analyzed by generalized linear models (GLMs) and standardized major axis tests to investigate the determinants of frond fertility and the differences between the sterile and fertile fronds. All the GLMs for predicting frond fertility contained NV, FL and BL, suggesting that NV had a specific role in fertility. Compared with the use of destructive measurements, the use of nondestructive measurements in the GLMs led to slightly lower prediction accuracies. The frond sizes and shapes in relation to fertility were not strongly related to the disturbance rank scores, while the populations in highly disturbed sites showed low numbers of fertile fronds. These findings strongly suggested that the shape- and size-related variables and NV affected frond fertility differently. NV provides more precise measurements of frond maturity and population dynamics for nondestructive long-term monitoring.

1. Introduction

More than 50% of wetlands have been lost worldwide since 1900, although this decrease is underestimated (Davidson, 2014). The major causes of deterioration are derived from human activities, such as peat-mining, drainage construction and land-use change. Therefore, detecting the effects of anthropogenic disturbances on populations and ecosystems is necessary to conserve wetland ecosystems. Bioindicators, which include any taxonomic group with populations that reveal the qualitative status of the environment, are often used to evaluate human disturbance intensity in wetlands and other ecosystem types (Taddeo and Dronova, 2018; Herlihy et al., 2019). However, the morphological and life-history traits of plants are differently affected by environmental and regional differences (McIntyre et al., 1995; Chitwood and Shinha, 2016).

Thelypteris confluens (Thunb.) C. V. Morton, also known as marsh fern, is a deciduous perennial fern often found growing in wetlands, including marshes, fens and bogs. This species is a Euro-Siberian boreo-temperate plant (Schweingruber et al., 2020). This species forms a caespitose population in moist, open sites in Japan (Iwatsuki, 1992) and has potential for use in the phytoremediation of wastewater or soil because of its establishment in a wide range of disturbed habitats (Anderson and Walsh, 2007). *Thelypteris confluens* forms dimorphic fronds: the fertile fronds have thin and curling pinnae whereas the sterile fronds have flattened pinnae (Kavak, 2014) when they produce sori between late summer and early autumn in Hokkaido, Japan (Takita, 2001). Because frond fertility is a key to anticipating the population dynamics of ferns, a suitable predictor of frond fertility is required (Tsuyuzaki, 2000). Furthermore, nondestructive methods for monitoring population dynamics are preferable (Hoyo and Tsuyuzaki, 2013). When we can evaluate frond fertility without destructive sampling, we can monitor population dynamics well over the long term.

Therefore, the objectives of this study were: 1) identifying the morphological variations in *Thelypteris confluens* established in variously disturbed wetlands by using destructive and nondestructive measurements, and 2) finding out the relationships between the morphological variations and frond fertility.

2. Materials and methods

2.1. Study sites

Four wetlands, from north to south in Hokkaido, northern Japan, were selected for the sampling sites: Sarobetsu, Nopporo, Utasai and Shizukari (Table 1). These four sites were distributed in a cool temperate region. The mean annual temperatures at the four sites increased from 6.1°C to 6.8°C with decreasing latitude. The annual precipitation ranged between 878 mm in Sarobetsu and 1684 mm in Utasai and generally decreased from south to north. To evaluate the human disturbance intensity, a disturbance intensity rank was assigned based on a disturbance gradient for ecological assessment of wetlands (Lopez and Fennessy, 2002), which was adjusted to this study by using peat mining, drainage (including ditches), road pavement and a vegetation buffer (Fig. 1). The chart lists 24 ranks of the intensities of human impacts based on 4 hierarchies (1st hierarchy = peat mining, 2nd = drainage, 3rd = paved road, and 4th = vegetation buffer).

The Sarobetsu wetland (or mire) is located in the northernmost part of Hokkaido, facing the Japan Sea. *Thelypteris confluens* fronds were sampled in peatlands mined in 1977. The peat was mined down to 6 m in depth. The site was enclosed by unmined bogs dominated by *Sphagnum* mosses, grasses and sedges, which functioned as a grassland buffer (Nishimura et al., 2009). The grasses, represented by *Molinia japonica* (Hack.) Hayata and *Phragmites australis* (Cav.) Trinius ex Steudel., were well established in the site. This wetland was affected by land-use change and conversion to pastures, with the construction of large drainage areas in the past (Ito and Wolejko, 1990). The foliage of *Thelypteris confluens* was sparsely distributed, and there were relatively few fertile fronds.

Nopporo is located in central Hokkaido. In Nopporo, the samples were collected adjacent to a semi-natural swamp near a parking lot. This wetland was close to a metropolitan city, Sapporo, and therefore has been greatly damaged by urbanization. However, due to natural and transplanted forests, the study site has remained a vegetation buffer. Since the foliage is established densely in a small area, the total number of samples was the lowest.

The Utasai bog is located in the southern part of Hokkaido. This wetland is surrounded by forests. A paved road with drainage areas was constructed across the wetland. *Thelypteris confluens* established in the drainage areas close to the paved road. Nonwetland and/or exotic species were common along the paved road.

Shizukari wetland, previously dominated by *Sphagnum* mosses, is located in the southern part of Hokkaido. This wetland is 1 km from a coast and was not enclosed by any vegetation buffers. The wetland was enclosed by farmlands, which had been converted from the wetland.

2.2 Sampling and analysis

Fronds were sampled from the four sites between late summer and early autumn in 2017 and 2018. Two to seven fronds of various sizes were collected from each plant. When fertile fronds were available, a few fertile were selected and collected along with a few sterile fronds. Samples of the foliage were collected at intervals more than 3 m apart. Therefore, the numbers of samples differed between the sites, depending on the distribution pattern and density of the foliage. In total, 1104 fronds were collected, 1013 of which were used to measure all the variables. The silhouette of each sample was scanned by an image scanner at 400 dpi (CanoScan LiDE 30, Canon, Tokyo) after pressing the frond specimens. Frond length (FL), blade length (BL), blade width (BW), frond area and blade perimeter were calculated by ImageJ software (ver. 1.52q) (Schneider et al. 2012). The number of midrib branches of the fronds (NV, number of veins) was counted visually (Sato, 1985ab). Then, the dry weight (DW) of the whole blade was measured after drying at 60°C for more than 3 days. The Dissection Index, aspect ratio and specific frond area were calculated based on these values. Two aspect ratios were examined, BW/FL and BW/BL. The Dissection Index was calculated by $P/(\pi \times \text{frond area})$ (Kincaid and Schneider, 1983). Therefore, aspect ratio and Dissection Index are composed of two variables and express the frond shape. The FL, BL, BW, NV and aspect ratio were measured or calculated nondestructively and the others variables required destructive sampling.

The differences in sizes or shapes between the fertile and sterile fronds were compared by generalized linear models (GLMs) with the assumption of a Gaussian distribution (Dobson and

Barnett, 2018). To detect the effects of frond characteristics on frond fertility, GLMs were used with the assumption of a binomial distribution; fertility was used as the response variable and frond characteristics and disturbance rank were used as the explanatory variables. The most suitable models were selected by a backward procedure with Akaike's Information Criteria (AIC) (Aho et al., 2014). Interactions between the variables were not examined because the objective was the selection of suitable parameters to predict frond fertility. To investigate the effectiveness of using nondestructive measurements to predict fertility, GLMs were tested by NV, FL, BL, BW, BW/FL, BW/BL and disturbance rank, and all the parameters from the four sites. GLMs were also developed by using three sites excluding Nopporo because fertile fronds were least abundant in Nopporo. To check the accuracy of the discrimination of frond fertility by destructive and nondestructive methods, the prediction accuracy was calculated as follows: (number of correctly discriminated fronds)/(total number of samples) × 100 (%).

The allometric relationship between the measured variables was selectively assessed by using standardized major axis tests (SMA) to observe the site-based differences in the allocation of the human disturbance rank scores (Warton et al., 2006). Since frond characteristics are related to BL in most examined ferns (Sato et al. 1989, Tsuyuzaki 2000), allometry was obtained between the BL and the other variables for fertile and sterile fronds. All the statistical analyses were performed by R software (version 3.6.2) (R Core Team, 2019) with the libraries MASS (Venables and Ripley, 2002) and smatr (Warton et al., 2012).

3. Results

The disturbance intensity ranks of the sites were ordered from high to low as follows: 22 in Nopporo, 20 in Sarobetsu, 10 in Utasai and 9 in Shizukari (Fig. 1). The numbers of sampled fertile fronds were 6, 38, 121 and 124 in Nopporo, Sarobetsu, Shizukari and Utasai, respectively, while the numbers of sterile fronds were 85, 259, 218 and 253. The ratios of fertile to sterile fronds were 7.1%, 14.7%, 55.5% and 49.0% in Nopporo, Sarobetsu, Shizukari and Utasai, respectively, and low ratios were observed in the highly disturbed sites. Of these samples, 0, 38, 121 and 124 fertile fronds were perfect, i.e., all the variables were measured, in Nopporo, Sarobetsu, Shizukari and Utasai, respectively, while 42, 259, 196 and 233 sterile fronds were perfect. Although frond fertility increased with increasing frond size (Fig. 2), the frequency distribution patterns differed between the variables. In particular, most sterile fronds showed low dry weight and blade perimeter length. Of the shape variables, BW/BL of aspect ratios was highly overlapped. This showed that, in relation to BW and BL, the frond shape did not differ greatly between the fertile and sterile fronds. BW/FL and the specific frond area were smaller for the fertile fronds than for the sterile fronds. These results indicated that the responses of the shape variables to fertility were different from those of the size variables.

The best model selected by the AIC consisted of seven of the eleven destructive and nondestructive variables for predicting frond fertility at the four sites (Table 2). The selected variables were NV, DW, FL, BL, frond area, Dissection Index and specific frond area. The two aspect ratios, out of the shape parameters, were not selected in the model, while another shape index, Dissection Index, remained. The variables selected for the models of three sites were the same as those for the models consisting of the four surveyed wetlands. The human disturbance rank scores were not included in these two models, indicating that the fertility patterns did not differ between the surveyed sites. Of the nondestructive variables, NV, FL, BL, BW/FL and BW/BL were included in the best models. These results meant that the shape index remained in all the models. Other than the two models that used all the variables, the rank was not retained in the models developed with the nondestructive variables.

NV, FL and BL were included in all the four models, indicating that these three size variables were required to predict the frond fertility. The models developed with all the variables showed higher accuracy of fertility prediction (i.e., > 92%) than the models made with nondestructive variables (ca 85%), although the difference was only 7%. The difference in accuracy was derived mostly from the prediction error of the sterile fronds. These results indicated that the sterile fronds showed more diverse sizes and shapes than the fertile fronds.

Except for the allometric relationship between BL and specific frond area for the fertile fronds, all the regression lines were significant (Fig. 3). The differences in the slopes of seven of the nine allometric relationships between BL and the other variables were significant. These results indicated that most frond size and shape variables were allometrically different between the fertile and sterile fronds. When sterile and fertile fronds had the same BL, fertile fronds had higher DWs, BWs, frond areas, and higher values of the Dissection Index, the BW/BL ratio and the specific frond area. NV was lower on the fertile fronds than on sterile fronds when BL was equivalent. The BW/BL ratio decreased with increasing BL, indicating that the frond shape became slimmer with increasing BL. Additionally, the fertile fronds were broader than the sterile fronds. The SMA showed clear differences in the slopes between the fertile and sterile fronds; that is, the SMA decreased with increasing BL for the fertile fronds but increased with increasing BL for the sterile fronds. This allometry pattern also indicated that the fertile fronds were denser than the sterile fronds. Nonsignificant differences were observed between the BL and FL and between the BL and blade perimeter, showing that the allocation to these two variables did not differ with frond fertility. Since the FL included the frond petioles, this also showed that the allocation to the frond petioles did not change with frond fertility.

4. Discussion

The fertility patterns of *Thelypteris confluens* predicted by the GLMs did not differ between the disturbance intensity rank scores, indicating that the fertility patterns predicted by the measured variables did not differ along the surveyed disturbance intensity range. Since the disturbance rank scores were different between the four sites, the site differences were also unlikely to affect the frond size or shape in relation to fertility patterns. Most lowland wetlands in Hokkaido have been disturbed to some extent by human settlements (Ito and Wolejko, 1990) and all the surveyed wetlands were affected by human-related disturbances, i.e., peat mining, drainage construction and/or pavement. The environment, including disturbances and stressors, affects frond size and shape (Chitwood and Sinha, 2016). Although the physical disturbances, such as trampling, directly affect the size and shape of leaves (Vujic et al., 2016), large-scale disturbances, such as peat mining, drainage construction and road construction, broadly and indirectly affect frond size and shape. These results suggested that the differences in disturbance characteristics among the four studied sites did not greatly affect the size or shape of fronds in relation to frond fertility.

Fertile fronds were less abundant in Nopporo and Sarobetsu where the disturbances had been more intense. Plants such as willows and poplars often undergo vegetative reproduction more than sexual reproduction to reduce seedling mortality in disturbed and/or stressful habitats (Thomas et al., 2012). Vegetative reproduction supports the population persistence of *Drosera anglica* Hudson on a postmined peatland in Sarobetsu mire, which is greatly disturbed by human activities (Hoyo and Tsuyuzaki, 2015). This fern has an intrinsic restriction on fertile frond production, which depends on the balance between habitat requirements and vegetative organ production (Shapre, 2005). The populations in Nopporo and Sarobetsu seemed to use this strategy, i.e., there was a low abundance of fertile fronds in highly disturbed habitats. The fertility ratio at the population level may be affected by the fate of population dynamics, although frond measurements have not been investigated at this level.

The fertility of *Thelypteris confluens* was predicted well by using multiple variables. Of the frond variables, NV, BL and FL were retained in all the GLMs to predict frond fertility. In models with single measurement variables, NV was the most powerful variable for detecting the frond fertility of ferns, such as five *Polystichum* ferns (Sato, 1985b) and three *Dryopteris* ferns (Sato and Tsuyuzaki, 1988). NV predicts the frond fertility of *Pteris mutilata* Burn., which develops dimorphic fronds depending on fertility, better than the other size and shape parameters, i.e., FL, BL and aspect ratios (Tsuyuzaki, 2000). NV is tightly related to the frond maturation of ferns, including *Thelypteris confluens*, as previously described. These results suggest that NV has a specific role in frond fertility, probably because the length of veins characterizes the frond shape (Cope et al., 2012) and therefore is related to the other frond size variables. The accuracy of the prediction by nondestructive measurements was not very low, 85-86%, but was slightly lower than that by variables including destructive measurements. Because measuring many parameters provides high identification accuracy, digital morphometrics with a computer has been applied to various researches, including research in plant taxonomy and ecology (Klein et al., 2017). However, such destructive measurements do not allow long-term monitoring of populations. The nondestructive methods used in this study measured only FL, BL, BW and NV. NV is easily counted and should be measured at any expense when nondestructive measurement is required.

When the Dissection Index was included in the best models (i.e., those developed with all the variables), the aspect ratios (BW/FL and BW/BL) were discarded from the models. The BW/FL ratio, BW/BL ratio and Dissection Index quantify frond shape. These results suggested that frond fertility was related to not only size but also shape. The slope of the allometric relationship between BL and specific frond area was negative for the sterile fronds and was not significant for the fertile fronds. Frond mass per area, which is equal to 1/(specific frond area), is dependent more on frond thickness than on dry weight for vascular plants, because dry weight is a more stable measurement than weight (Wilson et al., 1999). The specific frond area of the fertile fronds was lower than that of the sterile fronds because the weight of the sori was included in the measurement of the fertile fronds. This difference was observed in the fertile fronds because of sorus formation, which also seemed to be related to NV. In other words, frond shape may be a consequence of sorus development. The present study concluded that frond size and shape were both related to frond fertility and differently affected frond fertility.

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Table 1

Climatological and geographical characteristics (including the human disturbance intensity rank score) of the four surveyed wetlands. The climatological data are obtained from the Japan Meteorological Agency webpage (https://www.jma.go.jp/jp/amedas/) (accessed on March 25 2020). For information on the human disturbance rank scores, see Fig. 1.

Wetland	Sarobetsu	Nopporo	Utasai	Shizukari
Latitude (N)	45°06’	43°04’	43°63’	43°58’
Longitude (E)	141°43’	141°54’	140°34’	140°44’
Elevation (m)	8	8	27	10
Mean annual temperature* (^{o}C)	6.1	7.5	7.5	7.8
Annual precipitation (mm)	878	1018	1684	1369
Human disturbance rank score	20	22	10	9

*: based on records from 2017 and 2018

Table 2

Frond fertility predicted by frond parameters and disturbance ranks by GLM with the assumption of a binomial distribution. The values show the intercepts and estimates of the models. AIC was used to select the parameters that led to the best GLMs by a backward method. All models used all 11 measured variables and the disturbance rank scores. Three sites: data obtained from Nopporo, where the lowest number of fertile foronds, were not used. **: significant at $P < 0.01$, *: $P < 0.05$, NS: not significant. The numbers of fronds show the correct and incorrect discrimination on the left and right sides of slash marks, respectively. The AIC values are 422.19 and 422.07 for the best models created with all the variables from four and three sites, respectively. The AIC values are 675.19 and 674.31 for models created with nondestructively sampled variables from four an three sites, respectively. ×: not used for models developed with nondestructive measurements. -: discarded from the models according to the AIC values.

	Destructive		Nondestructive	
	Four sites	Three sites	Four sites	Three sites
Intercept	-3.881*	-3.894*	-9.526**	-9.473**
Number of veins	+0.070**	+0.070**	+0.152**	+0.151**
Dry weight	+5.797*	+5.830*	×	×
Frond length	+0.079**	+0.079**	+0.122**	+0.124**
Blade length	-0.199**	-0.199**	-0.220*	-0.224**
Blade width	-	-	-	-
Frond area	-0.035*	-0.036*	×	×
Blade perimeter	-	-	×	×
Dissection Index	+0.233**	+0.233**	×	×
Aspect ratio				
Width/(frond length)	-	-	-22.198*	-21.517*
Width/(blade length)	-	-	+8.430*	+4.199^{NS}
Specific frond area	-0.024**	-0.024**	×	×
Human disturbance rank score	-	-	-	-
Accuracy of fertile frond	225/17	225/17	164/24	163/24
Accuracy of sterile frond	713/58	671/58	706/119	664/120
Prediction accuracy (%)	92.6	92.3	85.9	85.2

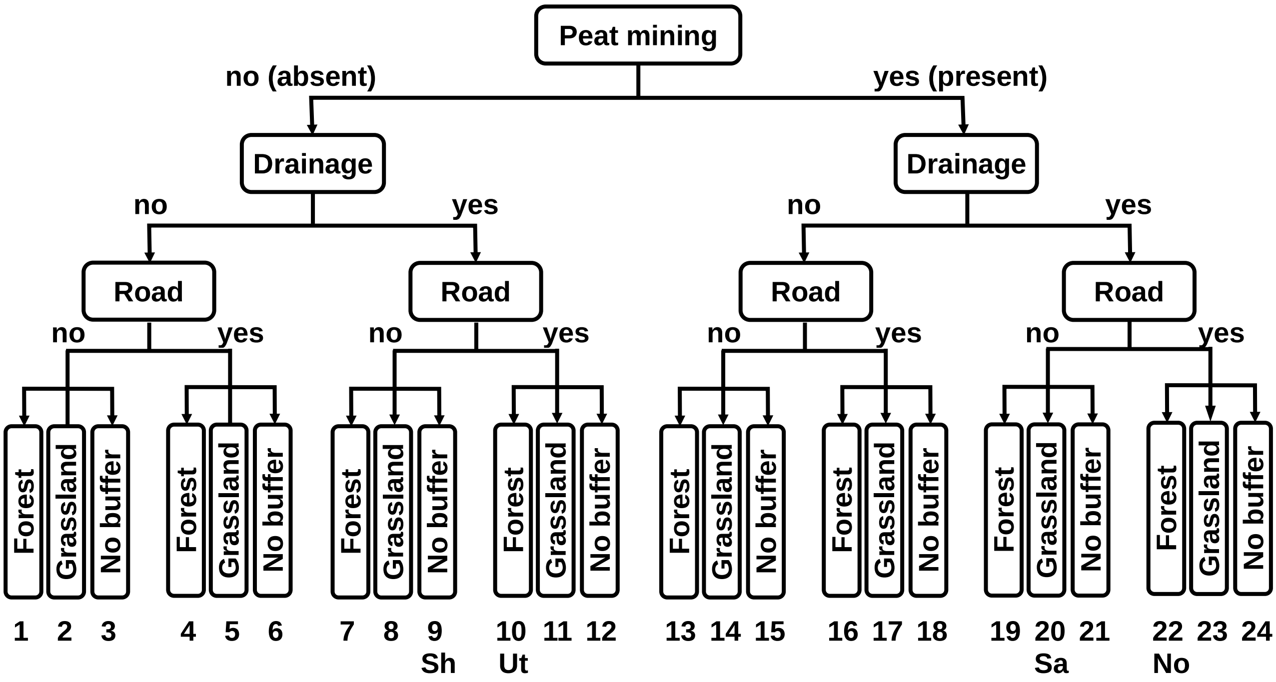

Fig. 1. Hierarchical classification procedures used to score the human disturbance rank. The hierarchical ranks are adjusted to the study sites. The following four types of human disturbances are considered: peat mining, drainage and ditches, paved roads and vegetation buffers. The vegetation buffers are classified into three categories: grassland, forest and no buffer. Each numeral indicates the disturbance rank score. The scores of the four sites are shown by the first two letters of the wetland names: Sa = Sarobetsu, No = Nopporo, Ut = Utasai and Sh = Shizukari.

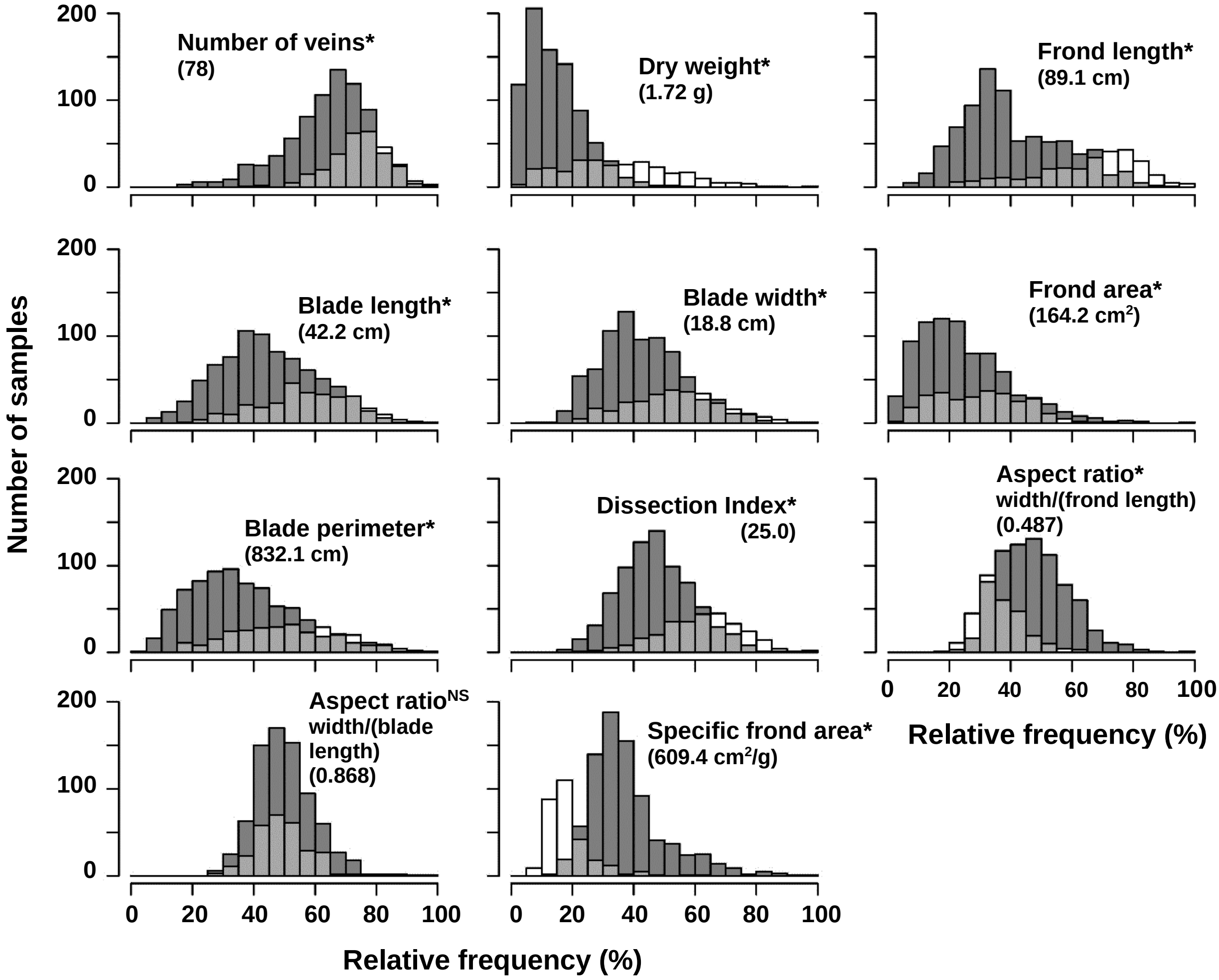

Fig. 2. Relative frequency distributions (%) of the size and shape variables in relation to frond fertility, examined for each of the four study sites. The X axis ranges between 0 and 1 for all the variables. The maximum value of each variable is shown in the parentheses on each panel. The dark gray and open bars indicate sterile and fertile fronds, respectively. The light gray bars indicate the overlaps between the fertile and sterile fronds. *: the variables are significantly different between the fertile and sterile fronds at $P < 0.01$ (GLM). NS: nonsignificant.

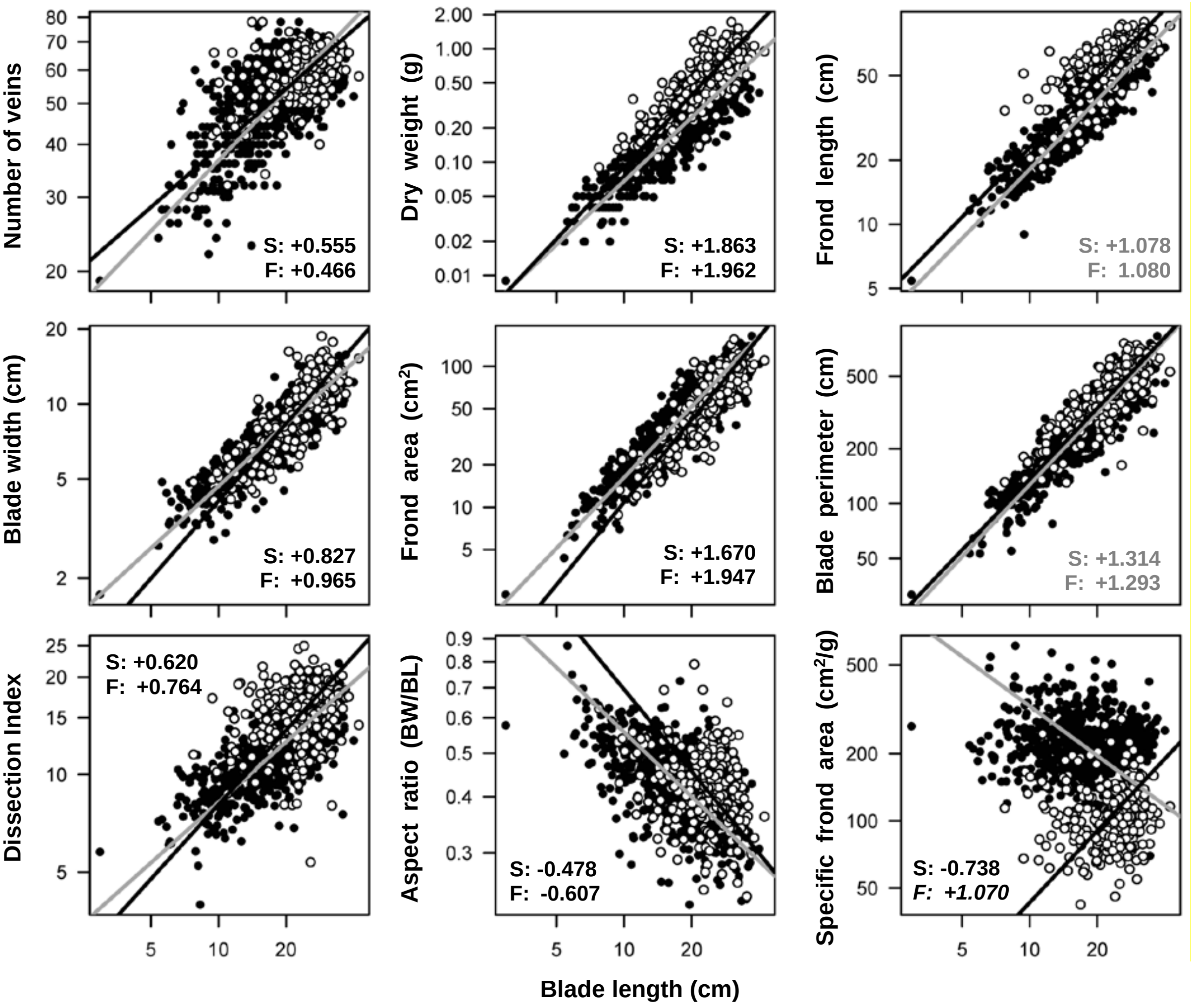

Fig. 3. Allometric relationships between blade length and the other nine variables in relation to frond fertility. The differences in slopes are examined by standardized major axis tests. The open and closed circles indicate fertile and sterile fronds, respectively. The black and gray lines indicate the allometric regression lines for fertile and sterile fronds, respectively. F and S: the slopes of the lines for fertile and sterile fronds, respectively. When the slopes of S and F are indicated in black letters, they are significantly different at $P < 0.01$. When the slopes of S and F are indicated in gray, they are not significantly different. All the regression lines are significant at $P < 0.01$, except for the specific frond area of fertile fronds, for which the slope value is shown in italics.