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Comparisons of life histories, morphological traits and habitats between endangered species, *Drosera anglica*, and common species, *D. rotundifolia*, in a post-mined peatland

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Summary

Clarifying relationships between survival, growth, reproduction and competitive ability is required for the conservation of species and biodiversity, in particular, for endangered species, through encouraging population persistence. *Drosera anglica* Hudson has been assigned as an endangered species by the Ministry of the Environment of Japan, because of decreasing the number of populations in boreal regions. This species in Japan has been reported only from three localities, Sarobetsu and Taisetsu in Hokkaido and Nikko in central Honshu. In contrast, *D. rotundifolia* L. is widespread in the boreal wetlands of the Northern Hemisphere. These two species are carnivorous plants often establishing in *Sphagnum* bogs with high water level and solar radiation.

The determinants of the establishment of *D. anglica* and *D. rotundifolia* have been considered to be water level, overstory vegetation and/or *Sphagnum* mat. However, these factors are spatially interacted to each other and therefore the prime determinants and mechanisms of population persistence have been controversial. Sarobetsu mire is located in Hokkaido Island, northern Japan (45°06’N, 141°42’E, 7 m elevation) and accumulates thick *Sphagnum* peat. The peat was annually mined for 40 years from 1970 to 2003 for commercial use. Here, I discovered that *D. anglica* established with *D. rotundifolia* in the post-mined peatland mined in 1972. In addition, *D. obovata* Mert. et Koch, a hybrid between *D. anglica* and *D. rotundifolia*, was likely to establish there.

Although a precise, convenient and non-destructive species identification is required to monitor plant populations for long-term, such identification has not been developed for species in *Drosera*. Firstly, I developed the identification method based on a hierarchical Bayesian model (HBM), using blade length and leaf width under assuming the size-dependent changes with individual variability. The method increased accuracy through the reduction of identification error on small-sized leaves, compared with a canonical discriminant analysis that represents multivariate analyses using a number of variables. I applied this new method to trace *Drosera* shoots in Sarobetsu mire.

D. anglica and *D. rotundifolia* reproduced by both seeds and vegetative propagations.

Vegetative reproduction tends to increase the offspring survival and does to decrease the migration, although the dependency to vegetative reproduction has not been quantified on these two species. I monitored recruitment, survival and reproduction on the two *Drosera* species for three years to detect spatio-temporal changes in reproduction. The production of flowers, seeds and daughters increased with increasing shoot size on both species. The production of flowers increased more sharply with increasing shoot size on *D. rotundifolia* than *D. anglica*, while *D. anglica* produced more daughters. When the flowers were produced, survival and growth were decreased on the parents on both species. The daughter production was not related to the survival and growth. These results indicated that *D. anglica* was dependent on vegetative reproduction more than *D. rotundifolia*.

The effects of water levels, *Sphagnum* mats and overstory vascular plants on the recruitment and survival of seedlings were examined for three years to investigate the determinants on the distribution of two *Drosera* species, because seedling stages are often critical for the distribution of populations. I also conducted transplanting and seeding experiments under manipulated water level or light intensity to quantify the effects of water level and light intensity on seedling survival and growth. Another seeding experiment examined seed germination and seedling growth with mortality in three habitats - bareground, *Sphagnum* mat and waterlogged surface. The establishment of *D. rotundifolia* seedlings was not determined by water level and plant cover, while the recruitment and survival of *D. anglica* was determined, i.e., decrease with high water level and high plant cover. The survival of overwintering seedlings was not related to water level and plant cover on both species. The daughters of *D. anglica* recruited more in low overstory plant cover. *D. anglica* seedlings grew slower on *Sphagnum* mat than *D. rotundifolia*. The seedling survival rates of both species were high at high water level in a greenhouse experiment but growth rates were low at high water level. *D. anglica* decreased the growth by shade more than *D. rotundifolia*, and thus was weak to compete against *Sphagnum* moss and overstory vascular plants. Therefore, the establishment of *D. anglica* was limited in areas at high water level and low plant cover, both of which reduced inter-specific competition.

D. anglica was pushed out to habitats not suitable for seedling establishment by

Sphagnum moss and overstory vascular plants because of low competitive ability at seedling stage. In waterlogged habitat, *D. anglica* maintained their population mostly by vegetative reproduction. *D. rotundifolia* reproduced mostly by seeds, and established in various habitats, independent of overstory plants, suggesting that seed dispersal was a key to determine the population dynamics. In conclusion, *D. anglica* is endangered because the established habitat was restricted in narrow area, i.e., on waterlogged surface. The habitat limitation of *D. anglica* was derived from the weak competitor to solar energy and weak seed-dispersal ability. For the conservation and restoration of *D. anglica* populations, therefore, the management of not only overstory vascular plants but also *Sphagnum* moss cover is required to reduce inter-specific competition at habitat scale. At wetland scale, the development of *Sphagnum* mat provides waterlogged surface where *D. anglica* persisted. These imply that *D. anglica* is vulnerable when *Sphagnum* mat is removed. I should mention scale-dependent inter-specific relations more to understand the mechanisms of population persistence on endangered species to conserve and restore populations.

Preface

To conserve plant populations, understanding the traits of survival, growth, reproduction and competition in respective habitats is prerequisite (Schemske et al. 1994; Münzbergová 2005). In particular, the conservation of endangered species is an urgent task for keeping biodiversity (IUCN 2012), because the extinction of species is accelerated owing to human activities (Takenaka et al. 1996). Bogs, broadly-distributed wetlands in boreal regions, suffer biodiversity loss derived from the extinction of rare species from global warming (Keddy 2010).

Endangered species often establish with the common congener(s), although the endangered species develop smaller populations and/or narrower distributional ranges than common species (Brook et al. 2008). Such comparable species are useful to detect the mechanisms of population maintenance (Young et al. 2007; Verhulst et al. 2008; Zietsman et al. 2008).

All species in *Drosera* are carnivorous plants establishing in poor-nutrient habitats (Juniper et al. 1989). *Drosera anglica* Hudson, a carnivorous plant, is widely distributed in bogs in the Northern Hemisphere (Crowder et al. 1990). The populations have declined throughout the world for the past 50 years, owing mostly to human activities (Jennings and Rohr 2011). In Japan, *D. anglica* species has been assigned as an endangered species in Red List (Ministry of the Environment Government of Japan 2012). *Drosera rotundifolia* L., a congener, overlaps the distributional range with *D. anglica* but is widespread (Wolf et al. 2006). These suggest that the comparisons between *D. anglica* and *D. rotundifolia* clarify the ecological traits and the mechanisms of population maintenance on *D. anglica.*

The study site was a post-mined *Sphagnum* peatland in Sarobetsu mire, Hokkaido, northern Japan, where the *Sphagnum* peat area was annually mined from 1970 to 2003 (Nishimura et al. 2009). *Drosera anglica* and *D. rotundifolia* established in an area mined 37-39 years before. In boreal peat bogs, *Sphagnum* mosses often determine the distributions of vascular plants (Malmer et al 1994; Pouliot et al. 2012). *D. rotundifolia* competes against *Sphagnum* moss at low water level (Svensson 1995), while *D. anglica* established at high

water level (Nordbakken 1996; Nordbakken et al. 2004).

The two *Drosera* species produce seeds and daughters, i.e., reproduce sexually and vegetatively (Murza and Davis 2003). Such reproductive traits determine temporal- spatial distribution patterns, including population sizes, although the dependencies to sexual and vegetative reproduction have not been evaluated (Walck et al. 2001; Burne et al. 2003). To clarify these, field census is required. However, one obstacle on the accurate monitoring is uncertainty of species identification.

Firstly, therefore, non-destructive, convenient identification technique is proposed by a hierarchical Bayesian model that identified two parental species, i.e., *D. anglica* and *D. rotundifolia*, and a hybrid, *D. obovata*, based on two variables - leaf length and leaf width. Secondly, the survival and growth of the two species were compared in relation to sexual reproduction (flowers and seeds) and vegetative reproduction (daughters). The survival and growth were monitored for three years from 2009 to 2011, using permanent plots (Figure 1). Finally, the microhabitat preferences of *D. anglica* and *D. rotundifolia* are clarified by monitoring population dynamics (density, recruitment, survival and growth), water level, overstory vegetation and *Sphagnum* mat. The effects of shade and peat moisture on the seedling emergence and survival were also experimentally measured. Based on these results, the conservation and restoration of endangered species were discussed.

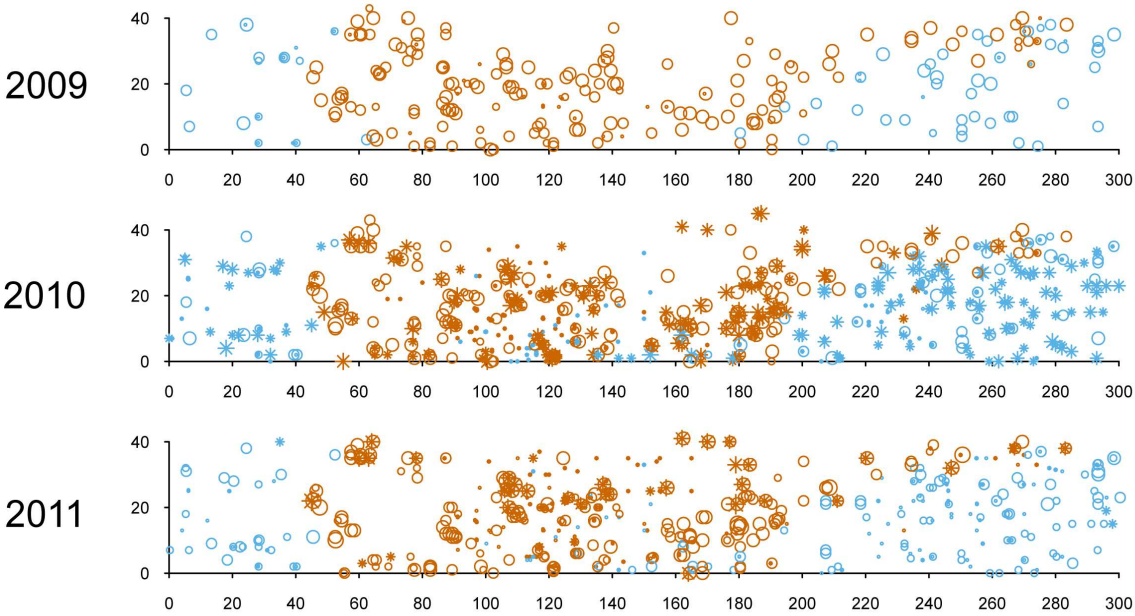

Figure 1. An example of monitoring *Drosera* shoots for three years from 2009 to 2011 in a representative 500 cm × 40 cm plot. Red and blue symbols indicate *D. anglica* and *D. rotundifolia* respectively. Asterisks show the recruits from seeds or by daughters. The symbol sizes are corresponded with the maximum leaf lengths.

Chapter 1. Characteristics of leaf shapes among two parental *Drosera* species and a hybrid examined by canonical discriminant analysis and a hierarchical Bayesian model

1-1. Introduction

Species identification is often confusing or misguided, even though species identification is a prerequisite for the field of ecology and the conservation of rare species. One problem with species identification is that interspecific hybrids often show complex phenotypes derived from the two parents (Rieseberg and Ellstrand 1993; Field et al. 2009). Furthermore, non-destructive identification is a prerequisite for long-term follow-up surveys (Beerling and Fry 1990; Tackenberg 2007). To solve these problems, geometric morphometrics have been applied to differentiate between groups based on morphological characteristics represented by leaf shape (Viscosi and Cardini 2011). Numerical taxonomy, including multivariate analysis, is also common for classifying species (Du et al. 2007). Canonical discriminant analysis (CDA), one of the most popular multivariate analyses, has also been used to classify species (Gurevitch 1992; Kothera et al. 2007). These techniques require measuring numerous variables and, often, destructive sampling. For field surveys, however, measuring fewer characteristics is desirable.

Because leaves can be measured conveniently, even when sexual reproductive organs are not developed, the ratio of blade length to width is often used to identify plant species (Albert et al. 1997; Neophytou et al. 2011). However, the shapes and sizes of leaves vary as a result of environmental, physiological and/or ontogenic factors (Sultan 2003). Analyses of leaf sizes and shapes should be performed carefully because of two types of errors: measurement errors (Sokal and Rohlf 1995; Jasieński and Bazzaz 1999) and non-linear errors (Phillips 1983; Jackson and Somers 1991).

The hierarchical Bayesian model (HBM) has recently been applied to compare continuous changes, such as allometric relationships, and to investigate bivariate changes (Price et al. 2009). One advantage of an HBM is the flexibility in constructing the model (Clark 2005). A number of random effects resulting from the variability of an individual and

measurement and non-linear errors can be included in the model. Therefore, an HBM is expected to provide high identification efficiency. In this study, I attempted to construct an HBM that identified two parental species and a hybrid using two simple variables—leaf length and leaf width. The accuracy of the HBM was evaluated by comparing it with a CDA based on 13 morphological traits. On the basis of these results, applications for the HBM in plant taxonomy, ecology, and conservation are discussed.

1-2. Materials and Methods

Plant materials and sampling

Three species of *Drosera* were examined: two parental species, a tetraploid *Drosera anglica* Hudson (2*n* = 40) and a diploid *D. rotundifolia* L. (2*n* = 20), and the triploid hybrid of them, *D. obovata* Mert. et Koch (2*n* = 30) (Crowder et al. 1990). These three species are typically identified by their leaf shapes: *D. anglica* has long-elongated leaves, *D. rotundifolia* has rounded leaves, and *D. obovata* has spoon-shaped leaves, which are often confused with the shapes of the two parental species (Tutin et al. 1993; Iwatsuki et al. 2001). Therefore, identification of these three species is often postponed, especially where these three species cohabit. Once the seeds are produced on the flowering stalks, *D. obovata* can be identified by the sterility of seeds (Rodondi et al. 2009).

The samples were measured in a post-mined peatland in the Sarobetsu mire (45°06’N, 141°42’E, 7 m a.s.l.), northern Hokkaido, Japan (Nishimura et al. 2009). *Sphagnum* peat was mined annually in the mire form 1970 to 2003. Two *Drosera* species, *D. rotundifolia* and *D. anglica*, co-occurred in an area mined 37-39 years ago. Because of the morphological traits of the parent species, the hybrid *D. obovata* was likely to establish there. The vegetation is dominated by *Carex middendorffii* Fr. Schm. and *Sphagnum papillosum* Lindb. A 300 cm × 50 cm plot was set up in an area where the putative hybrid was established. Additionally, four 300 cm × 50 cm plots were set up in areas where the two parental species were established.

Measurements of morphological traits

Sampling the entire *D. anglica* individual is prohibited in Japan. Because of the plant's small population in the southern region, it is an endangered species in Japan (Ministry of the Environment Government of Japan 2012). The samples were selected in three steps in early August 2010: (1) all fruiting individuals, whether the seeds were fertile or sterile, in the five plots; (2) individuals not producing flowers adjacent to the plots; and (3) putative *D. obovata* individuals inside and outside the plots. All the measurements were conducted *in situ*. First, the leaves were counted for each sample (Fig. 1.1). Since the *Drosera* species often develops rosettes, particularly in the spring and fall (Koyama and Tsuyuzaki 2013), measuring whole leaves was impractical. Thus, the longest leaf was chosen as the measurement for the size of each sample. Since the leaf turnover is rapid on these three species, the first leaves to emerge were mostly withered by the end of June. Leaves that became the longest usually emerged in June. For the longest leaves, the leaf length, blade length, and blade width were measured with a ruler with 1 mm divisions. The blade was defined as an area that bore tentacles. When flowers developed on the flowering stalks, the flowers was counted on each stalk.

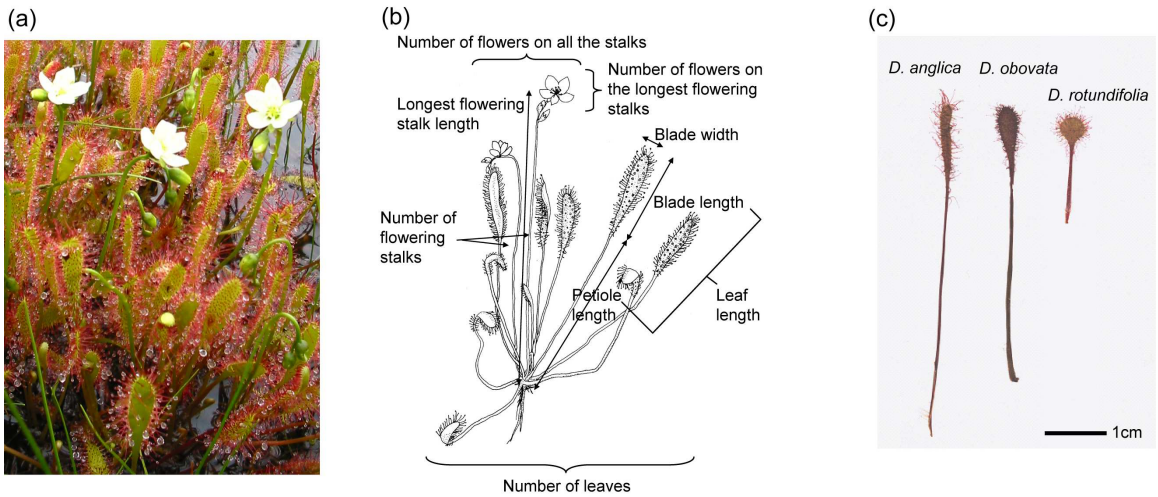

Fig. 1.1

Morphological variables measured for the classification analyses. (a) Flowering *D. anglica* samples established in the Sarobetsu post-mined peatland. (b) Schematic illustration of the nine variables measured in the field. Based on these measured variables, leaf area and the three ratios related to leaf shape were calculated. (c) Scans of dried leaf specimens of the three examined species collected from the study site in Sarobetsu mire during June 2012.

Species identification

All the sample individuals were identified by their relative DNA content in the leaf (Partec PA, Partec GmbH, Münster) (Miyashita et al. 2010) and/or the seed fertility (Rodondi et al. 2009). The samples were identified before the statistical analyses because complete identification is required to evaluate identification errors. The fertility of the seeds was confirmed by their firmness and solidness.

DNA content was measured for non-flowering individuals of *D. rotundifolia* and *D. anglica* and for all individuals of putative *D. obovata*. Two fully foliated leaves were separated from the shoot and were chopped in 0.2 mL nuclei-extraction buffer (CyStain UV precise P; Partec, Münster). More than 5000 cells were used for each flow cytometry measurement. After the samples were filtered through a 30-μm nylon mesh, the nuclear samples were stained with a 0.8 mL DAPI solution containing 10 mmol/L Tris, 50 mM sodium citrate, 2 mmol/L $MgCl_2$, 1% (*w*/*v*) PVP K-30, 0.1% (*v*/*v*) Triton X-100 and 2 mg/L DAPI (pH 7.5). After incubating the samples for 5 minutes at room temperature, the DNA content was measured using flow cytometry technique. The *D. rotundifolia* leaves that were identified by their fertility and morphological traits were used for the internal standard. Two replicates were measured for each individual.

Statistical analyses

Leaf area was calculated by assuming that the leaves have an oval shape. The ratios of leaf length to blade width, blade length to blade width and blade length to petiole length were also calculated. These three ratios express the slimness of the leaves and are related to leaf shape rather than leaf size (Tsuyuzaki 2000). Therefore, these ratios were used to quantify the leaf's shape (Corney et al. 2012).

A multivariate classification of species was attempted through CDA (James 1990). In CDA, canonical coordinates are created to establish separate categories, which are equivalent to species in this study, based on a linear function obtained by combining multivariate variables. In this study, the CDA was based on either 13 (full) variables or two variables

Table 1.1

Morphological characteristics of the three examined *Drosera* species and the standardized canonical coefficients for axes I and II of the canonical discriminant analysis (CDA) based on 13 variables. For each species, each numeral shows the median value with the range (minimum and maximum). Also see Fig. 1.3.

	Species			CDA	
Variables used for CDA	*D. anglica*	*D. rotundifolia*	*D. obovata*	Axis I	Axis II
Number of leaves	7 (3-18)	4 (2-11)	5 (2-25)	-0.101	0.048
Leaf size (of the longest leaf)					
Blade length (cm)	2.5 (0.4-5.2)	1.2 (0.2-2.5)	2.1 (0.2-3.6)	2.358	-10.111
Blade width (cm)	0.4 (0.2-0.7)	0.9 (0.2-1.5)	0.6 (0.2-1.2)	7.981	6.450
Petiole length (cm)	5.2 (0.8-8.4)	4.7 (0.5-10.6)	4.5 (0.5-9.0)	1.490	-10.329
Leaf length (cm)	7.7 (1.3 – 12.7)	5.9 (0.7 – 12.0)	6.7 (0.7 – 11.8)	-1.514	10.475
Leaf area (cm^2)	0.75 (0.06-2.09)	0.79 (0.03-2.59)	1.07 (0.03-2.83)	-3.365	-4.129
Leaf shape (of the longest leaf)					
Leaf length/blade width	21.0 (6.5-41.3)	6.5 (3.0-12.3)	10.0 (3.5-21.3)	-0.356	-0.221
Blade length/blade width	7.0 (2.0-13.7)	1.3 (0.8-2.4)	3.17 (1.0-5.0)	0.572	1.173
Blade length/petiole length	0.50 (0.21-1.22)	0.26 (0.09-0.90)	0.43 (0.13-0.97)	-6.764	-3.728
Flowering stalk					
Longest length (cm)	9.3 (2.5-16.3)	10.3 (4.9-21.2)	10.2 (3.6-21.2)	0.074	-0.011
Number of stalks	1 (0-4)	1 (0-3)	1 (0-5)	0.043	0.649
Number of flowers on all the stalks	3 (0-15)	5 (0-27)	3 (0-36)	-0.110	-0.310
Number of flowers on the longest flowering stalk	3 (0-9)	5 (0-13)	3 (0-24)	0.222	0.415

(blade length and width) (Table 1.1). The former CDA was applied to increase the detection sensitivity for the species classification, and the latter CDA was used as a comparison to an HBM that was developed using the same two variables.

Differences in the ratio of blade length to width between the three species were examined with an HBM that consisted of two processes (see Supplemental Data with the online version of this article): (1) a parameter estimation by linking the observed data to adjust the probability distribution with the consideration of size dependence (Price et al. 2009) and (2) a probability estimation on each individual that was classified into the three species.

For the first process, the ratio of the blade length to width is derived from the ratio of the observed blade length to the width for each sample. The observed data, $y_i^{(o)}$ and $x_i^{(o)}$, for sample i, were assumed to follow a normal distribution with two expected latent variables, blade length y_i, and width x_i. The superscript (o) denotes the observed quantities. y_i and x_i from the measured $y_i^{(o)}$ and $x_i^{(o)}$ were: $y_i^{(o)} \sim N(y_i\ ,\ 10^{-4})$ and $x_i^{(o)} \sim N(x_i\ ,\ 10^{-4})$, where 10^{-4} is assumed to be the variance of the measurement errors. y_i and x_i are defined as $y_i = L_i \times \sqrt{R_i}$, and $x_i = L_i/\sqrt{R_i}$, where L_i is the leaf area of sample i and is calculated by the geometric mean of blade length and width, i.e., $\sqrt{(y_i \times x_i)}$. The prior distribution of L_i is given by $L_i \sim N(0,\ 10^4)$. R_s is a function to consider the size-dependent changes of leaf shape on species s, given by $\text{mean}(R_s) = \exp(\beta_{1,s} + \beta_{2,s} \cdot L_i + \varepsilon_{i,s})$, where $\beta_{1,s}$ is the intercept on each species s and $\beta_{2,s}$ is the coefficient quantifying the dependence of leaf size, L_i, which is assumed to follow a logarithmic distribution. $\varepsilon_{i,s}$ is an inverse-variance parameter for a random effect on an individual following $N(0,\ \sigma_s)$, and σ_s represents the standard deviation of an individual's characteristics on the linear predictor of R_s. σ_s is assumed to follow a uniform distribution with a mean of 0 and a variance of 10^{-4}. A log link function was used to estimate the mean of R_s.

For the second process, the probability of a discriminated individual i on species s, $q_{i,s}$, was evaluated; v_s expresses the appearance intensity of isometric individuals on species s and is defined as $v_s = 1/\sigma_s \cdot \exp\{-[\ x - \text{mean}(R_s)]^2/2\sigma_s^2\} \cdot w_s$, where R_s and σ_s have already been estimated during the first process, and w_s is a weight on species s, depending on the number of samples (w_s for *D. anglica* and *D. obovata* are calculated with 1 substituted for w_s for *D.*

rotundifolia). The prior distribution of w_s is noninformative and follows $N(0, 10^4)$. $q_{i,s}$ follows a categorical distribution and is calculated by $q_{i,s} = v_{i,s}/\sum v_{i,s}$. Based on the highest $q_{i,s}$, individual *i* was determined to be species *s*.

Bayesian inference with a Markov chain Monte Carlo (MCMC) simulation was used to estimate the parameters and the posterior distribution (McCarthy 2007). The posterior samples were obtained by combining three independent MCMC chains in which 200 values were sampled at 10-step intervals after 1000 burn-in MCMC steps. The convergence of the MCMC calculation was confirmed for all the parameters (Gelman et al. 2003). WinBUGS 1.4.3 through R2WinBUGS was used to run the HBM in a software package R 2.15.1 (Spiegelhalter et al. 2003; Sturtz et al. 2005; R Development Core Team, 2012).

The relationships between leaf area and misidentification on the CDA and the HBM were examined for each species by a generalized linear model (GLM) with a binomial distribution, i.e., true assigned as 1 and false as 0.

1-3. Results

Species identification and morphological characteristics

The relative abundance of DNA was measured for 183 samples consisting of 124 flowering and 59 non-flowering individuals. The DNA content confirmed that all of the individuals were $2n$ = 20, 30 or 40 (Fig. 1.2). The flow cytometry identified 26 samples as *D. rotundifolia*, 68 as *D. anglica*, and 89 as *D. obovata*. Of the 59 non-flowering individuals, 27, 3, and 29 were *D. anglica*, *D. rotundifolia*, and *D. obovata*, respectively. All the flowering *D. obovata* individuals that were identified by their DNA content produced sterile seeds, while *D. rotundifolia* and *D. anglica* produced fertile seeds. Therefore, seed fertility was a perfect criterion for identification. Additionally, 321 *D. anglica* and 285 *D. rotundifolia* individuals were identified by their seed fertility. Therefore, 389 *D. anglica*, 89 *D. obovata*, and 311 *D. rotundifolia*, 789 in total, were used for the classification analyses (Table 1.2).

All the indexes on leaf size, except the leaf area of *D. obovata*, were intermediate between the two parental species (Table 1.1). The leaf area was larger for the hybrid, *D. obovata*, than for the two parental species. Since the blade length of *D. obovata* was closer

to that of *D. anglica* than to that of *D. rotundifolia*, the leaves of *D. obovata* became large. The leaf shapes of *D. obovata*, expressed by the three ratios, were also intermediate between those of the two parental species. On flowering stalks, the stalk lengths did not greatly differ between the three species. When the flowering stalks were developed, the number of stalks was usually one and less than five for all the species. *Drosera rotundifolia* produced more flowers than did *D. anglica* and *D. obovata*.

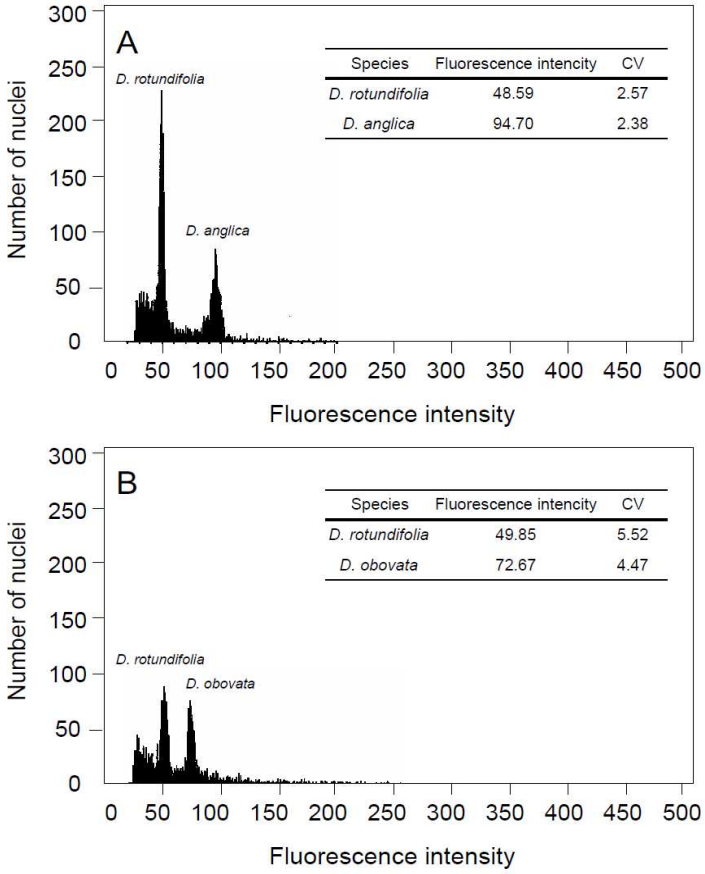

Fig. 1.2

The relative fluorescence intensity of nuclei isolated from the leaves of *Drosera anglica* (2*n* = 40) (A) and *D. obovata* (2*n* = 30) (B) with *D. rotundifolia* used as the internal standard (2*n* = 20).

Table 1.2

The species identification accuracies for the three *Drosera* species examined by the canonical discriminant analysis (CDA) based on all the measured leaf and flower characteristics (all), the CDA based on blade length and width (leaf) and the hierarchical Bayesian model (HBM) based on blade length and width. Numerals indicate the number of samples estimated by each of the three models. The identification errors (%) are shown in parentheses. The total number of samples is 789.

		Estimated			
Model	Correct	*D. anglica*	*D. rotundifolia*	*D. obovata*	Total
CDA					
All	*D. anglica*	369	0	20	389 (5.1)
	D. rotundifolia	0	304	7	311 (2.3)
	D. obovata	6	6	77	89 (13.5)
Leaf	*D. anglica*	376	0	13	389 (3.3)
	D. rotundifolia	0	301	10	311 (3.2)
	D. obovata	14	12	63	89 (29.2)
HBM	*D. anglica*	375	0	14	389 (3.5)
	D. rotundifolia	0	308	3	311 (1.0)
	D. obovata	11	9	69	89 (22.5)

Multivariate classification on three species

Using all 13 variables obtained from the leaves and flowers, the CDA clustered the samples into three groups (Fig. 1.3(a)). The contribution rates were 95.4% for the first axis and 4.6% for the second axis, indicating that the first axes explained most of the variation. The scores of the three species were arranged along canonical axis I, indicating that these three species were distinguished primarily by variables related to axis I. The two parental species were positioned on opposite sides on axis I, and the hybrid was located between the two parents, indicating that the overall characteristics of *D. obovata* were intermediate between the two parental species.

Three leaf size indexes (blade length, width, and area) and a shape index (ratio of blade length to petiole length) were strongly correlated with canonical axis I (Table 1.1). The other two size indexes, petiole length and leaf length, were also correlated with axis I. Along with axis I, these six indexes were correlated with axis II. Flowering indexes were more strongly correlated with axes I and II than with leaf size and shape, indicating that the leaves were mostly identified by their characteristics.

The overall identification error was 4.9% for the CDA based on all 13 morphological traits. Although the identification error was small for *D. rotundifolia* and *D. anglica*, 2-6%, when all morphological characteristics were used in the CDA, the identification error reached 13.5% for *D. obovata* (Table 1.2). Misidentification occurred more often with small leaves of *D. anglica* and *D. rotundifolia* (GLM, df = 387 and 309, z = 3.8 and 4.1, $P < 0.01$), indicating that the errors were size dependent. For *D. obovata*, however, the errors were not size dependent (GLM, df = 87, z = 0.3, $P > 0.01$).

Classification by blade length and width

When the CDA was based on two leaf variables, blade length and width, the species classification became difficult (Fig. 1.3(b)). The overall identification error increased from 4.9%, for the CDA based on 13 morphological traits, to 6.2%, for the CDA based on two leaf traits (Table 1.2). The identification error increased from 2.3% to 3.2% for *D. rotundifolia* and from 13.5% to 29.2% for *D. obovata*, while the error for *D. anglica* decreased from 5.1% to 3.3%. These results indicate that the CDA was ineffective when utilizing only a few variables and that CDA thus requires a number of variables to reduce the classification errors. The misidentified *D. anglica* and *D. rotundifolia* samples were classified as *D. obovata*, and *vice versa*.

The intercept of the ratio in the HBM, β_1, for *D. obovata* was intermediate between those for *D. anglica* and *D. rotundifolia* (Table 1.3). $\beta_{2,}$ in the HBM, was positive for all three species, demonstrating that the ratio of blade length to width changed non-linearly with changes in the leaf area (Fig.1.4). High standard deviations and credible intervals for *D. obovata* indicated that the hybrid had more variable leaf shape than the two parental species.

The HBM based on two variables had a 4.7% overall-identification error, which was 0.2% lower than the CDA based on 13 variables (Table1.2). In the HBM, the relationship between leaf area and misidentification was not significant for *D. rotundifolia* and *D. obovata* (GLM, df = 309 and 87, z = 0 and -0.2, $P > 0.01$), whereas it was significant for *D. anglica* (GLM, df = 387, z = 4.2, $P < 0.01$). Therefore, the size-dependent error for *D. obovata* was removed by the HBM, but the error for *D. anglica* was not.

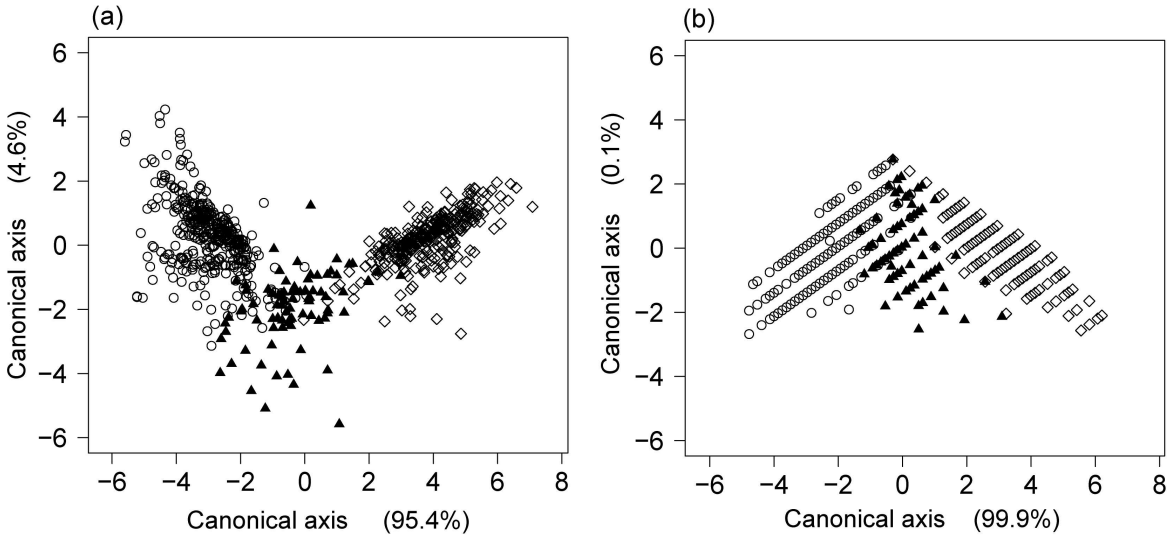

Fig. 1.3

The scores of individual samples of the three examined species, *Drosera anglica* (○), *D. obovata* (▲) and *D. rotundifolia* (◊), obtained by canonical discriminant analysis (CDA) on the first two canonical axes. The contribution percentage for each axis is shown in the parentheses. The CDA results were examined according to (a) 13 morphological variables and (b) two variables—blade width and length—for the three examined species.

Table 1.3

Mean, standard deviation (SD) and 95% credible interval (lower and upper limits) of the posterior distributions of the two parameters, β_1 (intercept) and β_2 (slope), for each species, obtained with the hierarchical Bayesian model.

Parameter	Species	Mean	SD	2.5%	97.5%
β_1	*D. anglica*	1.923	0.014	1.895	1.950
	D. rotundifolia	0.260	0.011	0.238	0.282
	D. obovata	1.041	0.030	0.985	1.098
β_2	*D. anglica*	0.584	0.052	0.482	0.685
	D. rotundifolia	0.383	0.040	0.306	0.460
	D. obovata	0.447	0.075	0.311	0.595

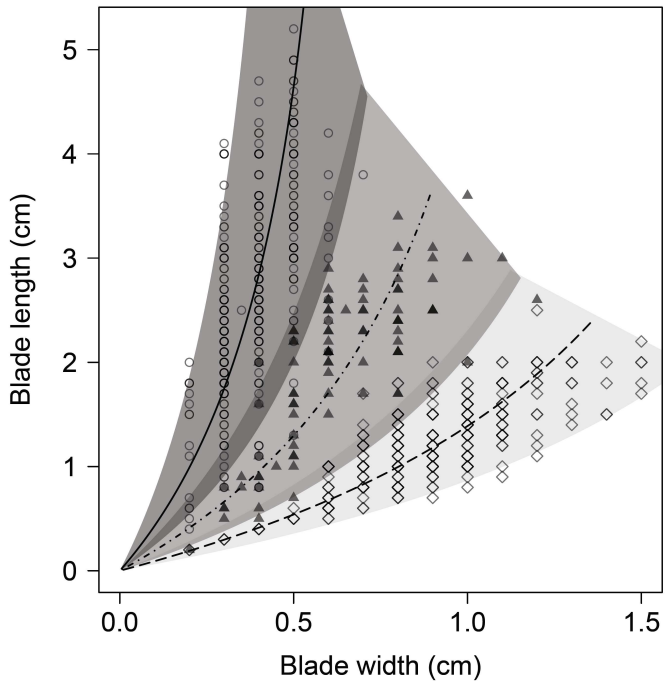

Fig. 1.4

Relationships between blade width and length for the three examined species, *Drosera anglica* (○), *D. obovata* (▲), and *D. rotundifolia* (◊), investigated by the hierarchical Bayesian model (HBM). Solid, dashed-dotted and interrupted lines indicate the relationships between blade length and leaf for *D. anglica*, *D. obovata* and *D. rotundifolia*, as estimated by the HBM. The shaded areas that cover the lines express 95% credible intervals on the respective species.

1-4. Discussion

The identification efficiency was higher with the HBM based on two leaf variables—the blade length and width of the largest leaf on an individual—than for the CDA based on 13 variables from leaves and flowers. The higher identification efficiency resulted from the improved identification efficiency for small leaves and the introduction of non-linear equations. Multivariate analyses, including CDA, are powerful tools to differentiate groups when numerous variables are being considered, even though these analyses are derived from unreliable assumptions, i.e., linearity, normality, and/or homoscedasticity (James 1990; Borcard et al. 2011). However, these functions are not interpreted by biological factors, except for their applications in quantitative genetics and in classification (Mitteroecker and Bookstein 2011). Identification errors when using the CDA occurred with smaller leaves for two of the three examined species, indicating that size dependency and shape variation should be considered (James 1990; Mitteroecker and Bookstein 2011). However, these errors cannot be removed when using a CDA because of the assumptions in the analysis.

Drosera obovata had intermediate values for most of the morphological traits between the parental species, except it had a larger leaf area than the parents. The enlargement of the leaves was considered to be a transgressive trait occurring in hybrids (Schwarzbach et al. 2001). Such transgressive traits increase the probability of emigration and establishment to new habitats (Rieseberg and Willis 2007), although the success of establishment depends on other circumstances. In addition, *D. obovata* is absolutely infertile. Therefore, the transgression is less conducive to population enlargement, owing to the incompatibilities. Since the HBM in this study was developed with two leaf-size variables, blade length and width, the transgressive traits of leaf sizes were a forethought.

The leaves of all the examined *Drosera* species showed a non-linear relationship between blade length and width. Leaf shapes often change with size or age in a species, e.g., *Salix viminalis* (Verwijst and Wen 1996), *Pteris mutilata* (Tsuyuzaki 2000), and *Quercus rubra* (Zwieniecki et al. 2004). For these three species, the ratio of blade length to width increases as the leaf size or age increase. Heteroblastic changes in leaf shapes can be considered (Kerstetter and Poethig 1998) in the present HBM if phenological assessments are performed,

although the ages of the *Drosera* were not determined. Considering the non-linear relationship between blade length and width and the measurement errors, the HBM succeeded in removing size-dependent errors for *D. obovata*, which was the most difficult species to identify.

The HBM is likely to have broad application in plant taxonomy, ecology, and conservation. Numerous morphological traits have been measured to identify interspecific hybrids that show the intermediate morphological traits of the parental species, such as *Carpobrotus* (Aizoaceae) (Albert et al. 1997), *Quercus* (Fagaceae) (Neophytou et al. 2011), and *Roscoea* (Zingiberaceae) (Du et al. 2012). The HBM may be able to identify not only interspecific hybrids but also intraspecific variations, including cultivars (Dornbusch et al. 2011), because of its high versatility (McCarthy 2007).

Currently, non-destructive measurement is highlighted for repeated monitoring (Tackenberg 2007; Wang and Zhang 2012). For example, non-destructive, long-term monitoring is required to detect morphological changes, including leaf size and shape, resulting from global climate change (Royer et al. 2008). Even though only two variables, blade length and width, were used in the HBM in this study, the overall identification error of the HBM was equal to or lower than the error of the CDA. These results suggest that non-destructive, convenient measurements, combined with an HBM, are possible for long-term monitoring of various species. Rare and/or endangered species generally develop small populations in restricted regions. In Japan, a classic example of such a species is the endangered species *D. anglica*, for which identification is particularly difficult when the hybrid, *D. obovata*, co-exists. This method using the HBM should be useful for monitoring the population dynamics of rare species. The present study provides convenient and non-destructive techniques to classify groups by introducing non-linear relationships and the errors of variables into the model.

Chapter 2. Sexual and vegetative reproduction and their population size on sympatric congeners, *Drosera anglica* and *D. rotundifolia*

2-1. Introduction

Differences in reproductive strategies may explain habitat differentiation between congeners (Grime 2001), and are related to population sizes and distributions (Espeland and Emam 2011). For example, short-distance seed dispersal and/or high clonal growth lead a narrow distributional range, and *vice versa* (Walck et al. 2001; Murray et al. 2002; Burne et al. 2003). *Drosera anglica* Huds. originates from hybridization between *D. rotundifolia* L. and *D. linearis* Goldie (Rivadavia et al. 2003). This species often establishes with *D. rotundifolia* on nutrient-poor wetlands in boreal regions (Wolf et al. 2006). These two species reproduce by seeds and daughters (sexual and vegetative reproduction). These two species separate their habitats in ombrotrophic bogs: *D. anglica* is established at higher water level than *D. rotundifolia* (Nordbakken 1996; Nordbakken et al. 2004).

The aims of this study were firstly to detect the differences of the two *Drosera* species in the sexual and vegetative reproduction, and secondly to clarify the relationship between the population dynamics, examined by recruitment, survival and growth, and reproductive characteristics. I hypothesized that *D. anglica* utilized vegetative reproduction more than *D. rotundifolia*, because the established habitats often have inundations.

2-2. Materials and Methods

Study sites and plant materials

The field survey was conducted in a post-mined peatland in Sarobetsu Mire (45°06’N, 141°42’E, 7 m a.s.l.), northern Hokkaido, Japan, where the *Sphagnum* peat was annually mined from 1970 to 2003 (Nishimura et al. 2009). *Drosera anglica* and *D. rotundifolia* establish in an area mined 37-39 years before. The vegetation is dominated by *Carex middendorffii* Fr. Schm. and *Sphagnum papillosum* Lindb. In the surveyed years of 2009-2012, the mean annual temperature was 6.3°C and mean annual precipitation was 1,056 mm (Japan Meteorological Agency 2012).

D. anglica is diploid of 2*n* = 40, whereas *D. rotundifolia* is of 2*n* = 20 (Crowder et al. 1990). These two species perform sexual and vegetative reproduction. The sexual reproduction is performed on scapes with a few hermaphroditic flowers of which pollination is self-compatible (Wolf et al. 2006). The vegetative reproduction is conducted by producing daughters, i.e., new ramets.

Sexual and vegetative reproduction

Five 300 cm × 40 cm permanent plots were established in an area where the two *Drosera* species co-occurred. The census was conducted from late July to early August during 2009 and 2011. The locations of all shoots were marked by numbered tags when the plots were established. At every census, the leaf length of the longest leaf on each shoot was measured by a ruler with 1 mm divisions. When seedlings and daughters recruited, the location and leaf length were recorded. The death was recorded when withered or disappeared. Based on the data, the recruitment and mortality were calculated at one year intervals from the preceding year to the early spring of the present year, because a number of seedlings died during overwintering owing to melting snow (Koyama and Tsuyuzaki 2013).

The leaf length was a surrogate for the shoot mass because the length was highly correlated to dry mass (r^2=0.80, n = 105). The shoots were harvested from the outside of the monitoring plots. The numbers of flowers and daughters were counted on each shoot in mid-summers. The longevity of each flower on the racemes did not exceed 2 days.

Leaf phenology

The leaf phenology of two species was recorded every three weeks from early June to middle September in 2012. On the outside of permanent plots, 29 and 23 shoots were randomly labeled on *D. anglica* and of *D. rotundifolia* in June 3 2012, respectively. Additionally, 15 shoots of *D. rotundifolia* were randomly marked in June 24 2012. The total numbers of shoots for analyses were 26 on *D. anglica* and 36 on *D. rotundifolia* because of the mammals attack or withering. At every survey, numbers of leaves, flowers and daughters were counted. The leaf length, blade length and leaf width were measured on all the leaves. The

total leaf area on each shoot was calculated from blade length and width by the equations obtained by 34 and 40 leaves of *D. anglica* and *D. rotundifolia*, respectively. The leaf area was measured by a flatbed scanner (CanoScan LiDE 90, 300 dpi) with Image-J image software (Rasband 2011). The leaf areas were estimated by $e^{-0.11+0.91\log(\text{blade length} \times \text{leaf width} \times 0.25\pi)}$ (r = 0.94, significant at $P < 0.001$) on *D. anglica* and $e^{-0.47+0.71 \times \log(\text{blade length} \times \text{leaf width} \times 0.25\pi)}$ on *D. rotundifolia* (r = 0.92, significant at $P < 0.001$).

Statistical analysis

Generalized linear mixed-effects model (GLMM) with the assumption of Poisson distribution was used to investigate the determinants on number of flowers or daughters. The lengths of longest leaf after log-transformation, species and their interaction were explanatory variables in the models and individual shoot was the random effect. To detect the determinants on the numbers of seeds and daughters, GLMM was used with the assumption of Poisson distribution. The numbers of flowers, species and their interaction were explanatory variables and individual code was the random effect. Akaike's Information Criteria (AIC) were used to select the best models of the GLMMs.

The determinants on number of daughters was analyzed by Poisson hurdle models, because of the excesses zero counts. Hurdle model consists of two sub-models: (1) a binomial model for the binomial distribution of daughters or flowers, and (2) a count model for the numbers, conditioned after the binomial model (Zeileis et al. 2008). The log-transformed lengths of longest leaf, number of flowers, species, observed year and the interaction between leaf length and observed year were explanatory variables. The sample code was used for the random effect.

The relationships between reproduction and survival were examined by GLMM with a binomial distribution. Plant size, number of flowers and number of daughters were explanatory variables, and the sample code was the random effect. The effects of sexual and vegetative reproduction on the relative growth rate in each year were evaluated by GLM with Gaussian distribution. Relative growth rate is expressed by: $\log(L_n / L_c)$, where L_n and L_c indicate leaf lengths in the next and current years, respectively. Number of flowers or

daughters was used as explanatory variables. All the analyses were conducted with the statistical package R (ver.2.15.1) (R Foundation for Statistical Computing, Vienna, 2012).

2-3. Results

Shoot phenology

In total, 2286 *D. anglica* and 2657 *D. rotundifolia* shoots were investigated in the five plots during the three surveyed years (Table 2.1). The total numbers of shoots were highest in 2010 on both species. On *D. anglica*, 559 seedlings and 125 daughters recruited to 2009, respectively, and 281 and 121 to 2011. On *D. rotundifolia*, 728 seedlings and 23 daughters recruited to 2009 and 396 and 34 to 2010. The seedlings contributed greatly to the mortalities on both species, i.e., 218 and 383 died on *D. anglica* to 2009 and to 2010, respectively, and 97 and 491 died on *D. rotundifolia* to 2009 and to 2010. Non-seedlings survived well, i.e., the mortalities on *D. anglica* were 66 in 2010 and 236 to 2011 and these on *D. rotundifolia* were 84 to 2010 and 374 to 2011. Therefore, the numbers of shoots fluctuated annually due mostly to the seedling recruitment and morality.

The flowering percentages annually fluctuated during the three surveyed years, ranging from 8.7% to 20.1% on *D. anglica* and from 6.8% to 27.0% on *D. rotundifolia*. The production of daughters per year was more than 2.9% on *D. anglica* for the three years, and less than 1.3% on *D. rotundifolia*.

The durations of flowering were four to six weeks on both species (Fig. 2.1). *D. anglica* flowered two to four weeks earlier than *D. rotundifolia* in all the examined years. Therefore, the flowering periods of these two species were overlapped for a few weeks. The productions of dormant buds that formed daughters began after flowering on the two species.

The seasonal changes in total leaf area on shoots resembled between the two species in 2012. The total leaf area gradually increased from June to July on both species, peaked in mid-July, and then decreased, i.e., defoliated (Fig. 2.1). Therefore, the peak of leaf area synchronized with flowering period on *D. anglica*, while it preceded flowering on *D. rotundifolia*. Shoots producing daughters were larger than shoots producing flowers on the two species at the peak of total leaf area (GLM, $P<0.05$). Shoots without any flowers and

daughters were smallest.

Table 2.1

The yearly fluctuations of number of shoots on *D. anglica* and *D. rotundifolia* in the five 300 cm × 40 cm plots from 2009 to 2011 in relation to reproduction.

Species	*D. anglica*			*D. rotundifolia*		
Year	2009	2010	2011	2009	2010	2011
Non-reproductive	461 (67.1%)	1073 (77.0%)	1114 (84.6%)	353 (69.1%)	1600 (87.2%)	1442 (91.6%)
Sexually reproductive	138 (20.1%)	216 (15.5%)	115 (8.7%)	138 (27.0%)	204 (11.1%)	107 (6.8%)
Vegetatively reproductive	25 (3.6%)	40 (2.9%)	61 (4.6%)	6 (1.2%)	22 (1.2%)	20 (1.3%)
Sexually and vegetatively reproductive	63 (9.2%)	65 (4.7%)	27 (2.1%)	14 (2.7%)	8 (0.4%)	6 (0.4%)
Total	687	1394	1317	511	1834	1575

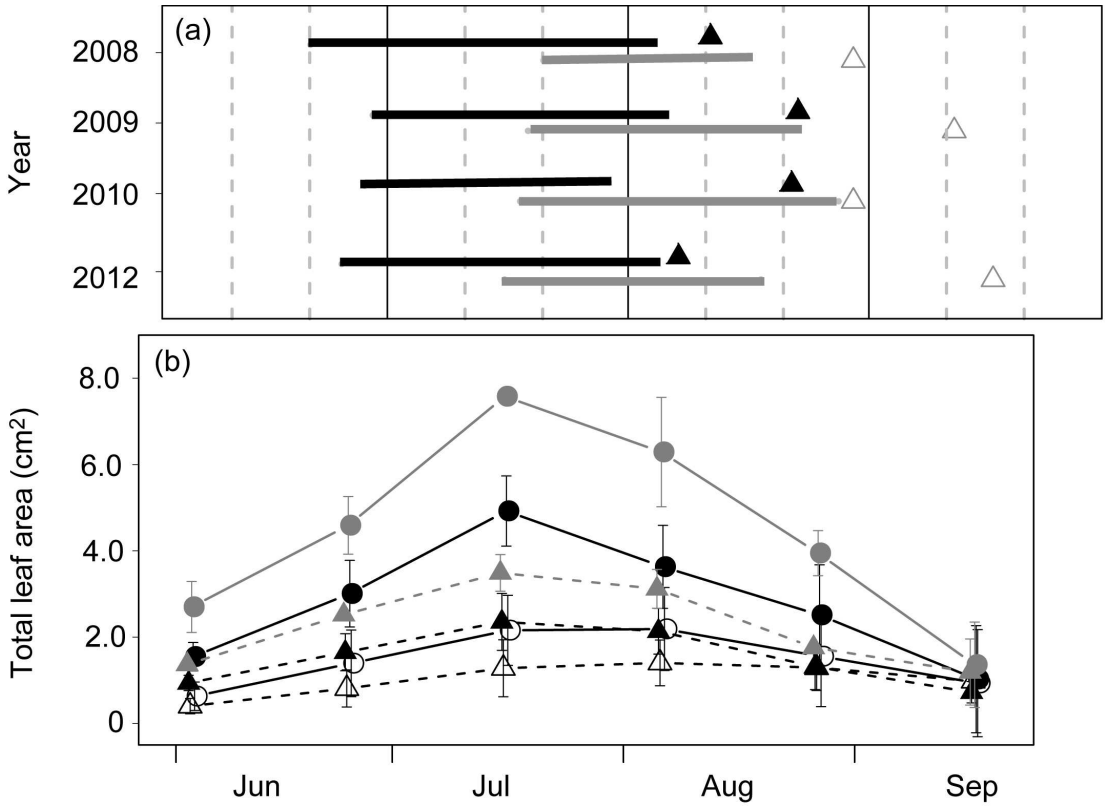

Fig.2.1

Phenology of flowering, dormant bud, and leaf area on the two examined *Drosera* species, *D. anglica* and *D. rotundifolia*. (a) The productions of flowers and dormant buds, which potentially became daughters, from 2008 to 2012 except in 2011. The black and grey lines indicate flowering periods on *D. anglica* and *D. rotundifolia*, respectively. The first observations of daughters in each year are shown by closed triangles on *D. anglica* and by open triangles on *D. rotundifolia*. (b) Temporal changes in total leaf area on shoots from June 3 to September 16 in 2012 on *D. anglica* (circles) and *D. rotundifolia* (triangles), with reference to producing daughters (grey symbols), flowers (solid) and no daughters and flowers (open). Means (symbols) are shown with standard deviations (error bars).

Sexual and vegetative reproduction

Increases in the number of flowers on shoots were exponentially with increases in number of flowers on the two species, and the regression slope was steeper on *D. rotundifolia* than *D. anglica* (Fig. 2.2). However, the relationships between numbers of flowers and seeds did not differ between the two species. Therefore, the seed production per shoot was lower on *D. anglica* than on *D. rotundifolia*, because of differences in number of flowers between them.

GLMM selected two exponential lines on relationship between numbers of flowers and daughters (Fig. 2.2). The increments of daughters were sharp on *D. anglica*. The sexual and vegetative reproduction on the two species were positively related to the shoot sizes, i.e., numbers of flowers and daughters increased with increasing the maximum leaf length (GLMM, $P < 0.05$), independent of species. The leaves of daughter-producing shoots were longer on *D. anglica* (range: 0.7-13.0 cm, 7.3 ± 2.4 cm) than on *D. rotundifolia* (0.8-10.5 cm, 5.4 ± 2.4 cm).

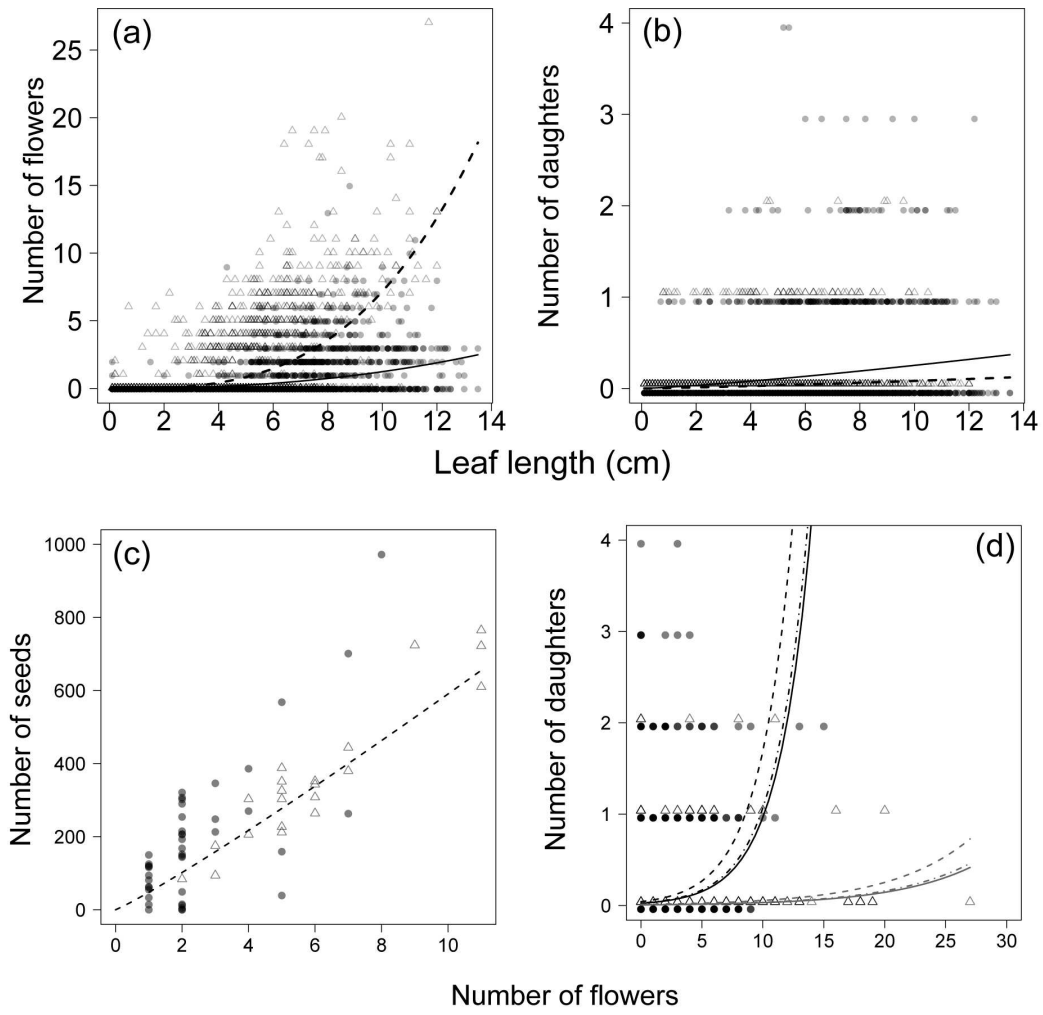

Fig.2.2

Relationships between maximum leaf length and number of flowers (a) or daughters (b), and between numbers of flowers and seeds in 2008 (c) or daughters in 2009-2011 (d) on *Drosera anglica* (filled circles) and *D. rotundifolia* (open triangles) examined by GLMM. (a) Two exponential lines explain the relationships: log$y$ = exp(-5.13 +2.32log$x$) on *D. anglica* (solid line), log$y$ = exp(-5.16 +3.10log$x$) on *D. rotundifolia* (interrupted line). (b) Two exponential lines explain the relationships: log$y$ = exp(-4.29 +1.26log$x$) on *D. anglica* (solid line), log$y$ = exp(-5.41 +1.43log$x$) on *D. rotundifolia* (interrupted line). Here, x indicates leaf length, and y indicates number of flowers in (a) and number of daughter in (b). Color strengths become darker when more shoots overlap the values. (c) An exponential line, log$y$ = exp(-3.80 +1.11log$x$), mostly explains the relationships. (d) Six exponential lines explain the relationships that mean the relationships differ between species and between years. *D. anglica* (black lines) and *D. rotundifolia* (grey lines) are: log$y$ = exp(-3.16 +0.36log$x$) and log y = exp(-4.40 -0.15log$x$) in 2009 (interrupted lines), log$y$ = exp(-3.72 +0.36log$x$) and log y = exp(-4.56 -0.15log$x$) in 2010 (solid lines) and log$y$ = exp(-3.62 +0.36log$x$) and log$y$ = exp(-4.86 -0.15log$x$) in 2011 (dotted lines),where x and y are the number of flowers and the number of daughters, respectively.

Daughter production

The numbers of daughters on the respective species were not explained by leaf length, number of flowers and year (count model in Table 2.2), while the presences of daughters on increased with increasing leaf length (zero-hurdle model). These indicated that the formation of daughters was determined by shoot sizes on both the species, although the number of daughters was independently determined by their sizes. The number of flowers was positively correlated to the formation of daughters on *D. anglica*, and was not on *D. rotundifolia*. These indicated that the production of daughters was synchronized with flowers only on *D. anglica*.

Table 2.2

Parameter estimated by hurdle models that predict number of daughters on *D. anglica* and *D. rotundifolia* respectively. ***: significantly different at $P < 0.001$, ** $P < 0.01$, *: $P < 0.05$, and NS: non-significant. ×: interaction.

	D. anglica		*D. rotundifolia*	
Estimate	Count	Zero-hurdle	Count	Zero-hurdle
Intercept	-1.25 $^{N.S}$	-3.58 ***	-2.75 $^{N.S}$	-7.02 ***
Leaf length	0.35 $^{N.S}$	1.00 ***	0.88 $^{N.S}$	2.50 **
Number of flowers	0.01 $^{N.S}$	0.21 ***	-0.03 $^{N.S}$	-0.01 $^{N.S}$
year 2010	-1.77 $^{N.S}$	-1.12 $^{N.S}$	-4.91 $^{N.S}$	1.80 $^{N.S}$
year 2011	-2.24 $^{N.S}$	-0.47 $^{N.S}$	-9.72 $^{N.S}$	2.05 $^{N.S}$
Leaf length × year 2010	0.65 $^{N.S}$	0.37 $^{N.S}$	2.47 $^{N.S}$	-1.16 $^{N.S}$
Leaf length × year 2011	0.40 $^{N.S}$	0.06 $^{N.S}$	-3.66 $^{N.S}$	-1.19 $^{N.S}$

Survival and growth rate

Larger shoots survived more on both species for the three years (Table 2.3). However, the shoots died more after flowered, except to 2010 on *D. rotundifolia*. *D. anglica* showed high survival when the shoots produced daughters to 2010. Flowering individuals died more than non-flowering ones on the two species, while *D. anglica* survived more after producing daughters.

The leaf lengths, surrogate for shoot size, on the two species were not adopted in the GLM models of growth rates (Table 2.3), indicating that the growth rates were not dependent on shoot sizes. The growth rates on both species slowed in the next years of flowering. The production of flowers, i.e., sexual reproduction, decreased the growth rates on the two species, while the production of daughters did not affect the rates.

Table 2.3
The effects of sexual and vegetative reproduction on the survival and growth of *D. anglica* and *D. rotundifolia*, examined by GLM on growth and GLMM on survival. Significance is determined by *t* scores on GLM and by *Z* scores on GLMM. ***: significantly different at $P < 0.001$, ** $P < 0.01$, and *$P < 0.05$. -: the variables are discarded by AIC.

Species	*D. anglica*		*D. rotundifolia*	
Coefficient	2009-10	2010-11	2009-10	2010-11
Survival				
Intercept	-13.40^{***}	-0.58^{***}	-0.47^{***}	-0.43^{***}
Leaf length in the previous year	6.76^{***}	0.26^{***}	0.33^{***}	0.31^{***}
Flower	-2.26^{*}	-0.16^{*}	-	-0.11^{***}
Daughter	18.45^{***}	$9.55^{N.S}$	$1.35^{N.S}$	$10.36^{N.S}$
Growth				
Intercept	0.31^{***}	0.16^{***}	0.31^{***}	0.07^{*}
Flower	-0.08^{***}	-0.06^{***}	-0.08^{***}	-0.06^{***}
Daughter	-	-	$-0.27^{N.S}$	-

2-4. Discussion

Size-dependent reproduction

Both sexual and vegetative reproduction was size-dependent on the two *Drosera* species, i.e., numbers of flowers and daughters increased with increasing shoot sizes. Shoot sizes often determine the productivities of flowers and daughters on perennial herbs, due to resource limitation or allocation (Worley and Harder 1996; Weiner et al. 2009).

Larger shoots produced more daughters on the two species. The size dependency was more conspicuous on daughter production than on flower production. Since the production of dormant buds for producing daughters followed the production of flowers, the production of daughters required plenty of resources. Large shoots afford the production of daughters, because they store nutrient resources in the dormant buds before defoliation (Wolf et al. 2006; Ott and Hartnett 2011). The production of dormant buds has not been reported from *D. anglica* and *D. rotundifolia* on a bog in southeast Norway (Nordbakken et al. 2004) and from *D. rotundifolia* in a German silting-up bog (Tumm 1988). The shoots in these two regions are smaller than in Sarobetsu mire. Probably because the shoots required resources more for producing daughters than for flowers, *D. anglica* that was larger than *D. rotundifolia* produced more daughters.

Effects of reproduction on growth and survival

The two species slowed their growths when they produced flowers, suggesting that the cost of flower production was compensated by reducing shoot growth. The trade-off between the productions of sexual reproduction and shoot growth often occurs when the resources are limited for perennial herbs (Karlsson and Méndez 2005). However, such relationship was not observed between shoot growth and daughter production. Since resources for daughter production was allocated the previous years, as seen in *Pinguicula vulgaris* L. (Worley and Harder 1996), the growth rate in the current years was not affected by the daughter production.

Flower-produced *D. anglica* decreased the survival more than *D. rotundifolia*, while daughter-produced *D. anglica* increased the survival. The flower production decreased not

only the growth rates but also survivals, probably because of high allocation to flowers (Karlsson et al. 1990; Aragón et al. 2009). Because shoots had to be large to produce daughters in the previous years, the survival was high on *D. anglica* daughter production in the current years. These results also suggest that the longevities of *Drosera* spp. are short, although plant longevity remains unclear for clonal perennial plants (de Witte and Stöcklin 2010).

Roles in the production of daughters

There are two strategies on reproduction for perennial plants; one is escape by sexual reproduction and another is anchor by vegetative reproduction (Kirkman and Sharitz 1994). A perennial forb, *Ranunculus reptans* L., prefers sexual reproduction to escape from the unfavorable habitats (van Kleunen et al. 2002). *D. rotundifolia* seemed to apply this strategy. *Mentha aquatic* L. and *Epilobium hirsutum* L. produce tough shoots in inundated habitats soon after snow-melt to anchor there (Lenssen et al. 2000). *D. anglica* seemed to utilize vegetative reproduction more than *D. rotundifolia* to maintain the populations in such inundated habitats.

Reproductive strategies are related to the distributions and sizes of plant populations (Espeland and Emam 2011). In conclusion, *D. rotundifolia* was broadly distributed by the dispersal of seeds in Sarobetsu mire (Nishimura et al. 2009) and others (Thum 1988), whereas *D. anglica* was not due to short-distance migration of daughters and small number of seeds.

Chapter 3. Differences in microhabitat selections between *Drosera anglica* and *D. rotundifolia* in a post-mined peatland

3-1. Introduction

Although phylogenetically-closed species resemble their ecological traits, they separate their habitat niches (Silvertown 2004). *Drosera anglica* Hudson and *D. rotundifolia* L. are carnivorous plants establishing on *Sphagnum* bogs in the circum-boreal regions of the Northern Hemisphere (Crowder et al. 1990), and overlap the global distributional ranges (Hulten 1968; Crowder et al. 1990). *D. anglica* populations are declining in various regions owing to habitat loss (Kapfer et al 2011), while *D rotundifolia* is widespread (Wolf et al. 2006). Because of the small populations, *D. anglica* has been assigned as a vulnerable species in Japan (Ministry of the Environment Government of Japan 2012). *D. anglica* originates from the hybridized amphiploidy between *D. rotundifolia* and *D. linearis* (Rivadavia et al. 2003). Therefore, the comparisons of ecological traits between these two species were expected to clarify the determinants on their population sizes.

At habitat scale, these two *Drosera* species separate their habitats by water level. *D. anglica* favors high water level, including open water, while *D. rotundifolia* inhabits at high and low water level (Nordbakken 1996; Nordbakken et al. 2004). Although seed germination and seedling survival are tightly related to the habitat preference and distribution patterns (Poorter 2007; ten Brink et al. 2013), the safe sites for seed germination often differ from these of seedling growth (Dalling et al. 2001; Eriksson 2002). Seed germination rates are often determined by water level in wetlands (Keddy 2010). Seedling growth is restricted by stresses, such as drought and inundation (Coops and van der Velde 1995; Anderson et al. 2009). Therefore, the stages of seeds and seedlings are focused in relation to water level.

The habitat preferences of plants in wetlands are determined not only by water level but also by interspecific interaction, including light and nutrient acquisition (McGraw and Chapin 1989; Nordbakken 1996; Keddy et al. 1998). *Sphagnum* mats support the establishment of vascular plants through increasing moisture and light in ombrotrophic bogs (Fenton and Bergeron 2006), but enhance nutrient competition between *Sphagnum* mosses and vascular

plants (Heijmans et al. 2002; Malmer et al. 2003). These indicate that the influence of *Sphagnum* mat on vascular plants differs between habitats and between wetlands. Therefore, *Sphagnum* mat should have an important role on the habitat differentiation.

Sphagnum mats develop heterogeneous habitats induced by microtopographical diversity represented by hollow-hummock or bulte-schlenke complex at wetland scale (Gore 1983). Hummock is covered with vascular plants while hollow develops less vegetation (Charman 2002). Therefore, I hypothesized that *Sphagnum* mat directly differentiated the establishment sites between *D. anglica* and *D. rotundifolia* and the resultant factors were related indirectly to the differentiations. To examine the hypothesis, the recruitment and survival of two *Drosera* species were monitored for three years, and experimentally examined the effects of water level, shade and habitat on the survival and growth of seedlings.

3-2. Materials and Methods

Study site

The field census was conducted in a post-mined peatland in Sarobetsu mire (45°06'N, 141°42'E, 7 m elevation), northern Hokkaido, Japan. Mean annual temperature was 6.4˚C at Toyotomi Town, six km far from the surveyed site, during 2008 and 2011, and annual precipitation was 962.4 mm (JMA 2012). The original peatland is predominated by *Sphagnum* mosses represented by *S. papillosum* Lindb. (Nishimura et al. 2009). Common vascular plants are *Scheuchzeria palustris* L., *Rhynchospora alba* (L.) Vahl, *Vaccinium oxycoccus* L. and *Carex middendorffii* Fr.Schm., although their cover was low. The *Sphagnum* peat was annually mined in the peatland from 1970 to 2003.

Field census

Two *Drosera* species, *D. rotundifolia* and *D. anglica*, established on the peatland mined 37-39 years before (Hoyo and Tsuyuzaki 2013). Five 300 cm × 40 cm plots, each of which was divided into 30 20 cm × 20 cm cells, were set up in an area where the two *Drosera* species established. Elevation was measured at the four corners of each cell on November 20 2010, a few days after rainfall. Level lines were set up by nylon strings stretched along the two

long sides on each plot. The distances between the lines and ground surface and between the lines and water surface were measured at each grid of cells. The elevation from the ground surfaces was calculated by the differences between the water surface and ground surface. The water surface was treated as 0 cm in height. The elevation in each cell was calculated by the mean of the four corners on each cell.

Three categories of growth stages were assigned on each shoot: seedling, daughter, and non-seedling. Seedling is defined as an annual shoot, daughter is as a ramet produced by vegetative reproduction, and non-seedling is as a seedling aged over two years. The location, length of the largest leaf and numbers of leaves and of flowers on each shoot were censused from late July to early August for three years during 2009 and 2011. Leaf length was measured with a ruler at 1 mm divisions. The vegetation in each cell was recorded based on Braun-Blanquet class (Braun-Blanquet 1964).

Seedling transplantation

The seeds used for seed transplantation experiments were randomly collected from more than 30 individuals on each species in the wetland during August and September in the previous years of experiments. The seeds were air-dried in paper bags for five days, and then were stored at 3˚C in an incubator until use. The seeds was cold-stratified in petri dishes placed in an incubator at 5/1˚C (12hr/12hr) for eight weeks to break down seed dormancy (Baskin et al. 2001), and then they were germinated in an incubator at 25/15˚C for five weeks.

Effects of water level on seedling survival and growth

The effects of water level on seedling survival and growth were experimentally examined in a greenhouse. Twenty-four plastic pots (12 cm in diameter and 10 cm in depth) were filled in peat moss and then placed into each of four plastic containers (42 cm × 31 cm in surface area, and 16 cm in depth). The water level was adjusted by holes drilled on the sides of containers at 1, 3, 5 or 9 cm deep. Water was supplied by sprinkling water 10 times every day. A hundred seedlings were grown in each pot in early August 2009. The survival of seedlings was monitored weekly under sunlight at 20-32˚C. Water content in peat moss was measured

by a time domain reflectometry (TDR) (Hydrosense, Campbell Scientific, Logan) with a 12-cm probe inserted vertically to the peat. All seedlings were grown for 63 days, were dried in an oven at 70^{o}C for 48 hours, and weighed. Three replications were used in each treatment.

Effects of shade on seedling recruitment and growth

The effects of shade on seedling recruitment and growth were investigated by a factorial-designed seed-sowing experiment in an area mined in 1972. Three intensities of shade were: 30% reduced by single a white sheer net, 50% by a single black net, and 70% by a doubled black net. A hundred seeds were sown into each degradable pot (100 cm^2 in surface area × 7.5 cm in depth) filled with *Sphagnum* peat on each species. The seeds were buried in the field on June 15 2011, and monitored monthly until September 10 2011. Six replicates were prepared on each shade treatment for each species. Two pots were not used for analysis due to trampling. Light intensity was measured on a pot selected in each treatment at 1 hour intervals from June 15 to September 10 in 2012 by an automatic data logger (HOBO, UA-002-XX, Onset Computer Corporation, Bourne, MA). The seedlings were excavated 87 days after the transplantation, dried in an oven at 70˚C for 48 hours, and weighed.

The effects of habitats on seedling establishment

Three habitats were selected for seed-sowing experiment, bareground, *Sphagnum* mat, and waterlogged surface, to confirm the habitat preferences. The distances were 30 m between bareground and *Sphagnum* mat, and 200 m between bareground and waterlogged surface. Three 100 cm × 30 cm plots were established in each habitat in late June 2010. All *Drosera* shoots were clipped out prior to the experiment. A total of 1000 seeds were sown to each plot. The seedlings of which maximum leaves were more than 3 mm were used for the analysis of seedling survival and growth, because of the accurate identification (Hoyo and Tsuyuzaki 2013).

To compare the growth and survival of seedlings on each species between habitats, a

hundred seeds were sown to each of 10 plastic cups (8 cm in diameter and 12 cm in height), and the cups were buried into 3 cm deep in each habitat on 3 July 2010. Ten blank cups were also set up for the control in each habitat. On July 30 2010 when most seeds completed the germination, I pulled up the cups carefully and set 8.5 × 8.5 cm wire frames to mark the locations. The seedlings were monitored monthly from July to September 2010 and on mid-summers in 2011 and 2012. The lengths of the longest leaf on each individual were measured in the late July of 2011 and 2012. The seedlings were excavated on July 25 2012, and the biomass was weighed after drying in an oven at 60˚C for 48 hours.

Volumetric water content was measured in July and October 2010 by using a TDR (Hydrosense, Campbell Scientific, Logan). Light intensity was measured at the three points of the ground surface in each plot at 1 hour intervals from June 2010 to July 2012 by automatic data loggers (HOBO, UA-002-XX, Onset Computer Corporation, Bourne, MA). When the sensors were inundated or covered with litter, the data was not used. The light intensity was converted to photosynthetic photon flux density (PPFD) by comparing between the light intensity and PPFD measured by a sensor established on a bareground (HOBO, S-LIA-M003).

Statistical analysis

Hierarchical Bayesian models (HBMs) estimated the determinants on density, recruitment and survival on the two species. The parameters of HBMs were estimated with a Markov chain Monte Carlo (MCMC) simulation by WinBUGS 1.4.3 through R2WinBUGS (Spiegelhalter et al. 2003). The density, recruitment and survival were estimated on each species in each year. The response variable, y_i, is the probability of number of individual or recruitment in cell i, following Poisson distribution, $y_i \sim P(\lambda)$, where λ is the observed number in cell i. Log-link function was applied to connect λ and the linear predictor ($\log\lambda_i$) as:

$$\log\lambda_i = \beta_1 + \beta_2 E_i + \beta_3 V_i + \beta_3 S_i + r_i + r_j \cdots \text{Eq. 1,}$$

where β_1 is the intercept, and β_2 and β_3 the coefficients, E_i elevation, V_i overstory vegetation cover, and S_i *Sphagnum* cover. r_i and r_j are the random effects on cell i and plot j, restrictively. The prior distribution of β is non-informative, and follows a normal

distribution, $N(0, 10^4)$. The inverse-variance parameters r_i and r_j are assumed to follow a prior distributions, $N(0, \sigma_c)$ and $N(0, \sigma_p)$. Here,σ_c and σ_p represents the standard deviation of individual characteristics on the linear predictor of λ_i. The σ_c and σ_p are assumed to follow a gamma distribution, $1/\sigma \sim \text{Gamma}(10^{-4}, 10^{-4})$. The spatial autocorrelation on the distribution of individuals was not considered, because a preliminary analysis detected that the autocorrelation was less.

The effects of elevation, *Sphagnum* cover and overstory vegetation on survival rate were examined on each species. The response variable, p_i, is the number of seedlings observed in cell i, following a binomial distribution, $p_i \sim B(q_i, N_i)$. q_i is the probability of survival from the last year in cell i, and N_i is the numbers of individuals in cell i in the last year. The logit-link function connects p_i and the linear predictors as:

$$\text{logit}p_i = \beta_1 + \beta_2 E_i + \beta_3 V_i + \beta_4 D_i + r_i + r_{j\,(i)} \cdots \text{Eq. 2,}$$

where D_i was density in cell i, and β, E, V and r are shown in Eq. 1. The prior distribution of β is non-informative following $N(0, 10^2)$. The posterior samples were obtained by combining three independent MCMC chains with 1000 samplings at 20-step intervals after 20000 burn-in MCMC steps. The convergence of the Markov chains was checked with $\hat{R}$ for each parameter (Gelman et al. 2003). The $\hat{R}$ values obtained were less than 1.1 for all the parameters.

The effects of water level, shade and habitat on seedling survival were analyzed by generalized linear mixed-effects model (GLMM) with a binomial distribution. The cell code was used as the random effect. The seedling biomass was compared between species, between treatments (water level, shade or habitat) and between their interactions, by generalized linear model (GLM) with a gamma distribution and log-link function. Akaike's information criteria (AIC) were used to select the best models. The statistical analysis was conducted by the statistical program R (ver. 2.15.1) (R Development Core Team 2012).

3-3. Results

Vegetation and microhabitat

The cover of *Drosera anglica* ranged from 1% to 15% in the five plots, and the cover of *D.*

rotundifolia 2% to 7%. In the five plots, the dominant species was *Rhynchospora alba*, of which cover was less than 25%, followed by *Carex middendorffii* and *Moliniopsis japonica* (Table 3.1). *Sphagnum* cover varied greatly between the plots ranging from 8% to 64%/ Open water area ranged from 10% to 67%.

The number of shoots in the plots fluctuated annually between 30 and 554 on *D. anglica* and between 82 and 546 on *D. rotundifolia* for the three years (Table 3.1). Of these, 43%, 1% and 56% of *D. rotundifolia* were in seedling, daughter and non-seedling, and 40%, 9% and 51% of *D. anglica* were in seedling, daughter and non-seedling. The daughters contributed to the density nine times higher on *D. anglica* than on *D. rotundifolia*, although the densities were low.

Temporal changes in density, recruitment and survival

The intercepts of densities on HBMs were significant for *D. rotundifolia* in 2010 and 2011 and the other intercepts were not significant (Fig. 3.1). *D. anglica* showed the high density at low elevations in 2010 and 2011 while *D. rotundifolia* showed the high density at high elevations. Under high overstory plant cover, *D. anglica* showed low density in 2009. *Sphagnum* mat decreased the density of *D. anglica*, and did not decrease that of *D. rotundifolia*. Therefore, the density of *D. anglica* was determined by overstory plant, *Sphagnum* mat and water level while that of *D. rotundifolia* was limited only by water level.

The intercept of *D. rotundifolia* seedling recruitment from 2010 to 2011 was significantly different from zero, and the other three intercepts were not (Fig. 3.2). *D. rotundifolia* recruited the seedlings more at higher elevation in 2010, but *D. anglica* recruited less under high overstory plant cover in 2010 and on *Sphagnum* mat in 2010 and 2011. *D. anglica* seedlings survived more on high elevation and less under higher overstory plant cover. *D. anglica* decreased the seedling survival with increasing density and *D. rotundifolia* did not. These results indicated that the sites for seedling establishment were limited more on *D. anglica* than on *D. rotundifolia*. The examined four factors, elevation, overstory plant cover, density and *Sphagnum* mat, did not affect the survival of non-seedlings on both the species,

Table 3.1

Microhabitat and vegetation in 30 cells in each of the five 300 cm × 40 cm plots. Mean is shown with standard deviation in parentheses on elevation. Open water is expressed by the percentage frequency of cells of which surface is covered with water.

Plot	1			2			3			4			5		
Year	09	10	11	09	10	11	09	10	11	09	10	11	09	10	11
Elevation (cm)		1.88 (±2.91)	0.63 (±3.29)		2.08 (±5.58)	0.65 (±4.27)		0.14 (±4.50)	-0.86 (±3.54)		1.85 (±2.81)	1.93 (±1.81)		-0.85 (±4.02)	0.03 (±2.62)
Vegetation cover (%)	60.7	77.0	77.6	70.5	79.5	75.4	42.8	64.5	70.0	81.9	67.7	73.6	59.8	71.4	73.9
Open water (%)		33.3	33.3		40.0	40.0		46.3	53.3		30.0	10.0		66.7	60.0
Drosera anglica Hudson	8.8	6.0	7.9	1.5	2.4	3.7	9.4	15.1	12.2	6.0	2.7	4.7	1.0	1.4	1.1
Drosera rotundifolia L.	2.7	4.2	7.0	2.2	5.8	6.4	1.5	1.7	3.5	7.3	3.2	7.3	3.0	4.0	5.5
Sphagnum papillosum Lindb.	43.7	57.7	61.0	54.0	68.8	63.5	15.7	17.9	33.2	12.7	20.0	16.8	16.7	5.0	8.2
Sphagnum fuscum (Schimp.) Klinggr	0	0	0	0	0	0	0	0	0	6.3	10.0	9.7	0	0	0
Rhynchospora alba (L.) Vahl	5.7	9.3	14.6	2.6	1.5	10.2	2.0	6.3	13.1	20.7	24.0	21.4	0.3	23.3	14.0
Carex middendorffii Fr. Schm.	11.8	4.1	5.6	30.7	17.0	10.0	7.3	14.2	9.0	25.3	3.0	4.5	0.8	0.1	2.9
Moliniopsis japonica (Hack.) Hayata	1.0	3.4	2.8	0.3	0.1	0.3	2.7	2.8	4.8	2.7	1.1	5.1	0.3	0.1	0.6
Scheuchzeria palustris L.	0.5	0	0	0	0	0	0	0	0	1.2	0.8	4.1	3.7	1.5	9.8
Pognia japonica Reichb. Fil	0.3	.0.3	2.1	0.3	0.3	0	0	0	0.1	0	0	0.1	0	0.6	8.0
Phragmites communis Trin.	0	0	0	0	0	0	2.2	5.7	4.0	1.1	1.5	1.4	.0.2	0.1	0.3
Eleocharis mamillata Lindb. f. var. *cyclocarpa* Kitag.	0	0	0	0	0	0	0	0	0	0	0	0	7.0	0.2	11.0
Number of individuals															
D. anglica	247	514	554	66	343	178	141	232	238	203	262	307	30	43	40
D. rotundifolia	92	417	273	82	546	506	84	163	135	144	432	409	109	276	252

Others: plots 1 and 6 = *Pogonia japonica* Reichb. fil, Plots 3 and 4 = *Lobelia sessilifolia* Nakai, Plot 6 = *Eleocharis mamillata* Lindb. f. var. *cyclocarpa* Kitag., *Scheuchzeria palustris* L.

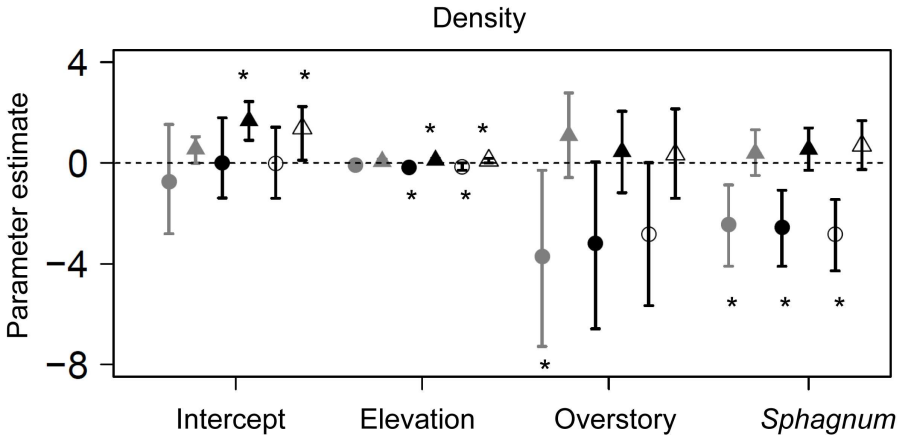

Fig. 3.1

Determinants on the densities of *D. anglica* and *D. rotundifolia*, estimated by a hierarchical Bayesian model. The coefficients are shown by medians (symbols) with 95% Bayesian credible intervals (vertical bars) on *Drosera anglica* (circles) and *D. rotundifolia* (triangles) in 2009 (grey), 2010 (closed) and 2011 (open). Asterisks show the variables do not overlay zero at 95% Bayesian credible intervals.

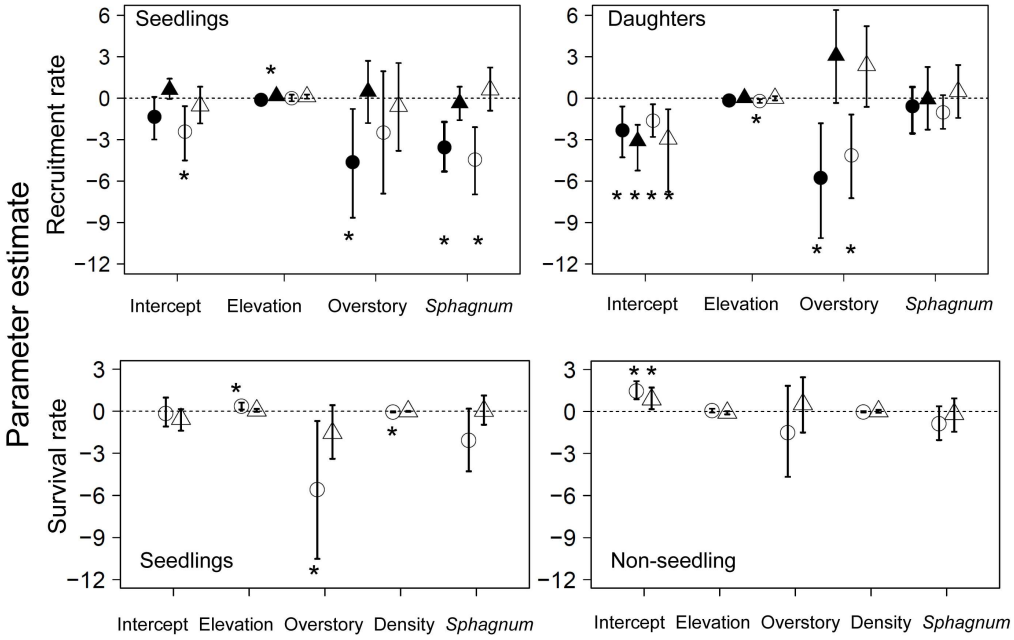

Fig. 3.2

Determinants of *D. anglica* (circles) and *D. rotundifolia* (triangles) on the recruitment rates of seedlings and daughters, and on survival rates of seedlings and non-seedlings in 2010 (closed) and 2011 (open), estimated by a hierarchical Bayesian model. Asterisks show the variables do not overlay zero at 95% Bayesian credible intervals.

showing that the fates of individuals were mostly determined before overwintering, i.e., in annual seedling stage.

All the intercepts of the production of daughters were significant (Fig. 3.2). *D. anglica* produced fewer daughters when the overstory was covered with more vascular plants, while *D. rotundifolia* did not alter the production with the vascular plant cover. *D. anglica* produced more daughters at low elevation in 2010. *Sphagnum* mat did not affect the daughter productions on the two species. Therefore, water level and overstory plant cover influenced only the daughter production of *D. anglica*.

Effects of water level on seedling survival and growth

The water content in peat decreased with decreasing water level, i.e., 68%, 63%, 61% and 56% at the water levels of 1, 3, 5 and 9 cm deep. The seedling survival two months after seed-sowing was lower at 5 and 9 cm deep than 1cm and was higher on *D. anglica* than on *D. rotundifolia* (Fig. 3.3). The interaction between treatments and species was not significant (Table 3.2). The seedling survival was lowest, 40%, at the lowest water level, 9 cm deep, on both species.

The seedling biomass was higher on *D. anglica* (0.472 mg ± 0.273, mean with standard deviation) than on *D. rotundifolia* (0.272 mg ± 0.136) (Fig.3.3). *D. anglica* developed larger seedlings at 5 and 9 cm of water level (Fig 3.3), while *D. rotundifolia* did not at 3 and 5 cm deep (Table 3.2). Therefore, the optimal water levels for seedling growth were present between 1 and 9 cm in depths under experimentally-stable water level.

Table 3.2

The effects of water level, shade and habitat on seedling survival and growth of *D. anglica* and *D. rotundifolia*. The survival was examined by GLMM, and the growth was done by GLM.

Variables	Survival		Biomass	
	Coefficient	*Z* value	Coefficient	*t* value
Water level				
Intercept	1.24	5.82***	-0.85	-22.88***
-3cm	-0.28	-1.03$^{N.S}$	-0.003	-0.05$^{N.S}$
-5cm	-1.03	-3.85***	0.29	5.29***
-9cm	-1.65	-6.17***	0.15	2.30*
D. rotundifolia	-0.33	-1.73*	-0.44	-8.44***
-3cm × *D. rotundifolia*	–	–	-0.16	-2.16*
-5cm × *D. rotundifolia*	–	–	-0.17	-2.15*
-9cm × *D. rotundifolia*	–	–	-0.09	-0.98$^{N.S}$
Shade				
Intercept	-0.42	-0.85$^{N.S}$	-0.67	-11.43***
50 %	0.40	0.61$^{N.S}$	-0.52	-5.81***
70 %	-0.01	-0.02$^{N.S}$	-0.74	-8.27***
D. rotundifolia	-0.87	-1.66$^{N.S}$	-0.78	-4.87***
50 % × *D. rotundifolia*	–	–	0.54	2.90**
70 % × *D. rotundifolia*	–	–	0.51	2.63**
Habitat type				
Intercept	-3.13	-19.84***	2.74	14.95***
Sphagnum mat	-1.69	-4.36***	-3.74	-15.55***
waterlogged surface	-2.17	-4.56***	-2.52	-6.01***
D. rotundifolia	-0.03	-0.11$^{N.S}$	-0.53	-2.09*
Sphagnum × *D. rotundifolia*	2.23	5.08***	–	–
waterlogged × *D. rotundifolia*	-0.49	-0.64$^{N.S}$	–	–

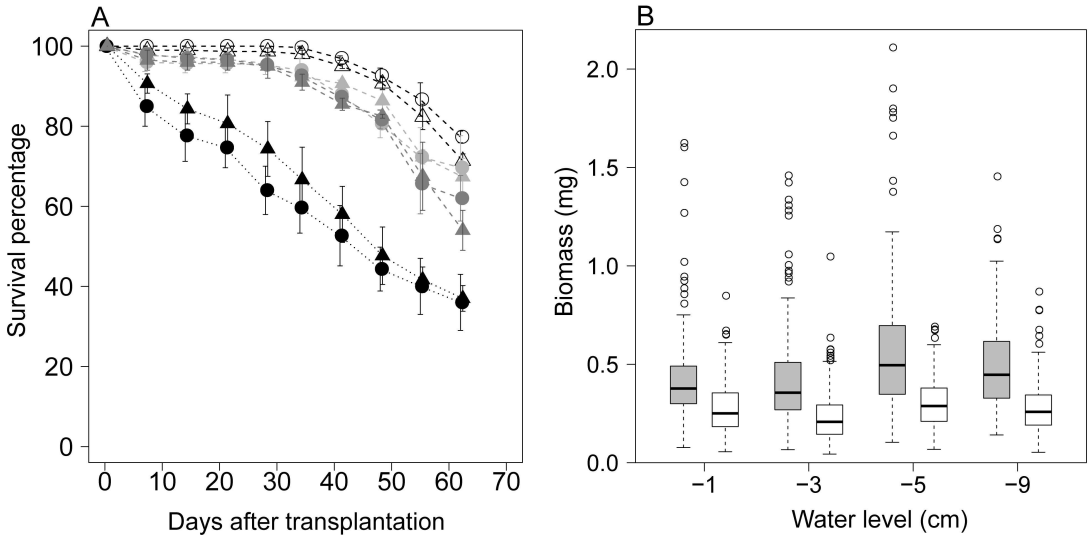

Fig. 3.3

The effects of water level on seedling survival and biomass in a greenhouse experiment. (A) Temporal changes in mean survivals of *D. anglica* (circles) and *D. rotundifolia* (triangles) shown with standard errors (vertical bars) at water levels of -1 cm, -3 cm, -5 cm and -9 cm. (B) Seedling biomass on *D. anglica* (greyed columns) and *D. rotundifolia* (open).

Effects of shade on seedling survival and growth

The numbers of seedlings increased three weeks after sowing seeds, and thereafter became stable or slightly decreased (Fig.3.4). Shade did not affect the number of seedlings (Table 3.2). *D. anglica* produced larger seedlings (0. 365 mg ± 0.527, mean with standard deviation) than on *D. rotundifolia* (0. 220 mg ± 0.174) (Fig. 3.4). Shade reduced the growth on *D. anglica* seedlings but did not on *D. rotundifolia* (Table 3.2). Shade kept the high numbers of seedling recruitments, survival and growth on *D. anglica* but decreased the biomass, and did not influence seedling recruitment, mortality and growth on *D. rotundifolia*.

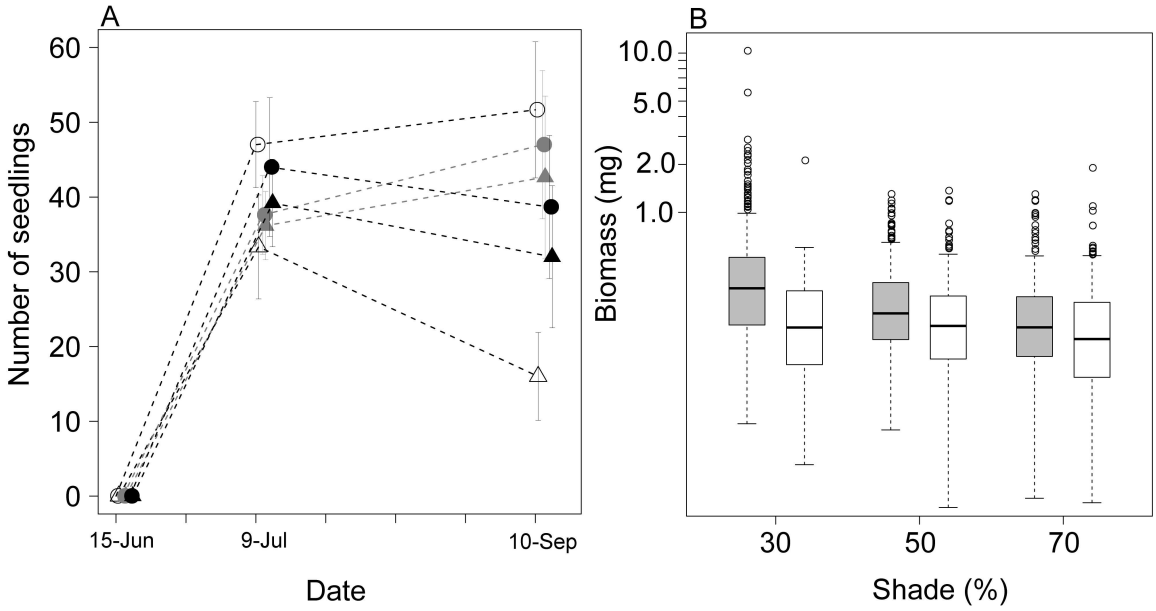

Fig. 3.4

Seedling survival and biomass on *Drosera anglica* and *D. rotundifolia* in a seed-sowing experiment to Sarobetsu mire. (A) Changes in numbers of seedlings of *D. anglica* (circles) and *D. rotundifolia* (triangles) with standard errors (vertical bars) at 30% (open symbols), 50% (grey) and 70% (solid) shade. (B) Seedling biomass of *D. anglica* (solid columns) and *D. rotundifolia* (open).

Survival and growth of seedlings

Water content averaged 93, 105 and 101% in the bareground, *Sphagnum* mat and waterlogged surface. Before the plant growth began in June, PPFD was high and was not different between the three habitats (Fig. 3.5). PPFD decreased thereafter in all the three habitats, in particular, on *Sphagnum* mat, showing that PPFD was reduced by the growth of overstory plant and *Sphagnum* mat. PPFD on *Sphagnum* mat was equivalent to 50% shade by shear nets. Precipitation was high in late July to early August 2010 (Fig. 3.5), therefore just germinated seedlings were heavily swept away.

On the seed-sowing experiment in the field, most seedlings originated from the sown seeds in the first year, because ten, three and eight seedlings emerged in the blank controls on bareground, *Sphagnum* mat and waterlogged surface. The seedlings of the two *Drosera* species recruited more on *Sphagnum* mat than on bareground and waterlogged surface in one

month (Fig. 3.6), showing that *Sphagnum* mat was the most suitable for seed germination. The seedlings of the two species died mostly until late September 2010, in particular, on *Sphagnum* mat. In the next year 2011, most seedlings of which species were unidentified occurred on *Sphagnum* mat. The numbers of survivors did not differ between the two species in the third year 2012, except that a large number of *D. rotundifolia* survived on *Sphagnum* mat (Table 3.2). *D. anglica* seedlings survived most on bareground, and the three of them flowered in 2012.

D. anglica grew larger than *D. rotundifolia* when seedlings were harvested in July 2012. The seedlings became largest on bareground for both species (15.35 mg ± 17.95 for *D. anglica* and 9.17 mg ±9.62 for *D. rotundifolia*) (Fig. 3.6). The biomass of *D. anglica* and *D. rotundifolia* was 1.81 mg ± 1.16 and 0.39 mg ± 0.10, respectively, on waterlogged surface and 0.18 mg ± 0.23 and 0.22 mg ±0.31 on *Sphagnum* mat. In summary, *Sphagnum* mat provided the seedling beds but inhibit the seedling growths and survivals, in particular, on *D. anglica*. Although waterlogged surface reduced the seed germination and growth of the two species, it allowed the establishment of *D. anglica* more than *D. rotundifolia*.

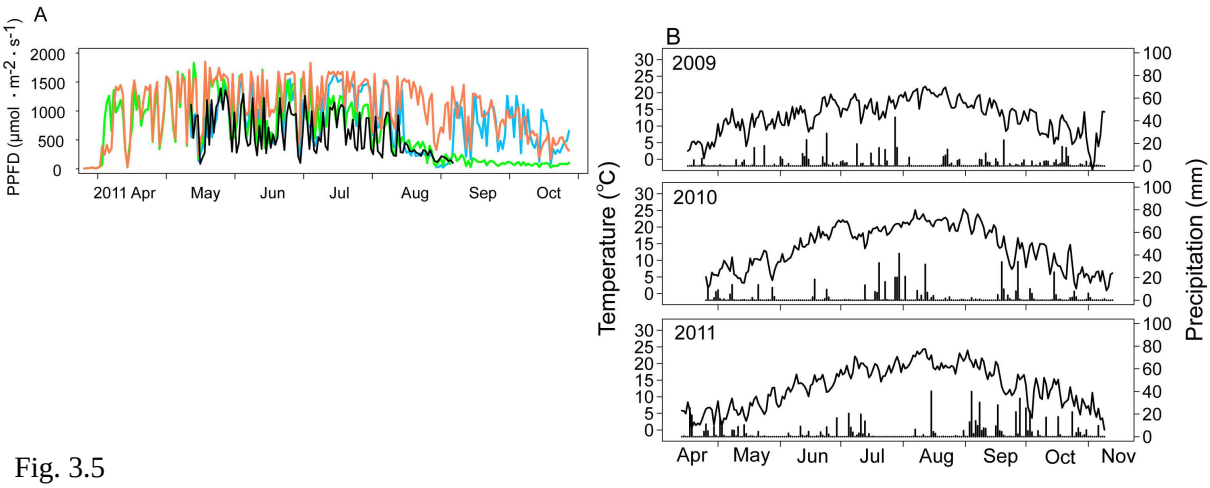

Fig. 3.5

(A): Temporal changes in daily maximum photosynthetic photon flux density (PPFD) in 2011 in the three habitats : bareground (red), *Sphagnum* mat (green) and waterlogged surface (aqua), and in the cell for three shading treatment adding out of the cell, i.e., 50% (black). (B) Daily mean temperature and cumulative precipitation in 2009, 2010 and 2011.

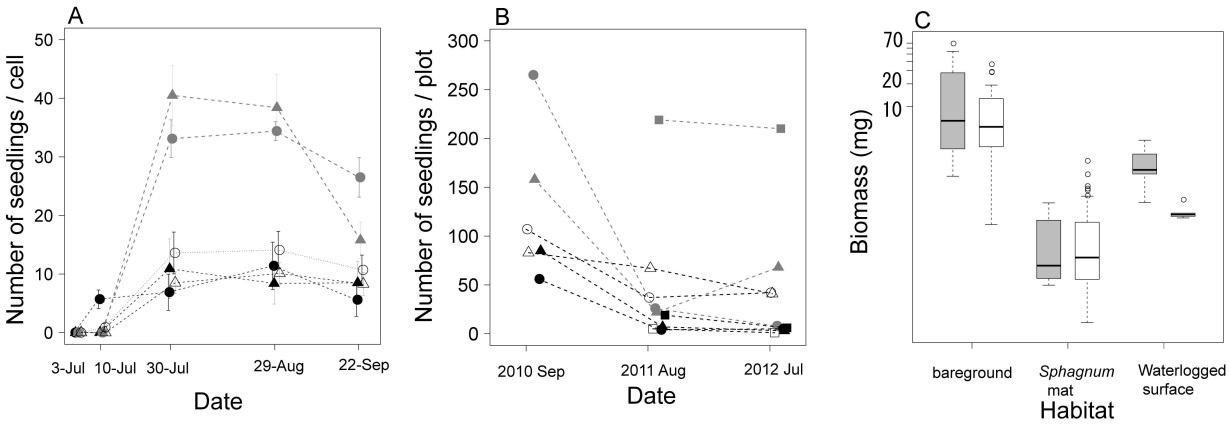

Fig. 3.6

Fluctuations of number of seedlings on *Drosera anglica* (circles) and *D. rotundifolia* (triangles) in the three habitats: bareground (open symbols), *Sphagnum* mat (grey), and waterlogged surface (closed). (A) Number of seedlings per cell from July 2010 to September. (B) Number of seedlings per plot from September 2010 to July 2012. Squares in (B) indicate number of unidentified seedlings of which leaves were less than 3 mm long. The numbers are shown by mean with standard errors. (C) Seedling biomass of *D. anglica* (grey columns) and *D. rotundifolia* (white).

3-4. Discussion

Growth stages on determining the distributions

Water level, overstory plants and *Sphagnum* mat restricted the density on *D. anglica* more than on *D. rotundifolia*. Because the survivals of overwintering shoots on the two species were not affected by the water level, overstory plant cover and *Sphagnum* mat, the distribution patterns were determined during the annual seedling stages before the next growing season.

D. rotundifolia preferred a wider range of water level than *D. anglica*. *D. rotundifolia* establish on the bases of raised mounds, such as tussocks, where peat moisture is low (Koyama and Tsuyuzaki 2013). However, most seedlings die on bareground in Sarobetsu mire when severe drought occurs (Egawa and Tsuyuzaki in press). Excess water including inundation also reduced the survival and growth of seedlings on the two *Drosera* species. The greenhouse experiments indicated that the optimal water level was present within a few

centimeters for the seedling growths on the two species when the water level was artificially stable. Such slight differences develop the elevational zonation of plants (Seabloom et al. 2001; Nordbakken et al. 2004). The optimal water levels for seedling growth and survival on the two species were lower than the ground surface, indicating that the preferable habitats were not waterlogged.

Habitat differentiation between D. anglica *and* D. rotundifolia

Shade by overstory vegetation weakly decreased *D. anglica* shoots and did not decrease *D. rotundifolia* shoots. The experimental shade decreased the seedling survival on both the species and decreased biomass on *D. anglica*. *D. anglica* was weaker to shade than *D. rotundifolia,* although both of the species are categorized into shade-intolerant (Crowder et al. 1990). The waterlogged surface developed less the cover of vascular plants and *Sphagnum* mosses. However, waterlogged surface was not suitable for the survival of *D. anglica*. The sites for seedling recruitment are not always the preferable site for seedling growth and survival due to the exclusion from the sites by biotic and/or abiotic factors (Poorter 2007).

The biomass of seedlings in the sowing experiment was higher on bareground and waterlogged surface than on *Sphagnum* mat for both the species, particularly *D. anglica*. Fast-growing biennial plants increase the reproduction after overwintering while most of them die after winter when stress induces the early defoliation (Rose et al. 2009). *D. anglica* showed slow growth and high mortality on *Sphagnum* mat and decreased biomass only by shade, suggesting that the slow growth and high mortality were derived from the competition to *Sphagnum*. Because *Sphagnum* mosses absorb nutrients in water from epidermis, they absorb the nutrients before vascular plants capture them (van Breemen 1995, Svensson 1995) except when *Sphagnum* mosses transport excess water to shallow-rooted vascular plants (Malmer et al. 1994). *Drosera* plants develop shallow roots (Adlassnig et al. 2005) that overlap with *Sphagnum* layer. Therefore, *Drosera* shoots should compete severely with *Sphagnum* mosses than deep-rooted vascular plants. On the seed-sowing experiment to detect habitat preferences, *D. anglica* seedlings grew faster than *D. rotundifolia* on bareground where the other plants did not establish. The restriction of growth was

conspicuous after the three years of treatments. These suggested that *D. anglica* is weaker to nutrient competition than *D. rotundifolia*. In contrast, *D. rotundifolia* established more on *Sphagnum* mat, because it was weak to inundation and was tolerate to shade and low nutrient.

Reproductive strategies

Another difference between the two species was reproductive strategy, i.e., *D. anglica* utilized vegetative reproduction by producing daughters than *D. rotundifolia*. Anchor by vegetative reproduction is one of the strategies for establishing in inundated habitats (Griffith and Forseth 2003; Sosnová et al. 2010). The populations of *D. anglica* persisted in inundated habitats by utilizing production of daughters as anchor, although the anchor abandoned long-distance migration. After *D. anglica* was pushed out by *Sphagnum* mosses to waterlogged surface, *D. anglica* was persistent by vegetative reproduction in the unsuitable habitat for growth. Waterlogged surfaced was derived from spatio-temporal heterogeneity developed by *Sphagnum* mat. *Sphagnum* mat was, therefore, prerequisite for developing waterlogged surface where *D. anglica* established.

Concluding remarks

This study clarified the morphology, life history and habitat preference of an endangered *D. anglica* on the basis of the comparisons with the common congener, *D. rotundifolia*. *D. obovata*, a hybrid between the two species, also established in Sarobetsu mire. Although the identification has been confusing without chromosome or DNA analysis, a convenient and non-destructive technique is required for long-term monitoring in field, based on nonlinear relationships between leaf length and width (Chapter 1).

D. anglica and *D. rotundifolia* reproduced by sexual and vegetative reproduction, both of which increased with increasing shoot sizes (Chapter 2). The seed production was lower on *D. anglica* than on *D. rotundifolia*, while the vegetative reproduction was higher on *D. anglica*. Daughter-produced shoots were larger than seed-produced shoos on both species. Therefore, *D. rotundifolia* populations were considered to expand its distribution mostly by seed dispersal. *D. anglica* populations were maintained more by vegetative reproduction, although the expansion of populations was slow or limited. Therefore, the small population sizes and/or distributional areas seem to be derived from the innate characteristic, i.e., reproductive strategy, of *D. anglica*. These results also suggest that the conservation of *D. anglica* populations should be focused more on providing preferable habitats than on increasing population sizes.

The distribution patterns of *D. anglica* and *D. rotundifolia* were determined at annual seedling stage (Chapter 3). Here I briefly summarize the establishment patterns of *D. anglica* and *D. rotundifolia* at two levels of scales, i.e., habitat and ecosystem levels (Figure 2), based on the findings shown in Chapters 2 and 3. *D. anglica* received shade stress more than *D. rotundifolia*. The ground surface at high water level was covered less with *Sphagnum* mat and vascular plant cover, and obtained high solar radiation. *D. anglica* was pushed out to areas at high water level by *Sphagnum* mat, and established with few competitors, and maintained the populations by vegetative reproduction. Because the safe sites for establishment of *D. anglica* are restricted by *Sphagnum* mat and its related factors, the population development should be sporadic. The niche separation originated mostly

from the relationships with *Sphagnum* mat. Therefore, the extinction of *D. anglica* populations should occur promptly when the habitats alter the characteristics. In particular, inter-specific interactions should be focused on seedling stage in a given habitat (Brewer 1998).

These findings suggested that the establishment patterns of *Sphagnum* mat is a key to conserve *D. anglica* populations (Figure 2.). Although *Sphagnum* restricted the establishment of *D. anglica* at habitat scale, *Sphagnum* reduced inter-specific competition between vascular plants and *D. anglica* by pushing out *D. anglica* to waterlogged surface. Furthermore, at ecosystem or wetland scale, the development of *Sphagnum* mat forms diverse topography, such as palsas and hummocks, in boreal regions (Charman 2002; Tsuyuzaki et al. 2008). The development of waterlogged surface was dependent on the development of *Sphagnum* mats, because the raised *Sphagnum* mat formed hollows that were waterlogged. If *Sphagnum* mat develops homogenously, then waterlogged surface does not develop. In this sense, *Sphagnum* is keystone taxa in bog-typed wetlands (Rochefort 2000).

Sphagnum mat has not completely recovered for 30 years after peat mining in the post-mined peatland (Nishimura et al. 2009), suggesting that the distribution and amount of *Sphagnum* are changeable. The artificial restoration of *Sphagnum* mat is also required, as needed, although the sophisticated restoration technique has not been proposed (Gorham and Rochefort 2003; Corson and Campbell 2013). Therefore, the careful investigations are required for the conservation and restoration of *Sphagnum* mats. In conclusion, *D. anglica* is endangered owing to its low abilities of competition and dispersal at seedling stage. For the conservation of *D. anglica* populations, the management of not only overstory vascular plants but also *Sphagnum* moss cover is required to reduce inter-specific competition to solar energy and nutrient at habitat scale.

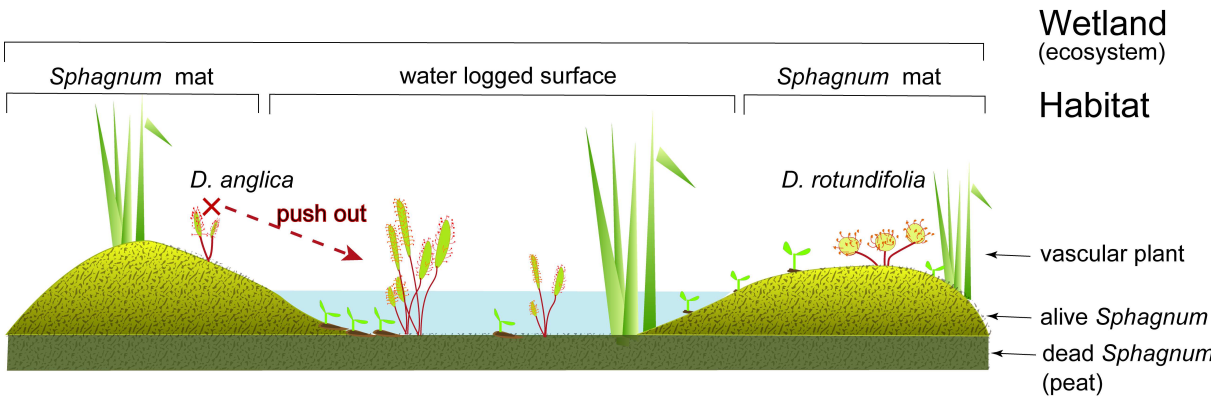

Figure 2. Schematic illustration summarizing the preferable habitats of *D. anglica* and *D. rotundifolia* in a post-mined peatland. *D. rotundifolia* establishes on *Sphagnum* mat because of tolerance to shade and/or competition more than *D. anglica*, while *D. anglica* is available on waterlogged surface where the competitors are less. Raised *Sphagnum* mat forms waterlogged hollows when the development of mats is patchy.

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