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Reproductive allocation of two dioecious *Rhamnus* species in temperate forests of northeast China

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When a plant increases resource allocation to reproduction from its limited reserves, the allocation to the other functions is reduced. Because of these trade-offs, differences in reproductive allocation are believed to result in relative differences in life history traits. Dioecious plants provide an excellent opportunity for detecting such possible trade-offs in resource allocation. This study aims to present a finding about the gender-based cost of reproductive allocation. The trade-off between reproduction and foliage biomass allocation was examined in *Rhamnus davurica* and *Rhamnus schneideri* at different modular levels (shoot/sub-branch, branch, and shrub/tree level). There were no intra-annual trade-offs between reproduction and foliage biomass in either sex of either species at shoot/sub-branch level, branch level and shrub level. Inter-annual trade-offs were detected in females for both species. Inter-annual trade-offs existed at all three different modular levels in *R. schneideri* females, while the evidence of inter-annual trade-offs was only detected at branch level in *R. davurica* females. At the population level, the sex ratio was female-biased in 2010, and it did not significantly deviate from 1:1 in 2011 in *R. davurica*. However, the sex ratios were significantly female-biased in both 2010 and 2011 in *R. schneideri*. This study show that the degree of autonomy of the different plant organs influences the trade-offs between reproduction and growth, which suggests a species- and sex-dependent modular autonomy.

Keywords: Inter-annual Trade-off, Intra-annual Trade-off, Dioecious Species, Modular Autonomy

Introduction

Individuals that have superior lifetime reproduction are often more successful in the face of natural selection. However, when reproductive events happen, these often lead to reduced foliage growth (Karlsson & Méndez 2005) and delayed future reproduction (Obeso 2002). The resources that plants obtain from their environment are allocated to growth, maintenance and reproduction (Willson 1983, Bazzaz et al. 1987). It may be assumed that, when a plant increases resource allocation to reproduction, the allocation to the other functions is reduced. Because of these trade-offs, differences in reproductive allocation are believed to result in relative differences in life history traits, like season-related flowering, fruiting and growth.

Dioecious plants provide an excellent opportunity for detecting possible trade-offs in resource allocation. In dioecious plants, the cost of reproduction in male individuals is different from that of females (Lloyd & Webb 1977). Females do allocate more resources to reproduction than males, and the trade-offs between growth and reproduction would be more obvious if there was a shortage of resources (Antos & Allen 1990, Barrett & Pannell 1998, Delph 1999). Several studies report that females, due to higher costs of reproduction, showed reduced vegetative growth (Bañuelos & Obeso 2004), put off flowering or reduced flowering frequency (Cipollini & Whigham 1994) and

had higher mortality rates if resources were insufficient (Delph 1999, Obeso 2002, Ueno et al. 2006). However, some authors found that females have growth rates equivalent to (Nicotra 1999) or even higher than males (Sakai & Burris 1985, Rovere et al. 2003). Females may have compensatory mechanisms for their higher reproductive costs (Dawson & Geber 1999).

There is no overwhelming evidence with regard to the trade-offs between reproduction and growth in plants (Delph & Meagher 1995). The degree of autonomy of the different plant organs might be an important reason for this (Obeso et al. 1998). In trees, if branches are autonomous units, the costs of reproduction may show up at lower modular levels, but may be compensated at higher combined modular levels, such as the crown, by non-reproductive branches (Hasegawa & Takeda 2001). Then we can detect the trade-offs at the branch level but not at shrub/tree level. The degree to which the individual (modular) plant organs act in response to reproductive costs is different among species (Bañuelos & Obeso 2004).

Trade-offs are also difficult to observe in plants because of the delayed cost of reproduction. Such delayed costs may appear in the form of lower frequency of flowering (Garcia & Antor 1995), higher mortality rates (Bierzychudek & Eckhart 1988, Lovett Doust & Lovett Doust 1988) or lower growth rates of females (Popp & Reinartz 1988). Fox & Stevens (1991) found a trade-off between current reproduction and subsequent reproduction in *Lindera benzoin*, Cipollini & Whigham (1994) in *Salix alaxensis*, while Nicotra (1999) detected a delayed cost of reproduction in both males and females of *Siparuna grandiflora*.

According to Delph (1999), only 29% of dioecious species have a 1:1 sex ratio, while 57% are male-biased and relatively few are female-biased. The reason for the male-biased sex ratios may be that males allocate fewer resources to reproduction than females and selection often tend to favor the sex with lower reproductive investment. However, some studies report that several populations in the genus *Salix* have female-biased sex ratios (Elmqvist et al. 1988, Dawson & Bliss 1989). Differences in the costs of reproduction between neighboring males and females competing for resources as well as genetic effects can also form biased sex ratios (Hamilton 1967, Taylor 1999).

This study examines the differences in reproductive costs between the sexes of two dioecious species, *Rhamnus davurica* and *Rhamnus schneideri var. manshurica*. We aim to show possible trade-offs between reproduction and vegetative growth. Accordingly, specific objectives are: (1) to evaluate the intra-annual trade-offs between repro-
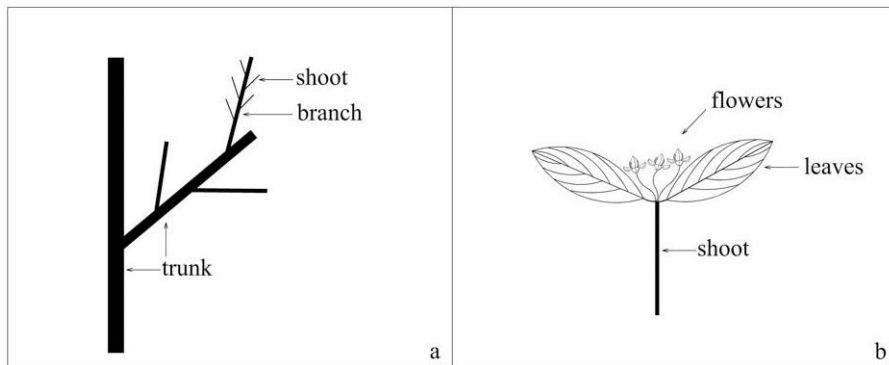
Fig. 1 - Sketch of phytomorph in dioecious *Rhamnus* species. (a): trunk and living branches; (b): a short current-year shoot.

duction and vegetative growth at different plant organ (modular) levels; and (2) to compare inter-annual trade-offs between females and males.

Materials and methods

Study sites

This research was conducted at the Jiaohe experimental forest in Jilin province, in northeastern China (43° 58' N, 127° 43' E; elevation 450 m a.s.l.). A permanent plot was established in 2009, covering a rectangular area of 23.76 ha (660 × 360 m). The forest type is a mixed broadleaf-conifer forest, in which the average annual temperature is 3.8 °C. The hottest month is July with a mean day temperature of 21.7 °C, while the coldest month is January with an average day temperature of -18.6 °C. The mean annual precipitation is 695.9 mm. The main coniferous species in the forest are *Abies holophylla* and *Pinus koraiensis*, while the dominant deciduous species are *Fraxinus mandshurica, Tilia amurensis* and *Acer mono* (Zhang et al. 2011).

The two species studied are *Rhamnus davurica* and *Rhamnus schneideri var. manshurica. R. davurica* occurs as a shrub or small tree, while *R. schneideri var. manshurica* only occurs as a shrub. Both species are common at the study site. *R. davurica* is usually taller than *R. schneideri var. manshurica*. Most individuals of both species

have a single trunk, a few have double or multiple trunks. Both sexes are similar in vegetative phytomorph, but not in flowers. The flower is yellowish green with four petals. There are four stamens in a male flower, but only one stigma in a female flower. The phytomorphs in the *Rhamnus* species are shown in Fig. 1. In order to understand the classification standard, we just show male flowers in Fig. 1. Female flowers grow on the shoot in the same way as male flowers.

Field measurements

The spatial distributions of the two studied species within the 360×660 m experimental plot are shown in Fig. S1 of the Supplementary Material. In order to examine the reproductive and vegetative allocation, female and male trees were observed in May 2010 and 2011. The numbers differ between years because not all plants were flowering and fruiting in both years. For this reason, a subset of trees was selected for analysis including only those individuals that were flowering and fruiting in both years. This involved 21 female and 18 male *R. davurica* and 57 female and 19 male *R. schneideri*. Five to ten reproductive branches were randomly selected in eight crown compartments (four horizontal sections and two vertical layers, following Henriksson 2001). The number of reproductive branches was different because some individuals were very small and had no more than 5 reproductive branches.

The trade-off between reproduction and growth was studied at four modular levels (shoot/sub-branch, branch, tree/shrub and population level). We counted the flowers, leaves and fruits on all selected branches in May (flowering season) and August (fruiting season). At shoot/sub-branch level, the number of flowers, leaves and fruits was assessed. At the shrub/tree level, the number of branches was counted in every sample tree. That number was multiplied by the mean number of flowers, leaves and fruits of randomly selected branches on each individual tree. This approach produced an estimate of the number of flowers, leaves and fruits on a single tree. At the time of flowering, one reproductive branch (including flowers and leaves) was harvested in each sample trees for both sexes. At the time of fruiting, 10-20 fruits were harvested on each female sample tree from the eight crown sections described above.

The harvested branches and fruits were weighed after oven-drying at 80 °C for 2 days to obtain an estimate of the average weight for each sample tree. The flower, leaf and fruit biomass estimates were calculated using eqn. 1-3 in Tab. 1. The relative reproductive ratio (*RR*) was calculated using eqn. 4-5 in Tab. 1. All calculations were done for different modular levels.

Growth diameter was estimated by year-ring analysis. Altogether, 64 trees (*R. davurica*: 20 females and 16 males; *R. schneideri*: 11 females and 17 males) were harvested in 2010. Across-sectional stem discs were cut 5 cm above ground level. Tree-ring widths were measured to the nearest 0.001 mm using the annual ring analyzer (Lintab 5). The tree ring samples were cross-dated using COFFCHA software.

Statistical analysis

Sometimes, it is difficult to decide if an effect is fixed or random (Crawley 2007). Therefore, differences in flower biomass, leaf mass and reproductive ratio at three levels were compared by factorial analyses of variance with two fixed-effects factors (sex and year). Pearson correlations were used to test the correlation between foliage biomass and reproductive biomass. Trees were grouped into diameter at breast height (DBH) classes. The sex ratio in each DBH class was determined during the study period. G-tests were used to analyze deviations from a 1:1 sex ratio for the two species in different years.
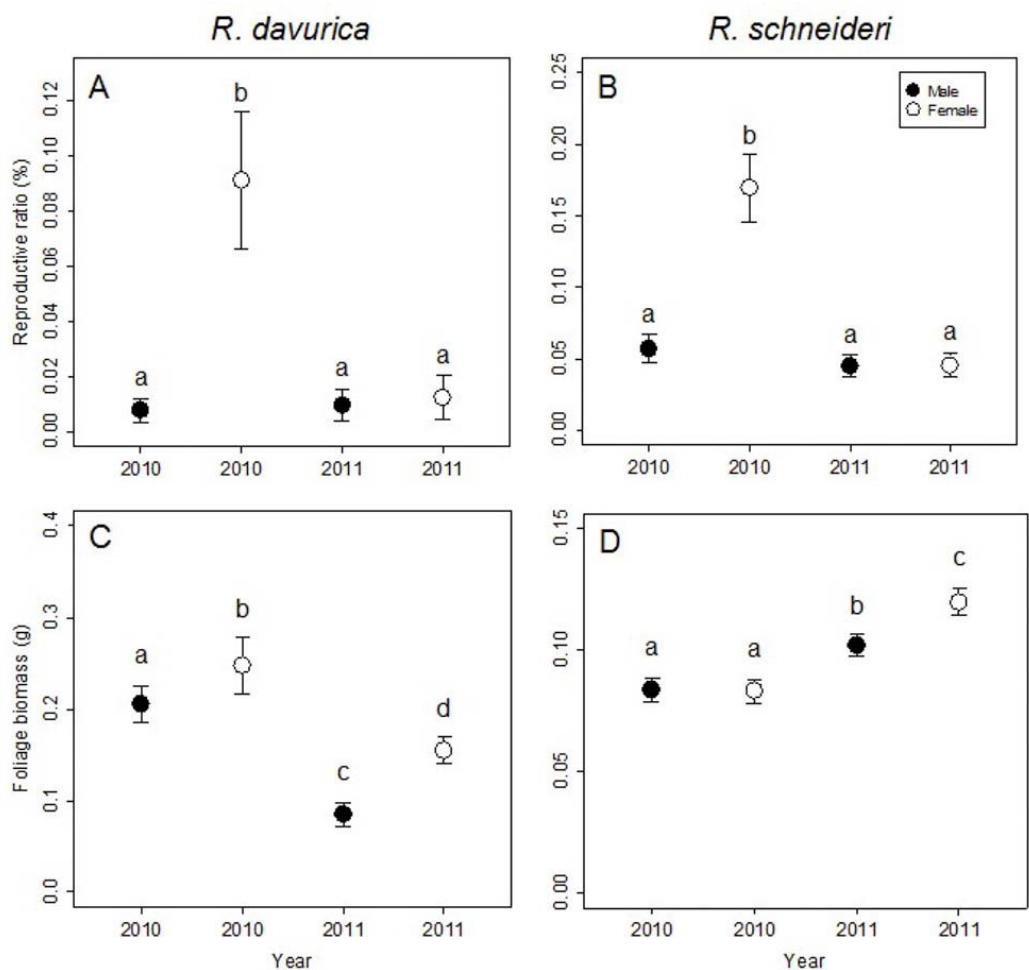
Results

Shoot/sub-branch level analysis

Both dioecious species, *R. davurica* and *R. schneideri*, show significant differences between females and males at the shoot level (Tab. 2). The differences between sexes in

Tab. 1 - Equations for calculating biomass and reproductive ratio (*RR*). DW_{fruits}, DW_{flower}, $N_{flowers}$ and N_{fruits} are the dry weight of one fruit, one flower, flower number and fruit number, respectively. B_{rep} is the sum of flower and fruit biomass for females or flower biomass for males; B_{veg} is the leaf biomass for both sexes.

Symbols	Equations
eqn. 1	Flower biomass = $N_{flowers} \times DW_{flower}$
eqn. 2	B_{veg} = number of leaves × average dry mass of one leaf
eqn. 3	Fruit biomass = $N_{fruits} \times DW_{fruits}$
eqn. 4	B_{rep} females = $DW_{fruits} \times N_{fruits} + (N_{flowers} - N_{fruits}) \times DW_{flower}$
eqn. 5	B_{rep} males = $DW_{flowers} \times N_{flowers}$
eqn. 6	$RR = B_{rep} / (B_{rep} + B_{veg}) \times 100$

Tab. 2 - Results of analyses of variance examining the effects of sex and year on the flower, foliage, and the percentage of reproductive ratio (*RR*) at different modular level in *Rhamnus* species . (***): $p<0.001$; (**): $p<0.01$; (*): $p<0.05$.

Modular level	Species	Source of variation	Flower		Foliage biomass		RR	
			Mean Sq	F value	Mean Sq	F value	Mean Sq	F Value
Shoot	*R. davurica*	sex	0	5.94*	3.6	18.63***	0.04	25.80***
		year	0	25.01***	12.86	66.61***	0.03	21.67***
		sex×year	0	0.04	0.23	1.2	0.03	23.22***
		Residuals	0	-	0.19	-	0	-
	R. schneideri	sex	0	46.60***	0.01	2.8	0.45	124.10***
		year	0	188.04***	0.12	27.38***	0.65	179.04***
		sex×year	0	142.49***	0.01	3.01	0.44	121.45***
		Residuals	0	-	0	-	0	-
Branch	*R. davurica*	sex	0	0.11	34.49	8.86**	0.12	56.80***
		year	0	0.26	210.06	53.96***	0.15	69.97***
		sex×year	0.01	8.80**	0.16	0.04	0.14	63.24***
		Residuals	0	-	3.89	-	0	-
	R. schneideri	sex	0	0	87.66	63.00***	0.46	58.10***
		year	0.32	65.95***	233.89	168.09***	0.83	103.73***
		sex×year	0.22	46.59***	78.51	56.43***	0.55	68.61***
		Residuals	0	-	1.39	-	0.01	-
Shrub	*R. davurica*	sex	30.6	4.22	122452.19	2.35	0.04	16.83**
		year	12.11	1.67	150.66	0	0.02	9.34**
		sex×year	9.54	1.31	33945.72	0.65	0.02	9.01**
		Residuals	7.26	-	52066.45	-	0	-
	R. schneideri	sex	9.18	0.32	53268.3	1.81	0.17	10.59**
		year	373.96	12.97***	137588.25	4.67*	0.24	15.60***
		sex×year	39.02	1.35	110844.39	3.76	0.13	8.29**
		Residuals	28.83	-	29456.93	-	0.02	-

Fig. 2 - Comparison of reproductive ratio and foliage biomass between males and females in 2010 and 2011 at shoot/sub-branch levels for the two *Rhamnus* species studied. Letters denote significant differences (p<0.05), calculated using multiple comparisons. Error bars represent standard errors.

Tab. 3 - Results of a correlation analysis between reproductive biomass and foliage biomass at the shoot/sub-branch and branch level based on the study data in 2011. Only the branches with eight or more shoots could be used in the analysis. Finally, 8 of 196 branches of female *R. davurica*, 26 of 178 branches of male *R. davurica*, 344 of 461 branches of female *R. schneideri* and 177 of 183 branches of male R. schneideri were used. Only the shrubs with 8 or more branches could be used in the correlation analysis. Finally, 12 of 21 *R. davurica* females, 18 of 18 *R. davurica* males, 57 of 57 *R. schneideri* females and 19 of 19 *R. schneideri* males were used.

Unit	Species	Sex	Significant negative	Significant positive	Non-significant	Total
Branch	*R. davurica*	Female	0	0	8	8
		Male	0	0	26	26
	R. schneideri	Female	3	28	313	344
		Male	5	51	121	177
Shrub	*R. davurica*	Female	0	4	8	12
		Male	0	8	10	18
	R. schneideri	Female	1	9	47	57
		Male	0	13	6	19

flower biomass and reproductive ratio were significant for both species, though differences between sexes in foliage biomass were significant only for *R. davurica* (Tab. 2). *R. schneideri* females invested more flower biomass than males in 2010, while *R. schneideri* males invested more flower biomass than females in 2011 (Fig. S2 in Supplementary Material). Females had higher reproductive ratios than males in 2010 in both species (Fig. 2a, b). *R. davurica* females invested more foliage biomass than males in both years, while in *R. schneideri* only females produced more foliage biomass than males in 2011 (Fig. 2c, d).

No significant correlations were found between reproductive biomass and foliage biomass at the shoot/sub-branch level in *R. davurica* (Tab. 3). In *R. schneideri*, only a few branches (female: 3 of 344 branches; male: 5 of 177 branches) showed significant negative correlations, while most branches exhibited significant positive correlations between reproductive biomass and foliage biomass (Tab. 3). This means that when a shoot shows higher growth rate, it also invests more in reproduction. The significant negative correlation can be considered as a trade-off between reproduction and vegetation. Therefore, there is no evidence of a trade-off at shoot level. The observation may be attributed to more resources being available at a higher level.

The differences between years in flower biomass, foliage biomass and reproductive ratio were significant for both species (Tab. 2). The reproductive ratio decreased for the females in both species. For the males in both species the differences in the reproductive ratio between years was not significant (Fig. 2a, b). A long-term cost of reproduction was detected at the shoot/sub-branch level just for females. The reproductive ratio decreased for females in both species (Fig. 2a, b). The foliage biomass decreased for both sexes in *R. davurica*, while foliage bio-
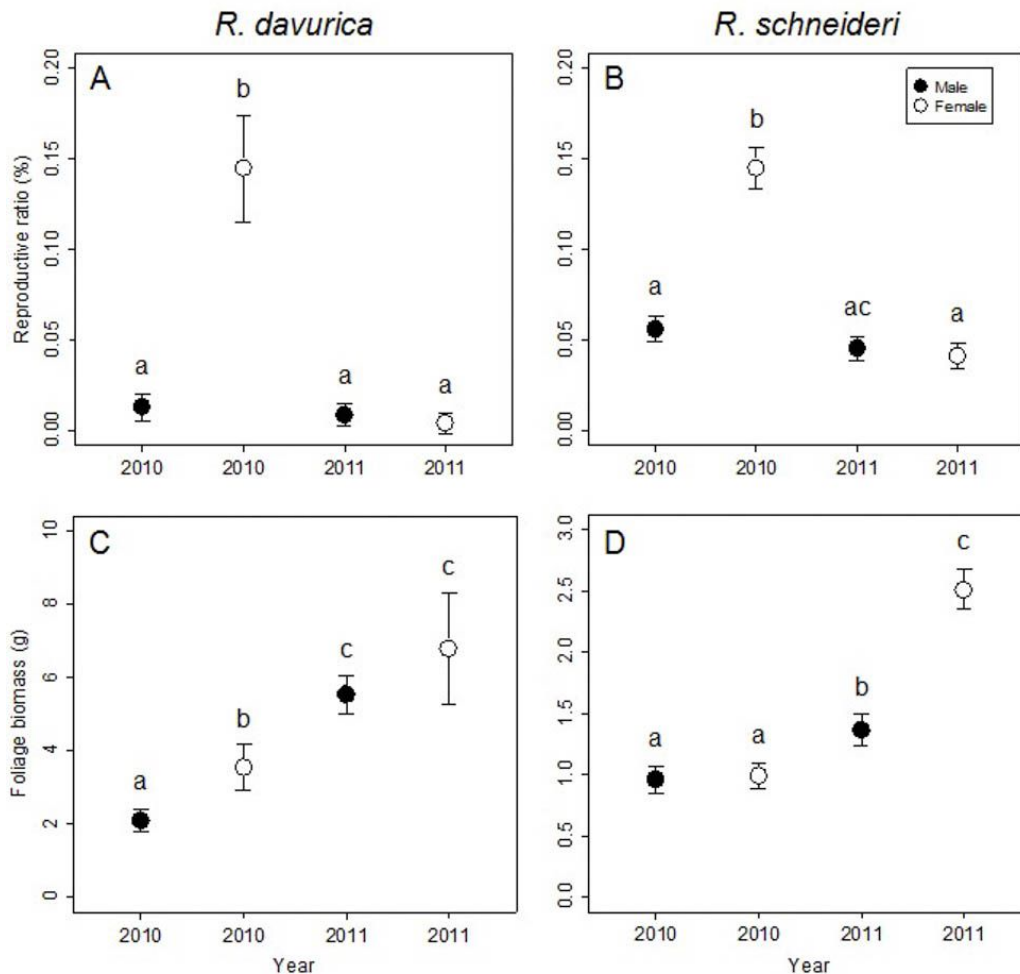

Fig. 3 - Comparison of reproductive ratio and foliage biomass between males and females in 2010 and 2011 at the branch level for the two *Rhamnus* species studied. Letters denote significant differences (p<0.05), and are calculated using multiple comparisons. Error bars represent standard errors.

Fig. 4 - Comparison of reproductive ratio and foliage biomass between males and females in 2010 and 2011 at the shrub/tree level for the two *Rhamnus* species studied. Letters denote significant differences (p<0.05), calculated using multiple comparisons. Error bars represent standard errors.
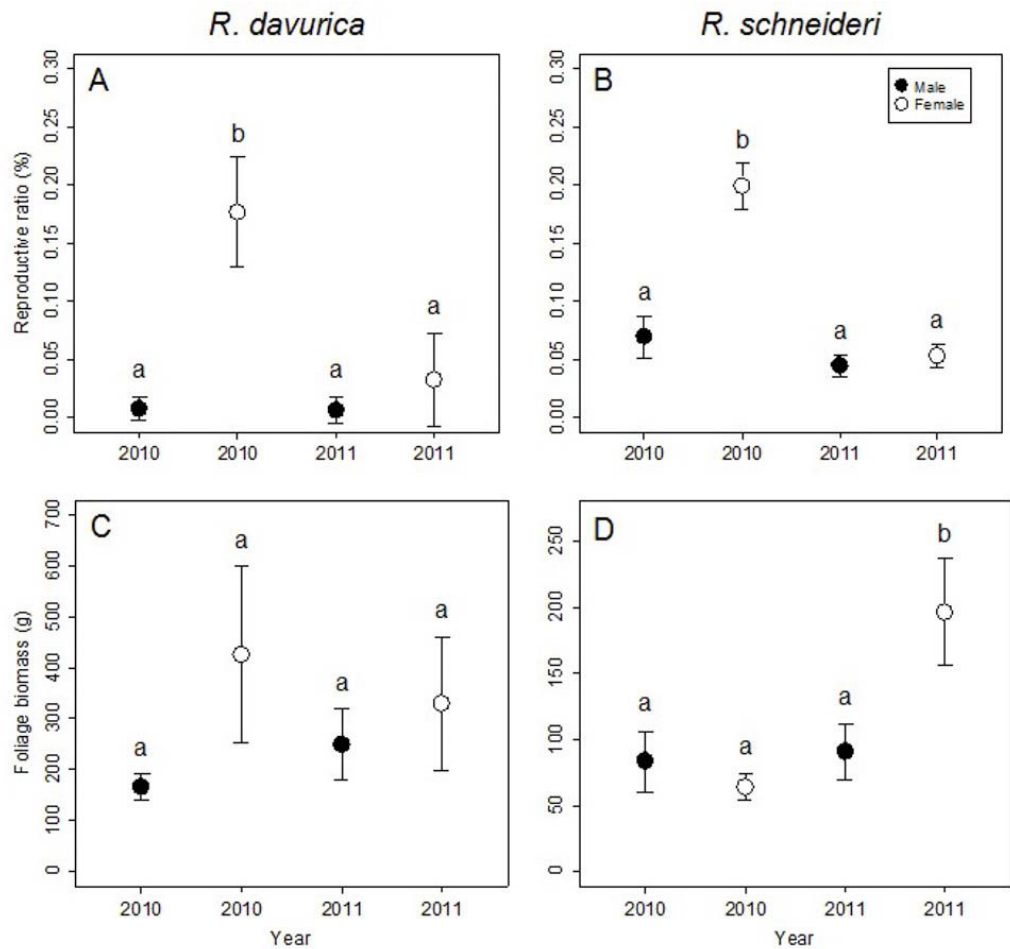
mass increased for both sexes in *R. schneideri* (Fig. 2c, d). Flower biomass and fruit biomass decreased for females of both species. Flower biomass decreased for *R. davurica* males, but there was not significant difference between years in the flower biomass for *R. schneideri* males (Fig. S3 in Supplementary Material).

Branch level analysis

At the branch level, no significant differences were detected between sexes in flower biomass for both *R. davurica* and *R. schneideri*. However, differences between sexes in both foliage biomass and reproductive ratio were significant for both species (Tab. 2). *R. davurica* (in 2010) and *R. schneideri* (in 2011) showed significant differences between sexes in foliage biomass. The difference in reproductive ratio between males and females was significant in both *R. davurica* and *R. schneideri* in 2010 (Fig. 3a, b).

At the branch level, almost all 106 shrubs/small trees (*R. davurica*: 12 females and 18 males; *R. schneideri*: 57 females and 19 males) showed significant positive or non-significant correlation between reproductive biomass and foliage biomass apart from one female *R. schneideri* (Tab. 3). This means no trade-off exists between reproduction and

Tab. 4 - Number of stems and sex ratios of two *Rhamnus* species in different DBH classes in the 360×660 m research plot. (DBH): diameter at breast height (cm); G(P): G-test results. (None): non-flowering trees; (***): p<0.001; (**): p<0.01; (*): p<0.05: (ns): p>0.05.

| Species	Parameters	DBH classes (cm)				
		0-2	2-4	4-6	6-8	>8
R. davurica in 2010	Male	3	22	18	11	0
	Female	9	78	136	62	19
	None	11	88	87	28	10
	Male/Female	0.33	0.28	0.13	0.18	0
	G(P)	3.01[ns]	33.08[***]	102.07[***]	39.04[***]	25.66[***]
R. davurica in 2011	Male	1	16	14	6	0
	Female	3	18	21	11	0
	Non-flowering	13	131	182	78	28
	Male/Female	0.33	0.89	0.67	0.55	-
	G(P)	0.93[ns]	0.12[ns]	1.39[ns]	1.45[ns]	-
R. schneideri in 2010	Male	1	21	1	-	-
	Female	11	73	4	-	-
	Non-flowering	1	10	1	-	-
	Male/Female	0.09	0.29	0.25	-	-
	G(P)	9.36[*]	30.29[***]	1.75[ns]	-	-
R. schneideri in 2011	Male	5	29	0	-	-
	Female	8	57	3	-	-
	Non-flowering	4	22	1	-	-
	Male/Female	0.63	0.51	0	-	-
	G(P)	0.67[ns]	9.23[*]	3.56[ns]	-	-
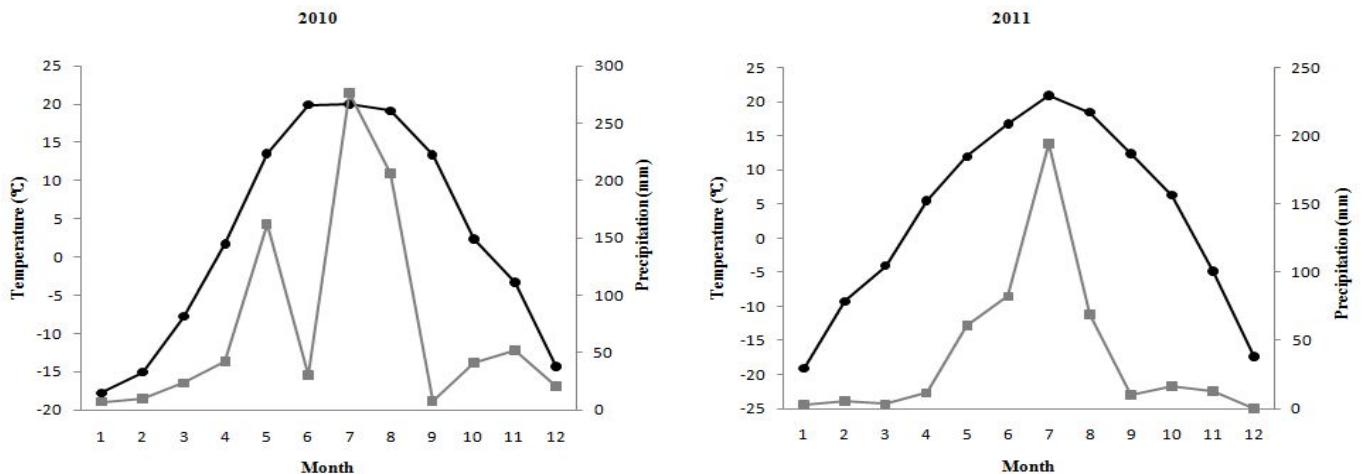
Fig. 5 - Monthly average temperature and monthly average precipitation in 2010 and 2011. The black dots indicate temperature, the grey squares indicate precipitation.

vegetation at branch level for two dioecious *Rhamnus* species.

The differences between years in foliage biomass and reproductive ratio were significant for both species, while the difference between years in flower biomass were significant in *R. schneideri* (Tab. 2). The reproductive ratio of females decreased in the subsequent year, while that of males was not significantly different for both species. This result is assumed to provide evidence of a delayed cost of reproduction for females at the branch level. The foliage biomass of males and females increased in the subsequent year for both dioecious species (Fig. 3c, d). The decrease of reproductive ratio and the increase of foliage biomass in females indicated inter-annual tradeoffs at branch level.

Shrub/tree level analysis

At the shrub/tree level, significant differences in reproductive ratio were detected between males and females for both *R. davurica* and *R. schneideri*, though no significant differences in flower biomass and foliage biomass were found (Tab. 2). Females invested more biomass into reproduction than males for both species in 2010, but there was no significant difference between sexes in reproductive allocation for both species in 2011 (Fig. 4a, b). The reproductive biomass correlated positively with the foliage biomass in the females of the two species at the shrub/tree level (*R. davurica*, Male: t = 1.12, correlation coefficient = 0.24, p>0.05; Female: t = 3.33, correlation coefficient = 0.74, p<0.01; *R. schneideri*, Male: t = 0.76, correlation coefficient = 0.15, p>0.05; Female: t = 2.73, correlation coefficient = 0.33; p<0.01). Thus, there was no trade-off between vegetative growth and reproduction in males and females of the two species.

Differences between years in flower bio-mass, foliage biomass and reproductive ratio were significant for *R. schneideri,* but the difference between years was significant only in the reproductive ratio for *R. davurica* (Tab. 2). Both dioecious species showed different reproductive ratios in 2010 and 2011. The reproductive ratios of females decreased in the subsequent year for both species, while no significant difference was detected between the two years in males. Interestingly, the leaf biomass in female *R. schneideri* also increased in the subsequent year (Fig. 4c, d). These differences in the reproductive ratios between the two years may be due to a delayed costs of reproduction.

Population level

The sex ratio of *R. davurica* in 2010 was significantly female-biased, but it was 1:1 in 2011. The sex ratios in each *DBH* class were consistent with the population sex ratio apart from the 1-2 cm *DBH* class (Tab. 4, Fig. S4 in Supplementary Material). The sex ratios of *R. schneideri* were significantly biased in both 2010 and 2011, apart from the 0-2 cm and 4-6 cm classes (Tab. 4, Fig. S4 in Supplementary Material).

The age of females and males was not significantly different (*R. davurica*: 36.92 ± 1.4 years for males, 38.5 ± 1.63 years for females, *t* = 0.74, *p* = 0.47; *R. schneideri*: 53.73 ± 1.46 years for males, 49.39 ± 2.02 years for females, *t* = -1.74, *p* = 0.09). The radial growth of *R. schneideri* showed inter-sexual differences with the growth rate of females being higher than that of males (*R. davurica*: 0.79 ± 0.03 mm · year^{-1} for males, 0.84 ± 0.03 mm·year^{-1} for females, *t* = 0.97, *p* = 0.34; *R. schneideri*: 0.31 ± 0.02 mm · year^{-1} for males, 0.37 ± 0.02 mm · year^{-1} for females, *t* = 2.67, *p* = 0.008643 - Fig. S4). The variation of monthly average temperature and precipitation was similar in 2010 and 2011 (Fig. 5).

Discussion

Intra-annual trade-off at different modular levels

Annual trade-off means that the correlation between foliage biomass and reproductive ratio is negative at 1 year. Namely, for each high reproductive biomass, there is a low foliage biomass. Unexpectedly, most males and females showed a non-significant correlation between foliage biomass and reproductive biomass for both species studied. Only a few individuals showed a trade-off at both the shoot/sub-branch level and branch level in *R. schneideri*. This findings indicate that, although some shoots and branches showed autonomy, the degree of modular autonomy was different between species, sexes and shrubs of the same sex.

The reproductive biomass was positively correlated with the foliage biomass at different levels for both sexes in *R. schneideri*. In *R. davurica*, the reproductive biomass was positively correlated with the foliage biomass among branches/shrubs, but neutral among shoots for both sexes. The positive correlation is probably due to the particular position of the shoots and/or branches. The better the position (*e.g.*, full light exposure), the more foliage growth and reproduction that can be expected (Bañuelos & Obeso 2004). Plant compensatory mechanisms may probably hide the trade-offs (Munetaka et al. 2009). During the investigation, we found that the flowers and immature fruits of both species were all green. These organs may also represent photosynthetic structures that can compensate the reproductive cost of a plant (Tozawa et al. 2009). Thus, females may have a greater compensatory advantage than males because of flower and fruit photosynthesis. Phenology patterns may provide another compensatory mechanism for reproduction. Both *R. davurica* and *R. schneideri*

grow leaves before flowers. If a leaf is growing before flowering, a plant will first allocate more resources to foliage growth than to reproduction. Trade-off can be detected only for a short time when flowers and leaves are both on a tree. Thus, the trade-off cannot be determined during the entire reproductive period, (Miyazaki et al. 2002). Interestingly, Verdú et al. (2007) found that the lower cost of reproduction for *Fraxinus ornus* males results in better vegetative growth than in the hermaphrodites of that species. Such differences in sex-specific reproductive costs do not seem to be anymore significant under stressful conditions.

Inter-annual trade-off at different modular levels

Delayed costs of reproduction were found at different modular levels for the two dioecious species. The delayed cost was detected in *R. schneideri* females and *R. davurica* females at shoot, branch and shrub levels, but not in males. The fruit biomass decreased at most modular levels in the subsequent year in *R. davurica* females. However, the fruit biomass decreased at shoot level but not at any other higher modular levels in the subsequent year in *R. schneideri* females. This indicates that different species may have different reproductive strategies. Every single shoot of *R. schneideri* female decreased in the fruit biomass, but the number of fruits at higher modular levels may increase in the subsequent year.

At three different modular levels, the reproductive ratio decreased, while the foliage biomass increased in *R. schneideri* females. This result suggests the existence of an inter-annual trade-off in *R. schneideri* females, being the performances consistent at three different modular levels. In *R. davurica*, the inter-annual trade-offs only existed at branch level for females. Some studies found that the reproduction pattern of the lower-level modules was the same as the higher-level modules (*e.g.*, Matsushita et al. 2011). Our study also confirmed the same performance in *R. schneideri*. Newell (1991) found that branches allocated more resources for reproduction and had less opportunity to flower or bear fruit in the subsequent year than on previously non-fruiting branches. Thus, reproductive activity in a given year may cause reduced fruit biomass in the subsequent year. Vaughton & Ramsey (2011) also found that *Leucopogon melaleucoides* females, which invest more energy in reproduction, will inhibit future growth in resource-limiting conditions and have no compensatory traits. No significant difference in monthly average temperature and precipitation was found between 2010 and 2011. In this study, climatic parameters may not be the key factors for the inter-annual trade-off.

Sex ratio is an important trait at the population level for dioecious species. The sex ratio is affected at germination by sex-linked genes (Shelton 2010), pollination intensity (Field et al. 2012) and soil nitrogen (Yu & Lu 2011). Sex ratios are affected by other factors as well, *e.g.*, sexually different mortality rates (Stehlik & Barrett 2005). The sex ratio changed at reproduction from a significant female-bias in 2010 to a non-significant bias in 2011, because more females did not flower in 2011 in *R. davurica*. The change in sex ratio within two reproductive years may be an indication of a delayed cost at the population level. The lower flowering frequency in females may be the evidence of a long-term cost of reproduction (Cipollini & Stiles 1991).

Conclusions

The intra-annual trade-off between foliage biomass and reproductive structure was not detected at different modular levels neither in sex nor in *Rhamnus* species. The performances of inter-annual trade-offs were different at two sexes and two *Rhamnus* species. The inter-annual trade-offs were just detected in females for both *Rhamnus* species, although the performances were different between two species at four modular levels. The intra-annual trade-off is hardly clear because of compensatory mechanism in both sexes, while the inter-annual trade-off is clear in females because of their higher reproductive allocation.

Acknowledgments

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Supplementary Material

Fig. S1 - Spatial distribution of *Rhamnus davurica* and *Rhamnus schneideri* var. *manshurica* in the 360 × 660m research plot.

Fig. S2 - Comparison of flower biomass and fruit biomass between males and females in *Rhamnus* shrub species at shoot level.

Fig. S3 - The annual DBH distribution in males, females and non-reproductive individuals in 2010 and 2011.

Fig. S4 - Accumulated annual ring width of males (hollow circles) and females (solid circles) for two species.

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