Do sexual dimorphisms in reproductive allocation and new shoot biomass increase with an increase of altitude? A case of the shrub willow Salix reinii (Salicaceae)

Do sexual dimorphisms in reproductive allocation and new shoot biomass increase with an increase of altitude? A case of the shrub willow *Salix reinii* (Salicaceae)[1]

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Based on the general tendency for females of dioecious plants to pay higher reproductive cost than males, it has been predicted that females should have much more reduced reproductive outputs and diminished vegetative production than males in energy-limited habitats. Nevertheless, this prediction has rarely been directly investigated. We investigated altitudinal changes in reproductive biomass and shoot production, normalized by plant size, for females and males of a shrub willow, *Salix reinii*, on Mt. Hakkoda, northeast Japan. Females maintained higher reproductive biomass than males at all altitudes; however, reproductive allocation for both sexes tended to decrease at a similar rate with an increase in altitude. Moreover, females vegetatively produced at the same rate as males at all altitudes. These findings suggest that females have a mechanism to compensate for the extra investment in reproduction irrespective of a changing environment. Shoot production did not change with altitude, suggesting that *S. reinii* gave priority to vegetative investment at the cost of reproductive output at higher altitudes. Inconsistent with general predictions, females did not respond more sensitively than males to severe environmental conditions in either reproductive allocation or shoot production, despite much higher resource investment in reproduction.

Key words: altitudinal gradient; annual shoot production; dioecious shrub; Mt. Hakkoda, Japan; reproductive cost; reproductive effort; Salicaceae; *Salix reinii*.

The manner of resource allocation between reproduction and somatic growth has long been a focus in ecological studies, because it is a fundamental component of life history strategy of an organism (Sterns, 1992). The manner in which plants change resource allocation to reproductive functions in response to environmental gradients is interesting because it is indicative of trends in life history evolution relating to environmental factors (Sterns, 1992; Thorén et al., 1996; Dawson and Geber, 1999; Delph, 1999; Obeso, 2002; Pickering and Hill, 2002; Sakai et al., 2003; Case and Barrett, 2004). In habitats with low availability of resources, reproductive allocation is generally small (Billings and Mooney, 1968; Bliss, 1971; Chester and Shaver, 1982; Thorén et al., 1996; Hemborg and Karlsson, 1998b), possibly because the cost of reproduction (i.e., its negative effects on vegetative production that influence future reproduction) would be most apparent in energy-limited conditions (Reznick, 1985; Primack et al., 1994; Obeso, 2002). In dioecious plants, this reduction in reproductive allocation is thought to be more pronounced in females than in males (Bierzychudek and Eckhart, 1988; Thomas and Lafrankie, 1993; Dawson and Geber, 1999; Pickering and Hill, 2002), because females generally pay higher costs for a given reproductive period (Delph, 1999; Obeso, 2002). Although this prediction is generally accepted,

there have been relatively few field studies (Dawson and Bliss 1989; Ortiz et al., 2002; Pickering and Hill, 2002; Case and Barrett, 2004; Pickering et al., 2004) that directly examined the manner in which sexual dimorphism in reproductive investment changes with a natural environmental gradient.

Because of the higher resource allocation to reproduction, it also could be predicted that females may be generally more sensitive to environmental conditions in terms of vegetative growth than males (Bierzychudek and Eckhart, 1988; Marion and Houle, 1996; Dawson and Geber, 1999; Delph, 1999). Accordingly, sexual differences in shoot growth rate increased in stressful habitats for some species (Dawson and Bliss, 1989; Dawson and Ehleringer, 1993; but see Marion and Houle, 1996). An alternative assumption, however, is also possible: females might suppress reproductive allocation to conserve vegetative allocation to ensure a certain level of survivorship (Jönsson and Tuomi, 1994), and thus sexual differences in vegetative investment might decrease in stressful habitats. To clarify which prediction has greater support, a simultaneous investigation of reproductive and vegetative investments on an environmental gradient is necessary, but the available literature on this is sparse (Popp and Reinartz, 1988; Ortiz et al. 2002).

In mountainous regions, environmental conditions dramatically change with altitude, providing an excellent situation to analyze variation in sexual dimorphisms in response to environmental differences. High altitude sites are representative of energy-limited habitats due to such factors as low temperature and a short growing season (Billings and Mooney, 1968; Bliss, 1971; Körner, 2003). Here we compare reproductive allocation and shoot production of male and female shrubs of *Salix reinii* Franch. et Savat. along an altitudinal gradient. We examined (1) whether females invest more resources in reproduction and fewer resources in shoot growth

Table 1.   Means (with SD in parentheses) for growth and reproduction measurements for females and males of *Salix reinii* at different altitudes: shrub size (basal diameter), dry mass of reproductive organs (fruiting inflorescences for females and flowering inflorescences for males), and dry mass of current vegetative organs (leaves and current twigs) for each sex and site.

		Female				
Site and altitude (m)	N	Basal diameter (cm)	Inflorescence (g)	Leaf (g)	Current twig (g)	Leaf + current twig (g)
Sarukura 850	25	4.4 (2.3)	25.7 (29.1)	150.2 (92.6)	42.1 (27.8)	191.3 (118.8)
Kasamatsu 1050	22	5.7 (1.9)	21.0 (14.9)	141.4 (59.1)	28.5 (18.4)	169.9 (76.5)
Jigokuzawa 1200	25	4.7 (2.6)	13.3 (13.4)	107.6 (60.9)	30.9 (24.0)	138.5 (83.4)
Tamoyachi 1300	12	4.3 (1.1)	7.4 (6.0)	203.7 (137.8)	62.8 (46.5)	266.5 (183.3)
Takada-Otake 1500	10	3.5 (1.6)	3.3 (4.2)	79.6 (46.9)	12.7 (8.0)	92.3 (54.5)
		Male				
	N	Basal diameter (cm)	Inflorescence (g)	Leaf (g)	Current twig (g)	Leaf + current twig (g)
Sarukura 850	30	3.6 (1.6)	4.5 (4.3)	165.8 (133.0)	52.6 (53.3)	218.4 (184.6)
Kasamatsu 1050	23	5.7 (2.0)	7.5 (5.8)	198.5 (123.2)	45.7 (28.5)	244.2 (150.5)
Jigokuzawa 1200	22	4.3 (1.7)	6.0 (6.3)	163.2 (98.1)	48.4 (34.0)	211.7 (128.8)
Tamoyachi 1300	16	4.2 (1.0)	1.2 (0.7)	182.5 (108.9)	61.1 (45.6)	243.6 (150.0)
Takada-Otake 1500	10	1.6 (0.6)	0.4 (0.3)	25.9 (19.4)	5.2 (4.9)	31.1 (24.2)

than males and (2) whether those sexual differences increase with an increase in altitude as per general predictions.

The best component of resources to use as a measure of resource allocation has been argued: biomass is the most basic, but nitrogen, phosphorous, or carbon may be more appropriate (Obeso, 2002). Nevertheless, proportional allocations of those components to reproduction are probably common (Abrahamson and Caswell, 1982; Reekie and Bazzaz, 1987; Hemborg and Karlsson 1998b). In a *Salix* species, Turcotte and Houle (2001) quantified those nutrients and concluded that higher reproductive effort observed in female shoots mainly resulted from their larger biomass than male shoots. We thus simply used biomass as a measure of resources in this study.

MATERIALS AND METHODS

Salix reinii is a dioecious willow common in sunny and nutrient-poor sites in subalpine and alpine areas of northern Japan. The maximum shrub height gradually decreases with an increase in altitude from ca. 3 m in lower subalpine areas to ca. 0.5 m in alpine areas (A. Sakai and A. Sasa, personal observations). From May to July, reproductive shoots emerge from some of the axillary buds on 1-yr-old shoots, and inflorescences, which terminate the apices of those reproductive shoots, then bloom and fruit. Current vegetative shoots develop from other axillary buds and from the terminal buds, leafing out successively until early summer.

The study was conducted during the growing season of 2002 on Mt. Hakkoda, Aomori Pref., northern Honshu, Japan (40°40′N, 140°25′E). We marked male and female willows of various sizes at five sites at different altitudes on this mountain (Table 1): Sarukura, in the upper montane area; Kasamatsu, Jigoku-zawa, and Tamoyachi, in the subalpine area; and Takada-otake, above the tree line. Basal stem diameter was recorded, and reproductive and vegetative production was estimated for each sample willow as detailed next.

At flowering, we counted the number of inflorescences for all marked males. During the period of 5–21 June, 130, 105, 30, 39, and 21 fresh inflorescences were collected from 13, 11, three, four, and three marked males at Sarukura, Kasamatsu, Jigoku-zawa, Tamoyachi, and Takada-otake, respectively. To elucidate the phenology, we observed the plants every few days and sampled just before pollen dispersal, which occurred later at higher sites. Each inflorescence was weighed after drying in an oven for 3 d at 70°C. We then calculated the mean dry mass of the inflorescences from each site. The total dry mass of inflorescences of each marked willow was estimated by calculating the product of the number of inflorescences and the mean dry mass of an inflorescence from the site.

At fruit maturation, we counted the number of inflorescences for all marked females. From 15 June to 6 July, all inflorescences were harvested from 10 randomly collected 1-yr-old shoots for each female, then weighed after drying in an oven for 3 d at 70°C. As for males, sampling was timed according to phenology, i.e., earlier at lower sites and later at higher sites. The mean dry mass were multiplied by the total number of inflorescences to yield the total dry mass of inflorescences for each female.

To compare the rate of vegetative production between sexes and among sites, we counted the number of all 1-yr-old shoots and randomly collected 20 of these from all marked female and male plants at completion of leaf development from 26 July to 8 September. Leaves and current twigs were separated and weighed after drying in an oven for 3 d at 70°C, and their total dry mass was estimated for each willow based on the mean mass and the number of shoots. For simplification, dry mass of leaves and current twigs was combined as vegetative investment, or shoot growth, in this study. There was little influence of herbivory.

Both reproductive and vegetative investments often depend on plant size, and size of mature plants often decreases with increasing altitude (Hemborg and Karlsson, 1998a, b; Sakai et al., 2003). In addition, the size effect may differ between sexes (Charnov, 1982) and may change with altitude (Sakai et al., 2003; Méndez and Karlsson, 2004). Therefore, in this study, to exclude the effects of size on reproductive and vegetative investments, the data of all sites and both sexes were pooled, and log-linear regressions weighted with sample size were conducted against the square of basal diameter. The residuals were subjected to ANOVA tests to compare reproductive and vegetative allocations among sites and between sexes. Fisher's protected least significant difference test (PLSD) was used for post hoc tests.

RESULTS

Total dry mass of reproductive organs per plant was larger in female willows than in males at all study sites (Table 1). Because this total dry mass was significantly correlated with shrub size (Fig. 1a and b, Table 2), the residual dry mass of reproductive organs was compared. It was significantly different between the sexes (Table 2) and larger in females (Fig. 2a), indicating that females invested greater resources in reproduction than the same-sized males. Residual dry mass also differed significantly among the study sites (Table 2) and tended to be smaller at higher-altitude sites (Fig. 2a). That is, shrubs at higher altitudes tended to invest less in reproduction than did same-sized shrubs at lower sites. The interactive effect of sex and site was not significant (Table 2), although the
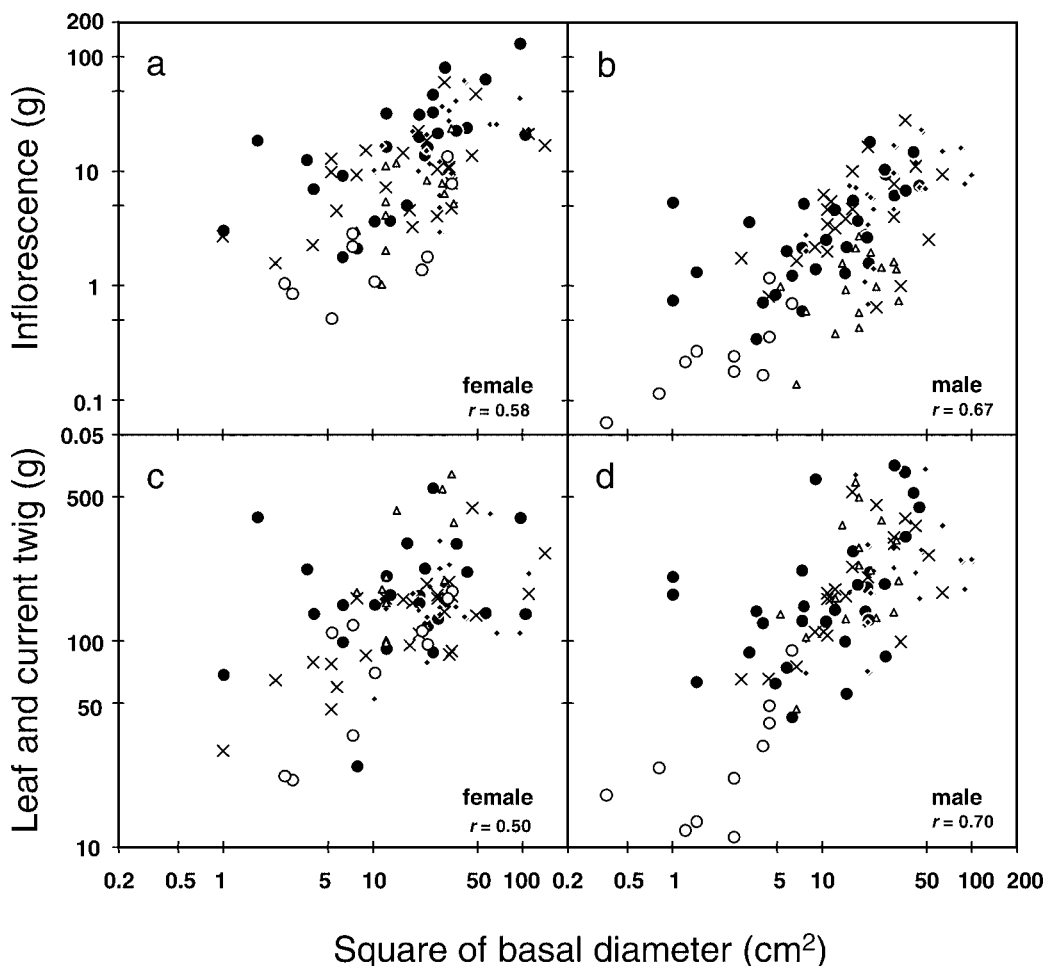
Fig. 1.   Whole-shrub reproductive biomass of females (a) and males (b) and shoot biomass of females (c) and males (d) plotted against shrub size of *Salix reinii*. Correlation coefficients are all significant ($P < 0.0001$, $N = 94$ and 101 for females and males, respectively). Symbols denote study sites: solid circles, Sarukura (850 m a.s.l.); solid diamonds, Kasamatsu (1050 m); multiplication symbols, Jigokuzawa (1200 m); open triangles, Tamoyachi (1300 m); open circles, Takada-Otake (1500 m).

TABLE 2.   Results of statistical analyses for *Salix reinii*. (A) The result of a linear regression of reproductive biomass (RB, i.e., inflorescences) and vegetative biomass (VB; i.e., sum of leaves and current twigs) against shrub size (*S*, square of basal diameter), where all values are log-transformed. Both sexes and five sites are pooled, and the regressions are weighted by each sample size denoted in Table 1. (B) ANOVA for the residuals of the regressions.

A) Biomass	Regression against *S*	*N*	R^2	*F*	*P*
Inflorescence (RB; g)	$\log(RB) = 0.937 \log(S) - 0.505$	195	0.48	181.6	<0.0001
Leaf and current twig (VB; g)	$\log(VB) = 0.557 \log(S) + 1.49$	195	0.56	247.4	<0.0001

		Inflorescence		Leaf and current twig	
B) ANOVA for residuals	*df*	*F*	*P*	*F*	*P*
Sex	1	75.5	<0.0001	2.8	0.099
Site	4	17.0	<0.0001	9.7	<0.0001
Sex × Site	4	1.9	0.105	1.5	0.207

sexual difference was not significant by the post hoc test at the highest site, Takada-Otake (Fig. 2a).

Total dry mass of leaves and current twigs showed similar trends between females and males against shrub size (Fig. 1c and d), and the residuals against shrub size were not significantly different between the sexes (Table 2 and Fig. 2b), indicating that same-sized female and male shrubs produce a similar dry mass of vegetative shoots. Total dry mass of leaves and current twigs was significantly different among sites (Table 2), but nevertheless was not an altitudinal trend (Fig. 2). For example, shrubs at the second highest site, Tamoyachi, produced as much in vegetative shoots as did the same-sized shrubs at the lowest site. Sex and site interaction was not significant (Table 2).

DISCUSSION

Whole-plant reproductive investment normalized by plant size tended to be smaller at higher sites for both sexes, indicating that reproductive allocation of observed females and males of *S. reinii* are both sensitive to the gradual change of
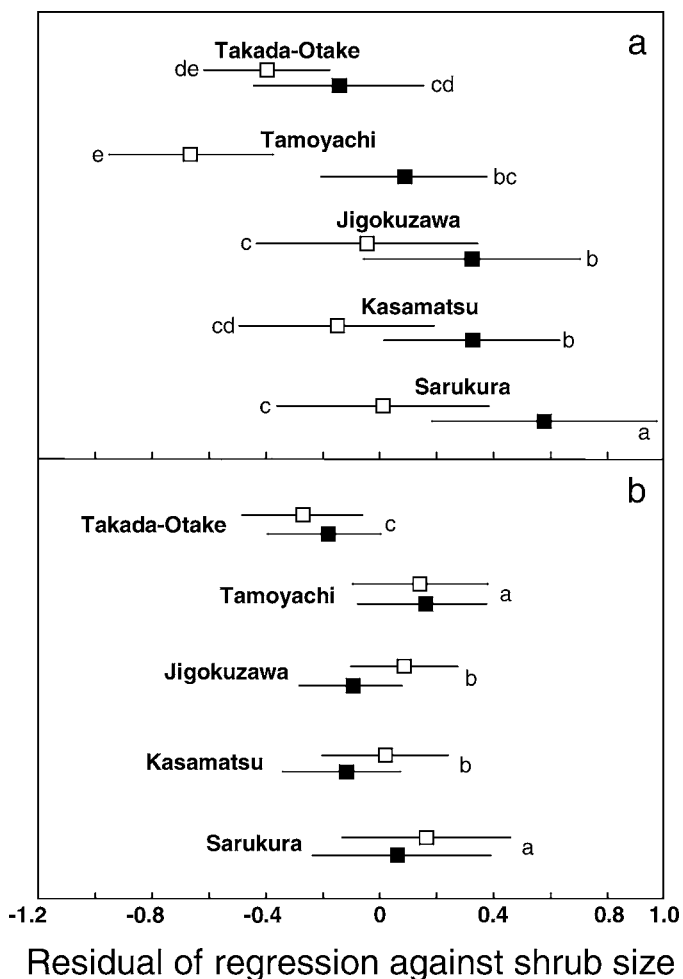
Fig. 2.   Residuals of regression against shrub size (see Table 2) of *Salix reinii* for each site and sex (mean and SD); solid and open squares denote females and males, respectively. (a) Whole-plant reproductive (inflorescences) biomass; (b) whole-plant shoot production (total biomass of leaves and current twigs). The same letters indicate no significant difference by post hoc test.

environment from lower subalpine to sites above the tree line. This is consistent with the fact that sexual reproduction is often small in alpine and arctic regions in comparison with the same or closely related species growing in warmer areas (Billings and Mooney, 1968; Bliss, 1971; Chester and Shaver, 1982). On the other hand, we did not find that annual shoot production normalized by plant size decreased with altitude, suggesting that shoot production is not affected by changes in altitude. This is inconsistent with the general prediction that reproductive cost of vegetative production is much more marked under stressful conditions (Reznick, 1985). Moreover, because producing the same amount of shoots at higher, more stressful sites may cost more, *S. reinii* seems to give priority to vegetative investment at the cost of reproductive output at higher sites. This result indicates that plants may evolve to reduce reproductive costs at energy-limited sites where resource investment in vegetative organs might be more important for survival (Jönsson and Tuomi, 1994; Hemborg and Karlsson 1998b).

Contrary to the common prediction for long-lived perennial plants (Harper, 1977; Lloyd and Webb, 1977), females did not

reduce shoot production compared with males growing at the same sites, in spite of the fact that overall reproductive allocation was much greater in females than in males, as per the general prediction (Lloyd and Webb, 1977) and many reported observations (Delph, 1999; Obeso, 2002), including *Salix* (Turcotte and Houle, 2001). This suggests that females of *S. reinii* have a mechanism to compensate for the extra reproductive cost. In *Salix*, females with larger reproductive allocation often have a growth rate similar to or even faster than males (Åhman, 1997), and physiological (Dawson and Bliss, 1989; Ueno and Seiwa, 2003) and phenological (Ueno and Seiwa, 2003) causative factors have been investigated. Ueno and Seiwa (2003) indicated that females of *Salix sachalinensis* produced a larger amount of N-rich leaves in the early growing season, when males temporarily pay a greater cost for reproduction. In this study, females tended to have greater relative dry mass of leaves compared to current twigs than did males at all sites (Table 1), though the differences were small and not significant. Although this might contribute to female compensation, further studies are necessary to elucidate this mechanism. Marion and Houle (1996) also reported that vegetative production of a dioecious shrub, *Junierus communis* var. *depressa*, did not differ between the sexes at all study sites on a latitudinal gradient.

There was no significant overall sexual difference in the response of reproductive allocation to altitudinal change, inconsistent with the general prediction that females reduce reproduction much more in stressful environments. This suggests that the ability of females to compensate is independent of altitudinal changes in environmental factors in the observed *S. reinii* populations. On the other hand, in *S. arctica*, Dawson and Bliss (1989) revealed that females performed better in terms of both reproductive output and shoot growth at wet, nutrient-rich sites (e.g., river banks), while males did so at dry, oligotrophic sites, according to an adaptive difference for optimum water availability. Such physiological differentiation between sexes is known as a female compensation mechanism (Delph, 1999; Obeso, 2002), which is ordinarily associated with habitat segregation between sexes (Case and Barrett, 2004). Sexual habitat segregation has been found or suggested for many species (Bierzychudek and Eckhart, 1988), including other willows (Ueno and Seiwa, 2003), suggesting that female compensation ability often depends on environmental conditions. It is seemingly inconsistent with the pattern of *S. reinii*. We assume that this is because almost all environmental gradients considered in the general prediction are ones relating to soil dryness, with which altitudinal gradients may not always be parallel (Körner, 2003). Ortiz et al. (2002) reported a similar tendency in *Juniperus communis* subsp. *alpina* on an altitudinal gradient in the Sierra Nevadas.

From a simultaneous investigation of reproductive and vegetative investments at a whole-plant level, we can conclude that, in *Salix reinii*, females did not respond more sensitively than males to severe environmental conditions in terms of reproductive allocation of biomass nor shoot production in spite of their much higher resource investment in reproduction. Although selection experiments manipulating reproductive traits are recommended (Jönsson and Tuomi, 1994; Delph, 1999; Obeso, 2002) to detect a reproductive cost, a key parameter to understand life history evolution, such a classical examination as this study is rare and thus informative.

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