

## Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (Lentibulariaceae) and its environmental correlates

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Several important fitness components in herbaceous perennial plants are commonly related to plant size: flowering probability, reproductive allocation and fecundity. However, evidence for such size-dependence of fitness components is mostly anecdotal and unconnected to other life history traits. Here we report size-dependence for flowering probability and reproductive allocation in 11 populations of *Pinguicula vulgaris* and relate it to environmental factors. Flowering probability was size-dependent in all populations of *P. vulgaris*, and indicated the existence of a threshold size for reproduction. Populations at low altitudes and in wet soils showed a significantly higher threshold size for reproduction. Reproductive mass was also size-dependent in all populations. We found considerable between-population differences in the slope and the intercept of the regression between plant size and reproductive mass. This variation was weakly related to the environmental factors measured. In general, relationships between different size-dependent fitness components were low. Instead of showing a covariation of traits, in line with interpretations in terms of life history “tactics”, *P. vulgaris* seemed to independently vary each size-dependent fitness component in each locality. In particular, no significant relationship was found between threshold size for reproduction and the slope of size-dependent reproductive allocation, as predicted by previous authors. Neither we found a significant influence of somatic cost of reproduction on size-dependent fitness components.

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In herbaceous perennial plants, several important fitness components are related to plant size. Flowering probability usually increases with size (Kawano 1985, Thompson 1987, Hanzawa and Kalisz 1993, Méndez and Obeso 1993, Schmid and Weiner 1993, García and Antor 1995, Piqueras 1999). Below a so called threshold size for reproduction flowering does not occur at all (Kawano 1985, Lacey 1986, Thompson 1987, Hanzawa and Kalisz 1993, Méndez and Obeso 1993, García and Antor 1995, Schmid et al. 1995, Bazzaz 1997). Typically, not all individuals above the threshold size reproduce; the percentage of reproductives is the percentage

of actual flowering plants with respect to the potentially reproductive plants in the population (Thompson 1987, Hanzawa and Kalisz 1993, García and Antor 1995). Mass of reproductive structures and fecundity (seed number) above the threshold size are also size-dependent (Kawano and Mikaye 1983, Samson and Werk 1986, Ohlson 1988, Weiner 1988, Hartnett 1990, Méndez and Obeso 1993, Schmid et al. 1995).

Size-dependence of fitness components in plants has both theoretical and practical interest. First, it plays a role in life history theory (Roff 1992, Stearns 1992). For example, several models for optimal age at maturity

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(Roff 1992, Stearns 1992), as well as models of the optimal allocation to growth, survival and reproduction from birth to death (Kozłowski and Uchmanski 1987), assume size-dependence of fecundity. Second, it is impossible to assess age for most herbaceous perennials, unless long-term demographic studies are conducted (but see Cochran and Ellner 1992). Hence, study of size-dependence of fitness components is often the only way to apply life history theory predictions to these organisms. Third, neglect of size-dependence of fitness components can potentially lead to misleading conclusions when comparing reproductive allocation among populations (Ohlson 1988, Coleman et al. 1994).

Consideration of size-dependence of fitness components in plant reproductive ecology is extensive but has an irregular coverage. Studies of threshold size for reproduction have mainly focused on herbaceous monocarpic plants (Wesselingh et al. 1993, 1997, Wesselingh and de Jong 1995, Wesselingh and Klinkamer 1996). Only in recent years have woody species been studied (Thomas 1996b, Davies and Ashton 1999). Evidence for a threshold size for reproduction in herbaceous perennials is scattered and mainly anecdotal. Rees and Crawley (1989) even denied the existence of a threshold size for reproduction in plants. Size-dependence of reproductive allocation and fecundity have been documented for a number of plants with various growth forms (Samson and Werk 1986, Weiner 1988, Thompson et al. 1991, Aarssen and Taylor 1992, Thomas 1996a, Davies and Ashton 1999). Phenotypic plasticity (Clauss and Aarssen 1994) and genetic component (Aarssen and Clauss 1992) of size-dependent reproductive allocation and fecundity have been studied mainly in annual plants (but see Schmid and Weiner 1993).

In order to integrate the currently scattered knowledge on size-dependence of fitness components in herbaceous perennials, two approaches seem useful. First, a better picture of the intraspecific variation of size-dependent fitness components in relation to environmental factors should be obtained (Briggs and Walters 1997). Life history theory predicts that the threshold size for reproduction should decrease in adverse environments (Stearns and Koella 1986, Berrigan and Koella 1994). In addition, Weiner (1988) and Schmid and Weiner (1993) predict that favourable environments should cause a higher slope or Y-intercept of the relationship between vegetative and reproductive biomass.

Second, patterns of covariation among different size-dependent fitness components and relationships between size-dependent fitness components and costs of reproduction should be explored. Schmid and Weiner (1993) predicted a negative correlation between the threshold size for reproduction and the slope of the regression describing the size-dependent reproductive allocation (Stearns and Koella 1986). Somatic costs of

reproduction (SCR hereafter) entail a loss in growth due to allocation of resources to reproduction. Hence, SCR interact with size-dependent fitness components: current reproduction will negatively affect future size and consequently future reproductive probability (through the threshold size and the percentage of reproductives) and fecundity (through size-dependent reproductive allocation). A negative relationship between reproductive allocation and cost of reproduction is theoretically predicted (Begon et al. 1996). SCR has been found to increase under stressful conditions (Biere 1995, Sandvik 2001), which gives further possibilities of interaction with size-dependent fitness components. Reekie (1998) has proposed that size-dependent reproductive allocation can result from size-dependence of SCR.

The present paper studies between-population variation in size-dependent fitness components in a carnivorous, herbaceous perennial, *Pinguicula vulgaris*, and how they are related to environmental factors and SCR. Eleven populations were investigated in northern Scandinavia, for which we quantified size-dependent fitness components, several soil variables, prey capture and SCR. The specific questions addressed are: (1) how broad is the variation in threshold size for reproduction, percentage of reproductives and reproductive allocation? (2) are those size-dependent fitness components related to each other according to the predictions above?, (3) can this variation in size-dependent fitness components be related to specific environmental variables and to SCR?

## Methods

### Study species and sampling sites

*Pinguicula vulgaris* L. (Lentibulariaceae) is a perennial, herbaceous, carnivorous plant with sticky leaves arranged in a rosette. Leaves and roots are annual and are shed at the end of the growing season. The plant overwinters as a "winter bud" or hibernaculum, consisting of tightly packed scales around a meristem. New leaves and roots develop from the winter bud in late May to early June, short after the snow cover disappears. Most flowering plants produce a single, blue flower bore on a ca 5–10 cm long peduncle.

The distribution of *P. vulgaris* is circumboreal. We carried out the present study during summer, 1997, at the Abisko Scientific Research station, northern Sweden (68° 21' N, 18° 49' E, 380 m a.s.l.), where *P. vulgaris* is common in a range of open habitats from poor mires to rich calcareous soils. We utilised 11 populations (Table 1) in the present study, which covered the range of habitats (roadsides, polygonal soils, mires and alpine areas) and altitudes (sea level to more than 1000 m) in which *P. vulgaris* is present in northern

Scandinavia. These populations were situated in the surroundings of Abisko (Abisko 1 to 3) and mountains nearby (Paddus 1 and 2, Njulla, Latnjajaure), and along the road E-10 from Abisko to Bjerkevik in Norway (Katterjåkk, Norway 1 to 3). The two most distant populations were about 80 km apart.

## Environmental variables measured

### Soil samples

At each site, we measured soil variables at three different occasions during the season. Due to phenological differences between sites, those occasions were defined according to the phenological status of the plants: flowering peak, during fruit development, and at the end of the growing season. Actual sampling dates ranged 23 June–23 July for flowering peak, 29 July–25 August for fruit development, and 7–10 September for the end of the growing season.

We took four soil samples per site and sampling date, using a core sampler of 7.5 cm diameter and 5 cm depth (volume ca 204 cm<sup>3</sup>). Two soil samples were utilised to measure soil N content (N), and the other two were utilised to measure water content (WATER), organic material content (OMC) and pH. Samples for N were frozen until analysis. Samples for WATER, OMC and pH were analysed on the same day of collection or one day later (Latnjajaure).

We sieved each soil sample through a 2-mm sieve and utilised 50 mg for the analyses. We extracted soil N by

shaking 20 g of fresh soil in 100 ml of KCl 0.2 M for 2 h (Binkley and Vitousek 1989). The extract was filtered and analysed using a Flow Injection Analyser (Tecator, Höganäs, Sweden). Due to the very heterogeneous nature of the soils among sites, N soil content was expressed on a per volume basis (excluding stones), as mg N/l. WATER was measured as the difference in mass of a soil sample (4 to 28 mg of fresh mass) before and after oven-drying it at 105°C for three days. WATER was expressed as % of fresh mass. Once dried, OMC was estimated as the difference in mass of the same soil sample before and after burning it in a furnace oven at 600°C for 1 h. OMC was expressed as % of dry mass. For pH measurements, 50 ml of distillate water were added to 15 g of fresh soil and shaken for 2 h. Measurements were taken with a pH-meter to the nearest 0.1 pH unit.

### Prey capture

From the 50 flowering plants harvested in each site during flowering (see “Reproductive allocation at flowering” below), we utilised a sub-sample of 19–21 to estimate prey capture. We counted and identified all animals present in the leaves of each plant. Five main categories were distinguished: Acarina, Collembola, Diptera, others (mainly Hymenoptera, Hemiptera and Araneae) and unidentified prey. We did not consider Acarina as prey, because some of them are sapro-fagous feeding on the corpses of prey on the leaves (Antor and García 1995). They also make a very small fraction of mass of the animals on the leaves (Karlsson et al. 1987).

Table 1. Location, description, altitude and average  $\pm$  SD values for the soil and prey variables measured in 11 populations of *Pinguicula vulgaris* in northern Scandinavia. For soil variables, n = 3 in all cases. For prey number, n = 19–21.

Population	Coordinates	Habitat	Alt <sup>1</sup>	N <sup>1</sup>	WATER <sup>1</sup>	OMC <sup>1</sup>	pH	Prey <sup>1</sup>
			(m)	(mg N/l)	(% fresh mass)	(% dry mass)		
Norway 1 (N1)	68°28.5'N 17°33.9'E	Roadside	5	42.8 $\pm$ 13.8	16.1 $\pm$ 3.6	1.1 $\pm$ 0.2	7.0 $\pm$ 0.3	0.22 $\pm$ 0.08
Norway 2 (N2)	68°28.3'N 17°42.9'E	Roadside	260	35.8 $\pm$ 5.4	19.7 $\pm$ 2.6	2.5 $\pm$ 0.4	6.5 $\pm$ 0.4	0.23 $\pm$ 0.11
Norway 3 (N3)	68°28.3'N 17°43.6'E	Mire	270	66.7 $\pm$ 6.8	89.9 $\pm$ 1.0	9.3 $\pm$ 0.9	6.1 $\pm$ 0.3	0.28 $\pm$ 0.26
Abisko 1 (A1)	68°21.5'N 18°48.7'E	Polygons	385	61.7 $\pm$ 20.3	14.7 $\pm$ 2.9	2.9 $\pm$ 1.5	4.9 $\pm$ 0.2	0.11 $\pm$ 0.06
Abisko 2 (A2)	68°21.2'N 18°48.6'E	Roadside	385	30.3 $\pm$ 7.5	11.0 $\pm$ 1.8	1.9 $\pm$ 0.1	7.2 $\pm$ 0.3	0.36 $\pm$ 0.12
Abisko 3 (A3)	68°21.4'N 18°47.8'E	Roadside	385	38.3 $\pm$ 4.0	13.6 $\pm$ 0.7	2.5 $\pm$ 0.4	7.2 $\pm$ 0.6	0.46 $\pm$ 0.12
Paddus 1 (P1)	68°19.5'N 18°50.9'E	Polygons	560	80.5 $\pm$ 35.8	15.9 $\pm$ 1.2	4.8 $\pm$ 1.3	5.4 $\pm$ 0.4	0.11 $\pm$ 0.04
Paddus 2 (P2)	68°19.0'N 18°52.0'E	Polygons	600	49.5 $\pm$ 15.8	22.8 $\pm$ 0.9	6.1 $\pm$ 0.2	4.8 $\pm$ 0.3	0.16 $\pm$ 0.09
Katterjåkk (KA)	68°25.0'N 18°09.9'E	Mire	510	89.7 $\pm$ 28.8	88.2 $\pm$ 1.1	10.8 $\pm$ 0.9	4.9 $\pm$ 0.2	0.42 $\pm$ 0.13
Njulla (NJ)	68°21.6'N 18°42.7'E	Alpine	940	87.2 $\pm$ 9.9	65.2 $\pm$ 14.9	11.7 $\pm$ 1.5	5.2 $\pm$ 0.1	0.41 $\pm$ 0.19
Latnjajaure (LA)	68°21.6'N 18°29.8'E	Alpine	1020	108.5 $\pm$ 9.6	44.0 $\pm$ 10.0	11.6 $\pm$ 1.7	5.6 $\pm$ 0.4	0.25 $\pm$ 0.07

<sup>1</sup> Alt: altitude, N: soil nitrogen content, WATER: soil water content, OMC: soil organic material content, Prey: prey number per unit plant size.

Table 2. Variables summarising size dependence of flowering probability in 11 populations of *P. vulgaris* in northern Scandinavia.  $\mu$  and  $\alpha$  are the parameters of the logistic regression relating flowering status to plant size index. SRI: size of the smallest reproductive individual. ATS: average threshold size. RAN: transition range; size difference between the smallest reproductive and the largest vegetative individuals. %R: percentage of reproductives. Sample size for each population is given in the last column.

Population	Size index range	$\mu \pm \text{S.E}$	$\alpha \pm \text{S.E}$	SRI	ATS	RAN	%R	n
Norway 1	2–279	$-7.689 \pm 1.122$	$0.060 \pm 0.009$	75	127.1	109	49.2	254
Norway 2	2–250	$-5.428 \pm 0.731$	$0.053 \pm 0.008$	60	102.8	108	51.4	224
Norway 3	8–315	$-6.348 \pm 0.816$	$0.036 \pm 0.005$	120	176.8	168	49.2	236
Abisko 1	6–153	$-4.598 \pm 0.658$	$0.054 \pm 0.008$	50	85.0	83	46.9	232
Abisko 2	2–203	$-5.817 \pm 0.768$	$0.068 \pm 0.009$	50	85.0	82	55.2	235
Abisko 3	2–200	$-4.609 \pm 0.614$	$0.058 \pm 0.007$	50	79.5	83	65.9	243
Paddus 1	4–133	$-4.926 \pm 0.699$	$0.053 \pm 0.009$	40	93.6	74	23.7	271
Paddus 2	4–171	$-4.325 \pm 0.654$	$0.050 \pm 0.008$	55	86.7	81	45.0	237
Katterjåkk	4–272	$-6.276 \pm 0.859$	$0.048 \pm 0.007$	85	130.5	111	53.7	249
Njulla	2–174	$-3.951 \pm 0.585$	$0.043 \pm 0.007$	48	91.4	92	44.2	247
Latnjajaure	6–161	$-2.773 \pm 0.507$	$0.032 \pm 0.006$	45	87.2	115	48.9	206

#### *Environmental characterization of the sites*

We characterized the sites according to their environmental variables by means of a PCA analysis, performed in SPSS 4.0. Variables considered were altitude, N, WATER, OMC, pH and prey capture (Table 1). As these variables were measured in different units, we run a correlation matrix PCA analysis. Sphericity of the data matrix was tested using a Bartlett's test of sphericity ( $p < 0.0001$ ). Varimax rotation was utilised to facilitate interpretation of the ordination axes obtained. Variables having a loading  $\geq 0.7$  in a factor were considered as related to that factor.

Prior to analysis, we tested within-site seasonal variation in soil variables by repeated measures ANOVA (Zar 1999). The average value of the two replicates per sampling date was utilised in the analyses. For N, OMC and WATER no significant variation ( $p \geq 0.352$ ) was found among sampling dates. Thus, we utilised the average value of the three sampling dates in the PCA. A significant seasonal variation was found for pH ( $p = 0.011$ ) due to an increased acidity along the season. As a PCA performed using the average values in each sampling date resulted in the same ordination as the one using average pH for the whole season, the last approach was preferred for simplicity.

In seven sites, prey capture increased with increasing plant size, estimated as the length of the longest leaf times the number of leaves. Thus, in all analyses the number of prey per plant was divided by plant size in order to normalise prey capture with respect to differences in plant size among populations.

#### **Size-dependent fitness components**

##### *Flowering probability and related variables*

We estimated flowering probability curves from a survey of the plant population structure, as recommended by Silvertown (1991). During full blooming, we non-

destructively estimated plant size of 206–271 plants at each site (Table 2) as the length of the longest leaf times the number of leaves (Svensson et al. 1993). Plants were haphazardly chosen and their reproductive status (vegetative or flowering) was noted. Three plant classes were distinguished: (a) juvenile plants; vegetative plants smaller than the smallest reproductive plant, (b) vegetative plants; vegetative plants equal or larger than the smallest reproductive plant, and (c) reproductive plants; plants with at least one flower.

The relationship linking flowering probability with plant size can be quantified using a logistic regression of reproductive status against plant size (Wesselingh et al. 1993, 1997). We utilised the formula

$$P = 1/(1 + e^{\mu + \alpha x})$$

Parameters  $\mu$  and  $\alpha$  of the fitted logistic curve determine the intercept with the X-axis and the slope of the curve, respectively, and can be related to the threshold size for reproduction and the percentage of reproductives (Wesselingh et al. 1993).

In addition to  $\mu$  and  $\alpha$ , the following variables summarising the size-dependent transition from vegetative to reproductive status were calculated: the size of the smallest reproductive individual (SRI hereafter); the average threshold size (Wesselingh et al. 1993; ATS hereafter), i.e. the size at which a plant has a 50% probability of flowering, obtained as  $ATS = -\mu/\alpha$ ; the transition range, i.e. the difference between the biggest vegetative and the smallest reproductive individuals (Wesselingh and de Jong 1995); and the percentage of reproductives (%R), calculated as the percentage of individuals equal or larger than SRI which were reproductive.

Logistic regression curves of reproductive status against plant size were compared between populations by including population as a categorical variable (deviation coefficient coding). We tested between-site differences in %R by means of G tests (Zar 1999).

### Reproductive allocation at flowering

During full bloom, we harvested 40 to 51 flowering individuals at each site (Table 3). Individuals were chosen to cover the full size range of reproductive plants within each population. Vegetative (leaves and roots) and reproductive (flowers) parts were oven-dried separately at 60°C for one week and the dry mass was obtained to the next 0.1 mg.

We studied reproductive allocation in two ways. First, we computed the percentage of total biomass into reproductive structures (RA, Bazzaz and Ackerly 1992) and compared it between populations using Kruskal-Wallis test and a posteriori Dunn tests (Zar 1999) due to violation of parametric assumptions. Second, we regressed reproductive biomass against vegetative biomass (Samson and Werk 1986) and differences in regression lines among populations were compared by ANCOVA.

### Relationships between variables representing size-dependent fitness components

We run a PCA analysis using nine variables representing size-dependent fitness components (Table 2 and 3): SRI, ATS, transition range, %R,  $\mu$ ,  $\alpha$ , RA and slope (b) and intercept (a) of the allometric regression between vegetative and reproductive biomass. As these variables had different measure units, we run a correlation matrix PCA analysis. Sphericity of the data matrix was tested using a Bartlett's test of sphericity ( $p < 0.0001$ ). Varimax rotation was utilised to facilitate interpretation of the ordination axes obtained. Variables having a loading  $\geq 0.7$  in a factor were considered as related to that factor.

### Relationships between size-dependent fitness components and environmental variables

We studied the relationships between size-dependent fitness components and environmental variables by means of redundancy analysis, RDA. RDA is an ordination method which summarises overall variation in

“species data” (size-dependent fitness components in this case) in a few ordination axes which are linear combinations of the measured environmental variables. This is equivalent to a multivariate multiple regression of the size-dependent fitness components on the environmental variables (ter Braak and Šmilauer 1998). We performed the analysis using CANOCO 4.0 (ter Braak and Šmilauer 1998). Nine size-dependent fitness components were included, six related to flowering probability (Table 2) and three related to reproductive allocation (Table 3). Variables were transformed by centering and standardising by “species” because size-dependent fitness components were measured in different units. Six environmental variables were considered (Table 1).

We used unconstrained Monte Carlo permutation tests to assess the statistical significance of the association between size-dependent fitness components and environmental variables. In all cases, we utilised 500 permutations. First, significance of the whole ordination, and of the first ordination axis, was assessed. Second, a forward stepwise method was used to choose the environmental variables significantly affecting the ordination. Due to collinearity among environmental variables (see “Environmental characterisation of the populations” in the Results section), we selected only three environmental variables in the final model. Results including the six environmental variables were similar to the simplified model.

### Somatic cost of reproduction

At the beginning of the flowering period, we labelled 100 reproductive plants at each site. We left half of the plants, haphazardly chosen, as a control and removed the flower bud to the other half. Usually, a single flower was produced by plant; in a few cases, we removed a second flower bud in a subsequent inspection. The average dry mass removed in deflowered plants ranged 0.5 to 2.4 mg. At the end of the growing season we

Table 3. Reproductive allocation at flowering (RA) in 11 populations of *P. vulgaris*. Slope (b) and intercept (a) values, percentage of variance explained ( $R^2$ ) and significance (p) are given for the regression analyses relating reproductive biomass at flowering to vegetative biomass. Intercepts significantly different from zero are indicated in boldface. Sample size in each population is indicated in the last column.

Population	RA $\pm$ SD <sup>1</sup>	b $\pm$ SE <sup>1</sup>	a $\pm$ SE <sup>1</sup>	R <sup>2</sup>	p	n
Norway 1	24.2 $\pm$ 5.5 abc	0.437 $\pm$ 0.045 a	-1.787 $\pm$ 0.910	0.66	<0.0001	50
Norway 2	21.1 $\pm$ 4.9 a	0.216 $\pm$ 0.027 b	0.552 $\pm$ 0.382 c	0.62	<0.0001	40
Norway 3	23.6 $\pm$ 5.5 abc	0.279 $\pm$ 0.030 b	0.976 $\pm$ 1.313 b	0.64	<0.0001	51
Abisko 1	22.0 $\pm$ 4.9 ab	0.255 $\pm$ 0.041 b	0.303 $\pm$ 0.443 c	0.45	<0.0001	49
Abisko 2	26.5 $\pm$ 6.5 c	0.411 $\pm$ 0.046 a	-0.518 $\pm$ 0.699 c	0.63	<0.0001	50
Abisko 3	25.1 $\pm$ 6.8 bc	0.328 $\pm$ 0.042 ab	0.254 $\pm$ 0.757	0.56	<0.0001	50
Paddus 1	24.8 $\pm$ 4.8 bc	0.252 $\pm$ 0.058 b	0.526 $\pm$ 0.398 c	0.28	0.0001	50
Paddus 2	21.6 $\pm$ 3.6 ab	0.212 $\pm$ 0.025 b	<b>0.581</b> $\pm$ 0.243 c	0.61	<0.0001	50
Katterjåkk	22.8 $\pm$ 3.7 abc	0.234 $\pm$ 0.020 b	<b>1.355</b> $\pm$ 0.555 a	0.75	<0.0001	51
Njulla	23.4 $\pm$ 5.9 abc	0.223 $\pm$ 0.053 b	1.300 $\pm$ 0.837 b	0.27	0.0001	49
Latnjajaure	25.4 $\pm$ 4.7 c	0.240 $\pm$ 0.020 b	<b>1.370</b> $\pm$ 0.360 a	0.75	<0.0001	50

<sup>1</sup> Values sharing the same letter were not significantly different.

harvested all the winter buds from control and deflowered plants. We took note of the production of capsules by the control plants, and excluded from analysis those which did not ripe a seed capsule. Winter buds were oven-dried at 60°C for one week and their dry mass was obtained to the next 0.1 mg. We calculated somatic cost of reproduction (SCR) according to Tuomi et al. (1983) as:

$$\text{SCR} = (V - R)/V$$

where V and R are the average dry mass of the deflowered, vegetative and control, reproductive winter buds, respectively.

We tested differences in winter bud mass between treatments (fixed factor) and populations (random factor) by mixed two-way ANOVA (Zar 1999). Significance was calculated by non-parametric methods, running 5000 permutations, due to lack of normality and homoscedasticity in winter bud mass.

We studied relationships between SCR and the size-dependent fitness components by RDA analysis as described above for environmental variables.

## Results

### Environmental characterization of the sites

There was a broad among-site variation in all the environmental variables measured (Table 1). PCA analysis extracted two factors explaining 81.6% of the variation in the data-set. The first factor, accounting for 60.1% of variation, was mainly related to pH in the negative side and altitude, N and OMC in the positive side (Fig. 1). The second factor, explaining 21.5% of variation, was mainly related to prey capture per unit plant size in the positive side (Fig. 1). WATER had similar loadings ( $\approx 0.6$ ) on the positive sides of both factors (Fig. 1). These factors can be interpreted as gradients in soil richness and prey capture.

Overall, three groups of sites could be distinguished (Fig. 1): (a) alpine sites and mires had positive values for both factors (acid pH, high prey capture and wet, rich soils), (b) polygons, with values for the first factor near to zero and negative values for the second factor (slightly acid pH, low prey capture and poor, dry soils), and (c) roadsides, with negative values for factor 1 (neutral pH, moderate to high prey capture and poor soils).

### Between-site variation in size-dependent fitness components

#### *Flowering probability and related variables*

Flowering was size-dependent in all populations (Fig. 2), as indicated by the highly significant relationship be-

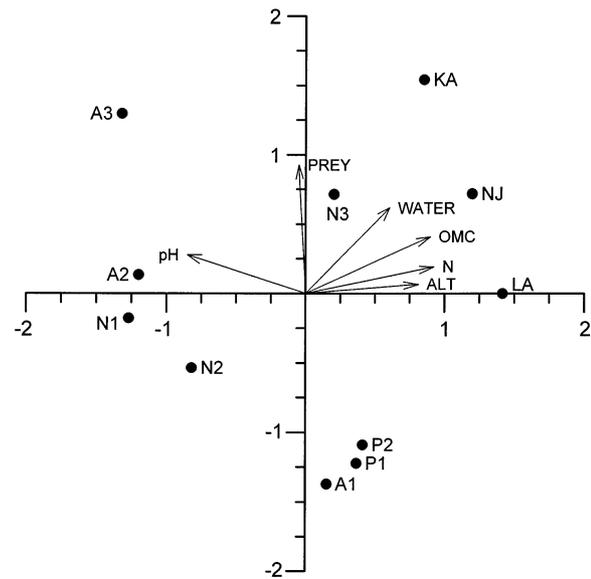


Fig. 1. Ordination of the 11 sites (dots) along the two axes extracted by a PCA analysis based on six environmental variables measured at each site. Arrows indicate the direction and strength of the relationship between each environmental variable and each axis. Relationship with one axis increases with the length of the arrow and decreases with increasing angle respect to that axis. Relationships between variables can be inferred from the angle between arrows (maximum for parallel arrows; minimum for perpendicular arrows). For abbreviations see Table 1.

tween plant size index and flowering probability (logistic regression;  $p < 0.0001$  for all populations). A joint logistic regression analysis with size index and population as independent variables showed that both populations on mires and Norway 1 had lower probabilities of flowering for a given size, compared to the other populations (results not shown). In agreement with the results above, SRI and ATS were higher in mires and Norway 1 (Fig. 2, Table 2).

Percentage of reproductives ranged from 23.7 to 65.9% (Table 2). Most populations clustered around a percentage of reproductives between 44–55%. Significant differences between sites in percentage of reproductives ( $G_{10} = 88.266$ ,  $p < 0.0001$ ) were due to very low percentage of reproductives in Paddus 1 and high percentage of reproductives in Abisko 3. When these two sites were excluded from the analysis, between-site differences in percentage of reproductives were no longer significant ( $G_8 = 7.154$ ,  $p = 0.520$ ).

#### *Reproductive allocation at flowering*

RA ranged from 21.1% to 26.4% (Table 3). There were significant differences between populations ( $\chi^2 = 50.750$ ,  $n = 540$ ,  $p < 0.0001$ ).

Reproductive biomass increased with vegetative biomass in all sites (Table 3). Percentage of variance

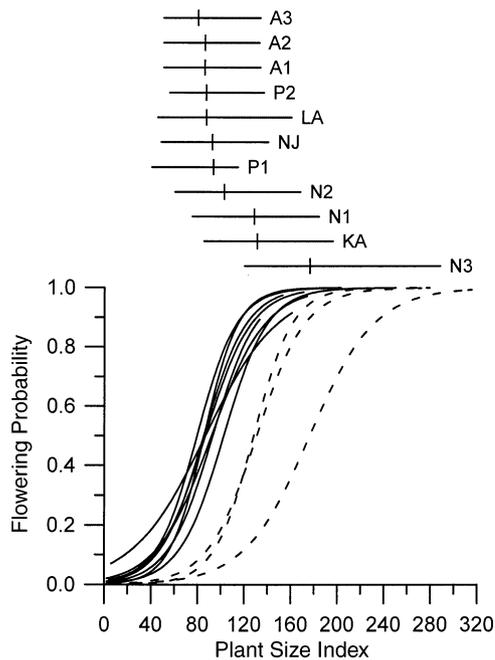


Fig. 2. Size-dependent flowering probability in 11 populations of *P. vulgaris*. Logistic curves were drawn according to the parameters in Table 2. Populations with the same line style had similar logistic curves. Segments above the curves indicate the transition range and the average threshold size (ATS) for each site. Sites abbreviated as in Table 1.

explained by the regression ranged from 27 to 75% and generally exceeded 60% (Table 3).

ANCOVA showed a significant heterogeneity of slopes among sites ( $F_{10, 518} = 3.86$ ,  $p < 0.001$ ; Fig. 3). Three roadside sites (Norway 1, Abisko 2 and Abisko 3) were responsible for these differences (Table 3). Significant differences in intercept were found among the eight sites with smallest slope ( $F_{7, 381} = 2.43$ ,  $p = 0.019$ ). In general, mire and alpine sites showed significantly higher intercepts than polygons and Norway 2 (Table 3).

### Relationships between size-dependent fitness components

PCA analysis extracted three factors, explaining 85% of variation. First factor, accounting for 37.9% of variation, was mainly related to variables related to flowering probability (SRI, ATS and transition range) in the positive side (Fig. 3). Second factor, accounting for 34.4% of variation, was related to the parameters of the equations describing flowering probability and size-dependent reproductive allocation ( $\mu$  and  $b$  in the positive side and  $\alpha$  and  $a$  in the negative side) (Fig. 3). Third factor, accounting for 12.7% of variation, was mainly related to RA in the positive side (Fig. 3). % R also had a positive loading (0.55) in the third factor.

Similar amount of variation explained by the two first factors indicates that PCA analysis did not perform very well and that relationships between variables representing size-dependent fitness components were not very tight. Some trends are worth noting, however. SRI, ATS and transition range were strongly related to each other, but not to %R,  $\mu$  or  $\alpha$  (Fig. 3). Parameters  $\mu$  and  $\alpha$  were negatively correlated to each other (Fig. 3). This indicates that in this data-set populations which began to increase in flowering probability at smaller sizes also increased at a higher rate in flowering probability with increasing size, and vice versa. This is consistent with the positive relationships between SRI, ATS and transition range.

Parameter  $b$  was negatively related to  $a$  and weakly, positively related to RA (Fig. 3). The only relationships between variables related to flowering probability and those related to reproductive allocation were a positive relationship between  $b$  and  $\alpha$  (Fig. 3). Sites in which reproductive biomass increased in a steeper way with plant size were also those in which flowering probability increased in a less steep way with plant size. RA and  $b$  were positively, weakly related to %R (Fig. 3). Thus, there was a trend in the data-set for populations exhibiting higher proportional reproductive allocation to also show higher percentage of reproductive individuals above the threshold size for reproduction.

In the space defined by the three factors extracted by PCA, sites were arranged in a way consistent with the results above (see “Between-site variation in size-dependent fitness components”, Fig. 3). Mires showed high threshold size for reproduction and broad transition range, but intermediate values for the remaining variables. Latnjajaure showed the highest  $a$  and  $\mu$ , while Norway 1 showed the highest  $b$  and the second highest  $\alpha$ . RA was highest at Abisko 2, Abisko 3 and Latnjajaure and lowest at polygons and Norway 2.

### Relationships between size-dependent fitness components and environmental variables

The ordination produced by RDA was significant ( $F = 2.482$ ,  $p = 0.014$ ), as well as the first canonical axis ( $F = 2.378$ ,  $p = 0.006$ ). Forward selection of environmental variables identified WATER ( $F = 3.24$ ,  $p = 0.020$ ), altitude ( $F = 5.04$ ,  $p = 0.002$ ) and pH ( $F = 2.60$ ,  $p = 0.049$ ) as significantly influencing the ordination of size-dependent fitness components.

The sum of the canonical eigenvalues in the simplified model (including only the three significant environmental variables) explained 67.1% of the variance in the data-set. The first axis of the ordination explained 34.6% of the variance and the intersite correlations of the environmental variables with this axis showed a correlation with altitude ( $-0.575$ ) and WATER

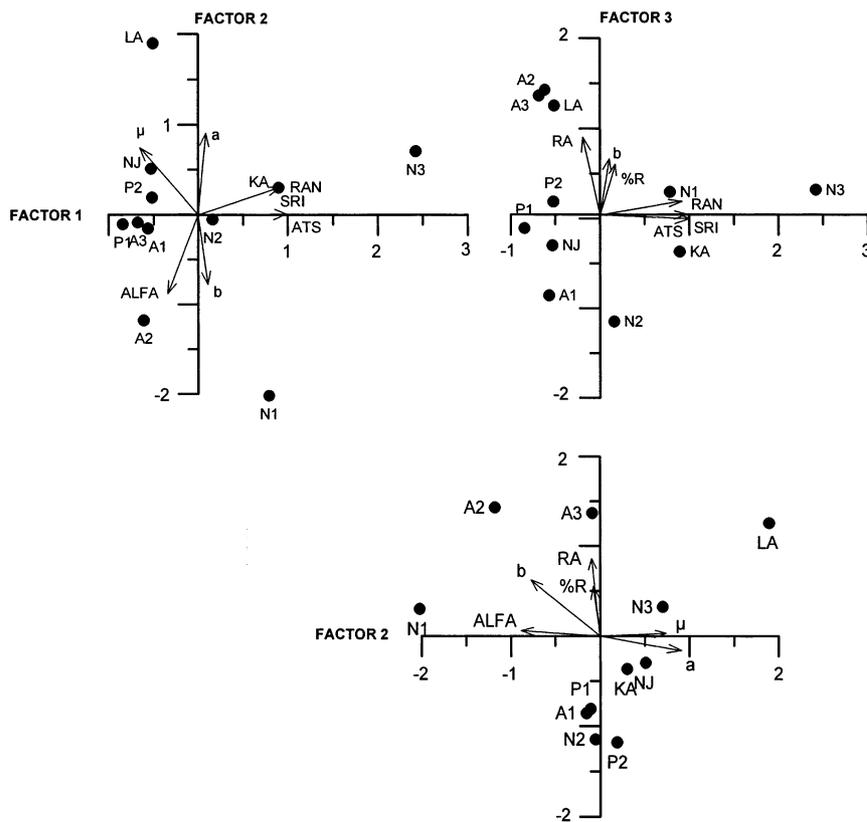


Fig. 3. Ordination of the 11 sites (dots) along the three axes extracted by a PCA analysis based on nine variables related to size-dependent fitness components, measured at each site. Arrows indicate the direction and strength of the relationship between each variable and each axis. Relationship with the axis increases with the length of the arrow and decreases with increasing angle respect to the axis. Relationships between variables can be inferred from the angle between arrows (maximum for parallel arrows; minimum for perpendicular arrows). For abbreviations see Table 1–3.

(0.580). The second axis of the ordination explained 23.4% of the variation in the data-set, and was correlated to WATER (0.622), pH (-0.706) and altitude (0.562).

SRI, ATS1 and transition range were positively related to WATER, while parameter  $\mu$  was positively related to altitude (Fig. 4). Parameters  $\alpha$  and  $b$  were positively related to pH (Fig. 4). Results from Fig. 4 also agree with the correlation table between size-dependent fitness components and environmental variables (results not shown) as well as with results in previous sections.

### Somatic cost of reproduction

Reproductive individuals produced winter buds 16 to 53% lighter than those in which flower buds were removed (Table 4). The mixed two-way ANOVA showed significant differences in mass of the winter bud between treatments ( $p = 0.0002$ ) and between populations ( $p = 0.0002$ ). The interaction between treatment and population was also significant ( $p = 0.0020$ ).

RDA did not show any significant relationship between size-dependent fitness components and SCR ( $F = 0.492$ ,  $p = 0.740$ ). Similar results were obtained

when RDA was repeated including only variables related to flowering probability ( $F = 0.513$ ,  $p = 0.649$ ) or reproductive allocation ( $F = 0.450$ ,  $p = 0.677$ ).

### Discussion

In agreement with previous results of Worley and Harder (1999), we found a size-dependence of flowering probability in *Pinguicula vulgaris*. Between-site variation in flowering probability components was relatively low. Notwithstanding, a higher threshold size for reproduction was related to high soil water content and low altitude, indicating favourable growing conditions. This agrees with qualitative predictions of life history theory (Stearns and Koella 1986, Berrigan and Koella 1994).

Variation in size-dependent flowering probability has been mostly studied in monocarpic species. Between-site differences in threshold size for reproduction have been reported for the monocarpic *Cynoglossum officinale* (Wesselingh et al. 1993). These differences were consistent with the predictions of life history theory regarding survival and growth probabilities (Wesselingh et al. 1997). Between-site variation in size-dependent flowering probability or environmental correlates of

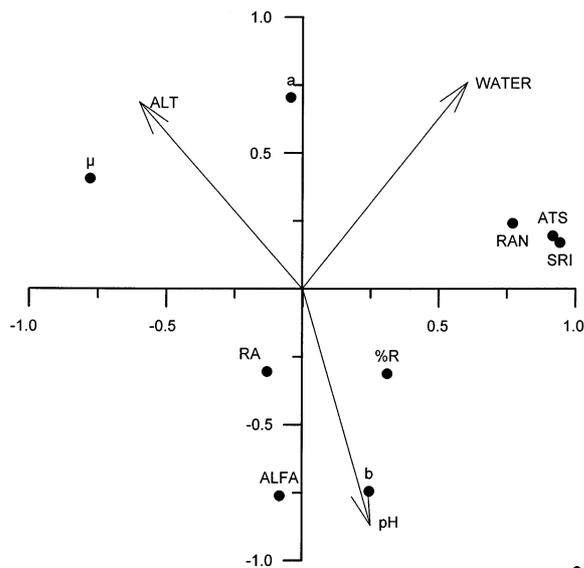


Fig. 4. Bi-plot summarising the relationships between the size-dependent fitness components and the environmental variables according to a redundancy analysis. Only three environmental variables selected by a forward stepwise procedure are represented. For abbreviations see Table 2 and 3.

that intraspecific variation have been very little studied in herbaceous perennials. Schmid and Weiner (1993) found differences between families in flowering probability for *Solidago altissima* in one of four experiments, but no connection was made with environmental factors. Relationship between age and size at first reproduction in *Plantago major* partially supported the hypothesis of adaptation to local conditions (Lotz 1990).

A significant positive relationship between vegetative and reproductive mass has been well documented for herbaceous perennial plants (see references in the Introduction). Such relationship can be considered as

a constraint on reproductive allocation (Reekie 1998, Weiner 1988) caused by proximate architectural mechanisms like production of single flowers vs inflorescences, axillary vs terminal flowers or inflorescences, and so on (Chiariello and Gulmon 1991, Niklas 1993). In addition, it can be looked at from an allocation perspective in connection to the reproductive strategy of the plant. In this sense, it reveals how efficiently resources acquired or stored can be converted into reproductive structures (Weiner 1988). Intraspecific variation in the relationship between size and reproductive mass indicates flexibility in the allocation pattern that can be connected to the reproductive strategy of the species (Weiner 1988).

In the populations of *P. vulgaris* studied in the present paper most individuals produced a single flower (only 4.6% of individuals included in data showed in Table 3 bore more than one flower). Thus, size-dependent reproductive allocation in *P. vulgaris* entailed almost exclusively variation in flower mass, which is strongly correlated with ovule number (M. Méndez, unpubl.). Substantial intraspecific, between-site variability in size-dependent reproductive allocation, involving both slope and intercept of the regression line, was found for *P. vulgaris*. Between-population variation in slope (Ohlson 1988, Hartnett 1990, Welham and Setter 1998), intercept (Méndez and Obeso 1993) or both (Hartnett 1990) has been documented for size-dependent reproductive allocation or fecundity in other herbaceous perennials. Such between-site variation is probably common, as it has been found even in the limited comparisons available, usually involving less than five sites. This begs the question about whether those changes have any biological meaning. Changes in slope or intercept had been related to environmental gradients: soil richness in Ohlson (1988), disturbance in Welham and Setter (1998), fire regime in Hartnett (1990). In the present study, a relationship between slope and pH was found, but it must be interpreted cautiously because it

Table 4. Average  $\pm$  SD (n) dry mass of winter buds for reproductive and deflowered individuals, and somatic cost of reproduction (SCR), in 11 populations of *P. vulgaris* in northern Scandinavia.

Population	Winter bud mass reproductive plants	Winter bud mass deflowered plants	SCR
Norway 1	13.9 $\pm$ 10.2 (47)	19.7 $\pm$ 8.3 (46)	0.29
Norway 2	12.8 $\pm$ 9.8 (27)	15.2 $\pm$ 6.7 (36)	0.16
Norway 3	18.0 $\pm$ 9.9 (43)	31.1 $\pm$ 12.4 (45)	0.43
Abisko 1	8.4 $\pm$ 5.4 (12)	15.0 $\pm$ 6.4 (44)	0.44
Abisko 2	8.2 $\pm$ 7.3 (40)	14.4 $\pm$ 7.6 (41)	0.43
Abisko 3	13.6 $\pm$ 8.6 (40)	21.1 $\pm$ 11.5 (45)	0.35
Paddus 1	5.8 $\pm$ 2.7 (46)	10.8 $\pm$ 2.8 (46)	0.46
Paddus 2	7.4 $\pm$ 3.2 (45)	12.8 $\pm$ 4.4 (48)	0.42
Katterjäkk	15.6 $\pm$ 9.4 (40)	26.8 $\pm$ 9.7 (46)	0.42
Njulla	8.0 $\pm$ 3.1 (45)	16.8 $\pm$ 4.8 (45)	0.53
Latnjajaure	9.2 $\pm$ 5.8 (32)	15.0 $\pm$ 11.1 (32)	0.39

was mainly related to the second, weaker axis of the RDA analysis. The scarce evidence available points to a connection between a higher slope in favourable environmental conditions, which intuitively agrees with the hypothesis of higher allocation efficiency. It also gives tentative support to life history predictions of higher investment in reproduction as survival probabilities decrease (Welham and Setter 1998). Further progress in this topic will require documenting variation in size-dependence of reproductive allocation and fecundity as a response to experimental manipulations of environmental factors, as done for some annual species (Sugiyama and Bazzaz 1998).

A steeper slope of reproductive allocation was positively related to a more gradual transition from vegetative to reproductive status and a larger percentage of reproductive individuals. However, in general there was no strong relationship between the measured size-dependent fitness components. Schmid and Weiner (1993) postulated a trade-off between the threshold size and the slope of the regression line describing size-dependent reproductive allocation. Neither these authors, based on genetic correlations, nor the present paper, based on phenotypic correlations, found such negative correlation. On the contrary, our results indicated that variables related to flowering probability and those related to reproductive allocation defined two independent phenotypic "axes" of variation. Instead of showing a covariation of traits, in line with interpretations in terms of life history "tactics" (Stearns 1976), *P. vulgaris* seemed to independently vary each size-dependent fitness component in each locality. Similar conclusions have been drawn in other studies. Elle (1996) utilised PCA analysis to define life history strategies in different genotypes of *Vaccinium macrocarpum*. Different genotypes segregated along two PCA axes, indicating different strategies, but some of them were against common wisdom in life history theory (i.e. many big fruits).

No relationship was found between size-dependent fitness components and somatic cost of reproduction. Several models have postulated a lower reproductive allocation in habitats in which cost of reproduction is higher (Begon et al. 1996). This prediction was not confirmed in the present paper.

This study, as many others previously (Welham and Setter 1998), was based in a snapshot and it could be argued that the results would have been different in a different year. For example, between-year variation in the percentage of reproductive individuals (Ehrlén and van Groenendael 2001), and size-dependent reproductive allocation (Kawano et al. 1982, Ohlson 1988) has been found in other species. Some evidence points to the consistency of the environmental and population patterns in our study area.

Our soil data were consistent with past research in Katterjåkk (Karlsson 1987) and later research in Padus 2, Abisko 1, Katterjåkk and Njulla (Lang 2001). At least in Abisko 1, Katterjåkk and Njulla, the size structure and flowering probability has remained similar to the one during the study year (Karlsson 1987, Méndez, pers. obs.).

In conclusion, intraspecific variation was found for size-dependent fitness components in *P. vulgaris*. For threshold size and percentage of reproductives variation was rather limited but not for variables related to reproductive allocation. This variation was related to environmental factors in ways mostly consistent with current life history expectations. However, weak links existed among different components, as well as between those fitness components and cost of reproduction. Study of the plastic and genetic character of variation in size-dependent fitness components, and its potential adaptive value, in perennial plants deserves further attention.

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## References

- Aarssen, L. W. and Clauss, M. J. 1992. Genotypic variation in fecundity allocation in *Arabidopsis thaliana*. – *J. Ecol.* 80: 109–114.
- Aarssen, L. W. and Taylor, D. R. 1992. Fecundity allocation in herbaceous plants. – *Oikos* 65: 225–232.
- Antor, R. J. and García, M. B. 1995. A new mite-plant association: mites living amidst the adhesive traps of a carnivorous plant. – *Oecologia* 101: 51–54.
- Bazzaz, F. A. 1997. Allocation of resources in plants: state of the science and critical questions. – In: Bazzaz, F. A. and Grace, J. (eds), *Plant resource allocation*. Academic Press, pp. 1–37.
- Bazzaz, F. A. and Ackerly, D. D. 1992. Reproductive allocation and reproductive effort in plants. – In: Fenner, M. (ed.), *Seeds: the ecology of regeneration in plant communities*. CAB, pp. 1–26.
- Begon, M., Harper, J. L. and Townsend, C. R. 1996. *Ecology: individuals, populations and communities*, 3rd ed. – Blackwell.
- Berrigan, D. and Koella, J. C. 1994. The evolution of reaction norms: simple models for age and size at maturity. – *J. Evol. Biol.* 7: 549–566.
- Biere, A. 1995. Genotypic and plastic variation in plant size: effects on fecundity and allocation patterns in *Lychnis flos-cuculi* along a gradient of natural soil fertility. – *J. Ecol.* 83: 629–642.
- Binkley, D. and Vitousek, P. 1989. Soil nutrient availability. – In: Pearcy, R. W., Ehleringer, J., Mooney, H. A. and Rundel, P. W. (eds), *Plant physiological ecology: field methods and instrumentation*. Chapman and Hall, pp. 75–96.

- Briggs, D. and Walters, S. M. 1997. Plant variation and evolution, 3rd ed. – Cambridge Univ. Press.
- Chiariello, N. R. and Gulmon, S. L. 1991. Stress effects on plant reproduction. – In: Mooney, H. A., Winner, W. E., Pell, E. J. and Chu, E. (eds), Response of plants to multiple stress. Academic Press, pp. 161–188.
- Clauss, M. J. and Aarssen, L. W. 1994. Phenotypic plasticity of size-fecundity relationships in *Arabidopsis thaliana*. – J. Ecol. 82: 447–455.
- Cochran, M. E. and Ellner, E. 1992. Simple methods for calculating age-based life history parameters for stage-structure populations. – Ecol. Monogr. 62: 345–364.
- Coleman, J. S., McConnaughay, D. M. and Ackerly, D. D. 1994. Interpreting phenotypic variation in plants. – Trends Ecol. Evol. 9: 187–191.
- Davies, S. J. and Ashton, P. S. 1999. Phenology and fecundity in 11 sympatric pioneer species of *Macaranga* (Euphorbiaceae). – Am. J. Bot. 86: 1786–1795.
- Ehrlén, J. and van Groenendael, J. 2001. Storage and the delayed costs of reproduction in the understorey perennial *Lathyrus vernus*. – J. Ecol. 89: 237–246.
- Elle, E. 1996. Reproductive trade-offs in genetically distinct clones of *Vaccinium macrocarpon*, the American cranberry. – Oecologia 107: 61–70.
- García, M. B. and Antor, R. J. 1995. Age and size structure in populations of a long-lived dioecious geophyte: *Bor-derea pyrenaica* (Dioscoreaceae). – Int. J. Plant Sci. 156: 236–243.
- Hanzawa, F. M. and Kalisz, S. 1993. The relationship between age, size, and reproduction in *Trillium grandiflorum* (Liliaceae). – Am. J. Bot. 80: 405–410.
- Hartnett, D. C. 1990. Size-dependent allocation to sexual and vegetative reproduction in four clonal composites. – Oecologia 84: 254–259.
- Karlsson, P. S. 1987. Seasonal pattern of biomass allocation in flowering and non-flowering specimens of three *Pinguicula* species. – Can. J. Bot. 64: 2872–2877.
- Karlsson, P. S., Nordell, K. O. and Eirefelt, S. 1987. Trapping efficiency of three carnivorous *Pinguicula* species. – Oecologia 73: 518–521.
- Kawano, S. 1985. Life history characteristics of temperate woodland plants in Japan. – In: White, J. (ed.), The population structure of vegetation. Dr W. Junk, pp. 515–549.
- Kawano, S. and Mikaye, S. 1983. The productive and reproductive biology of flowering plants. X. Reproductive energy allocation and propagule output of five congeners of the genus *Setaria* (Gramineae). – Oecologia 57: 6–13.
- Kawano, S., Hiratsuka, A. and Hayashi, K. 1982. The productive and reproductive biology of flowering plants. V. Life history characteristics and survivorship of *Erythronium japonicum*. – Oikos 38: 129–149.
- Kozłowski, J. and Uchmanski, J. 1987. Optimal individual growth and reproduction in perennial species with indeterminate growth. – Evol. Ecol. 1: 214–230.
- Lacey, E. P. 1986. Onset of reproduction in plants: size-versus age-dependency. – Trends Ecol. Evol. 1: 72–75.
- Lang, S. 2001. Growth of *Pinguicula vulgaris* in northern Sweden, in relation to nitrogen compounds and other soil nutrients. – Masters Thesis, Univ. of Karlsruhe.
- Lotz, L. A. P. 1990. The relationship between age and size at first flowering of *Plantago major* in various habitats. – J. Ecol. 78: 757–771.
- Méndez, M. and Obeso, J. R. 1993. Size-dependent reproductive and vegetative allocation in *Arum italicum* (Araceae). – Can. J. Bot. 71: 309–314.
- Niklas, K. J. 1993. Ontogenetic-response models and the evolution of plant size. – Evol. Trends Plants 7: 42–48.
- Ohlson, M. 1988. Size-dependent reproductive effort in three populations of *Saxifraga hirculus* in Sweden. – J. Ecol. 76: 1007–1016.
- Piqueras, J. 1999. Herbivory and ramet performance in the clonal herb *Trientalis europaea* L. – J. Ecol. 87: 450–460.
- Reekie, E. G. 1998. An explanation for size-dependent reproductive allocation in *Plantago major*. – Can. J. Bot. 76: 43–50.
- Rees, M. and Crawley, M. J. 1989. Growth, reproduction and population dynamics. – Funct. Ecol. 3: 645–653.
- Roff, D. A. 1992. The evolution of life histories: theory and analysis. – Chapman and Hall.
- Samson, D. A. and Werk, K. S. 1986. Size-dependent effects in the analysis of reproductive effort in plants. – Am. Nat. 127: 667–680.
- Sandvik, S. M. 2001. Somatic and demographic costs under different temperature regimes in the late-flowering alpine perennial herb *Saxifraga stellaris* (Saxifragaceae). – Oikos 93: 303–311.
- Schmid, B. and Weiner, J. 1993. Plastic relationships between reproductive and vegetative mass in *Solidago altissima*. – Evolution 47: 61–74.
- Schmid, B., Bazzaz, F. A. and Weiner, J. 1995. Size dependency of sexual reproduction and clonal growth in two perennial plants. – Can. J. Bot. 73: 1831–1837.
- Silvertown, J. 1991. Modularity, reproductive thresholds and plant population dynamics. – Funct. Ecol. 5: 577–580.
- Stearns, S. C. 1976. Life-history tactics: a review of the ideas. – Q. Rev. Biol. 51: 3–47.
- Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press.
- Stearns, S. C. and Koella, J. C. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. – Evolution 40: 893–913.
- Sugiyama, S. and Bazzaz, F. A. 1998. Size dependence of reproductive allocation: the influence of resource availability, competition and genetic identity. – Funct. Ecol. 12: 280–288.
- Svensson, B. M., Carlsson, B. Å., Karlsson, P. S. et al. 1993. Comparative long-term demography of three species of *Pinguicula*. – J. Ecol. 81: 635–645.
- ter Braak, C. J. F. and Šmilauer, P. 1998. CANOCO reference manual and user's guide to CANOCO for Windows: software for canonical community ordination (version 4). – Microcomputer Power.
- Thomas, S. C. 1996a. Reproductive allometry in Malaysian rain forest trees: biomechanics versus optimal allocation. – Evol. Ecol. 10: 517–530.
- Thomas, S. C. 1996b. Relative size at onset of maturity in rain forest trees: a comparative analysis of 37 Malaysian species. – Oikos 76: 145–154.
- Thompson, B. K., Weiner, J. and Warwick, S. I. 1991. Size-dependent reproductive output in agricultural weeds. – Can. J. Bot. 69: 442–446.
- Thompson, J. N. 1987. The ontogeny of flowering and sex expression in divergent populations of *Lomatium grayi*. – Oecologia 72: 605–611.
- Tuomi, J., Hakala, T. and Haukioja, E. 1983. Alternative concepts of reproductive effort, costs of reproduction, and selection in life-history evolution. – Am. Zool. 23: 25–34.
- Weiner, J. 1988. The influence of competition on plant reproduction. – In: Lovett Doust, J. and Lovett Doust, L. (eds), Plant reproductive ecology: patterns and strategies. Oxford Univ. Press, pp. 228–245.
- Welham, C. V. J. and Setter, R. A. 1998. Comparison of size-dependent reproductive effort in two dandelion (*Taraxacum officinale*) populations. – Can. J. Bot. 76: 166–173.
- Wesselingh, R. A. and de Jong, T. J. 1995. Bi-directional selection on threshold size for flowering in *Cynoglossum officinale* (hound's-tongue). – Heredity 74: 415–424.
- Wesselingh, R. A. and Klinkhamer, P. G. L. 1996. Threshold size for vernalization in *Senecio jacobaea*: genetic varia-

- tion and response to artificial selection. – *Funct. Ecol.* 10: 281–288.
- Wesselingh, R. A., de Jong, T. J., Klinkhamer, P. G. L. et al. 1993. Geographical variation in threshold size for flowering in *Cynoglossum officinale*. – *Acta Bot. Neerl.* 42: 81–91.
- Wesselingh, R. A., Klinkhamer, P. G. L., de Jong, T. J. et al. 1997. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. – *Ecology* 78: 2118–2132.
- Worley, A. C. and Harder, L. D. 1999. Consequences of preformation for dynamic resource allocation by a carnivorous herb, *Pinguicula vulgaris* (Lentibulariaceae). – *Am. J. Bot.* 86: 1136–1145.
- Zar, J. H. 1999. *Biostatistical analysis*, 4th ed. – Prentice-Hall.