

Resource allocation in the dioecious shrub *Rhamnus alpinus*: the hidden costs of reproduction

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ABSTRACT

We know little about the demographic consequences of reproductive investment in woody plant species, especially dioecious ones. We analysed the sexual dimorphism in the reproductive investment of the dioecious shrub *Rhamnus alpinus* at different hierarchical levels (shoot, branch, plant, population), and the consequences of such investment for both males and females. We found no short-term reproductive costs at shoot or branch levels for either sex. This lack of costs can be attributed to translocation of resources from other shoots or branches. We found evidence for reproductive costs in female shrubs at the plant and population levels. Dendrochronological analyses showed a connection between the estimated age of first reproduction and a decrease in growth rate of females over time. The annual ring growth of females differed significantly from that of males in periods with low annual precipitation, which could exacerbate the costs of reproduction. Populations with older individuals exhibited male-biased sex ratios, which might be due to higher mortality rates of females because of the cumulative effects of reproductive costs. Evidence for the costs of reproduction at lower modular levels depends on the balance of autonomy and integration of branches at the whole shrub level. Cumulative effects may be seen at the whole shrub and population levels after a number of reproductive episodes.

Keywords: growth rate, hierarchical structure, sex ratio, sexual dimorphism, trade-offs.

INTRODUCTION

The ultimate aim of all reproductive processes is to preserve the genetic material of each individual through time in future generations. This goal is usually achieved by producing viable offspring. In most species, each embryo includes the same amount of maternal and paternal genetic material, so when comparing male and female adults we are comparing individuals obtaining the same benefit (i.e. *one* viable seed) but expending very different

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amounts of biomass and energy in its acquisition. These differences contribute to sexual dimorphisms in life histories.

Life-history theory in plants depends heavily upon assumptions regarding the trade-offs between reproductive and vegetative processes, although clear evidence for such trade-offs is often lacking (Lovett-Doust and Lovett-Doust, 1988; Obeso, 2002). Research on these trade-offs has largely focused on the documentation of differences between the sexes in life history and patterns of resource allocation in dioecious species. Females of woody dioecious plants usually allocate more resources to reproduction than do males (Delph, 1999; Obeso, 2002; but see Marion and Houle, 1996). If resources become limiting, females may bear higher costs of reproduction, which should be measurable in terms of a reduced vegetative growth rate (Cipollini and Whigham, 1994), later flowering, lower flowering frequency (Thomas and LaFrankie, 1993) and/or reduced survival (Allen and Antos, 1993). However, despite the sizeable amount of information published on the reproduction of dioecious plants, the link between differential reproductive allocation and costs of reproduction is not clearly understood.

The trade-off between reproduction and growth in plants is not easily detected (Obeso and Grubb, 1993; Delph and Meagher, 1995). This might be related to: (1) the different responses obtained from experimental manipulation versus estimates of genetic correlations (Bailey, 1992; Reznick, 1992); (2) strong selection against individuals incurring high costs (Bailey, 1992; Jönsson and Tuomi, 1994); (3) pollen limitation, which can confound effects (Dudash and Fenster, 1997); (4) increased resource availability that might uncouple vegetative and reproductive costs (Tuomi *et al.*, 1983); and (5) the degree of autonomy of the modular levels considered, which can affect our ability to detect the trade-offs (Watson and Casper, 1984; Obeso *et al.*, 1998). The first two pitfalls can be avoided through careful experimental designs. Pollen and resource availability (3 and 4) can confound the consequences of the reproductive investment. The hypothesis of costs of reproduction has a slimmer chance of being supported when reproduction is pollen-limited (Delph and Meagher, 1995; Ramsey, 1997). Studies testing this hypothesis should include experiments with additional pollen supply (Dudash and Fenster, 1997). In a complementary approach, trade-offs could also be examined by modifying the demand of resources and measuring the effect on subsequent growth and reproduction. This can be done by limiting resources via defoliation (Marquis, 1992; Ruohomäki *et al.*, 1997), or relaxing the demand by suppressing reproduction in otherwise reproductive branches or individuals (Fox, 1995; Houle, 2001). Finally (5), trade-offs may be obscured because resource allocation at lower modular levels might respond to a small-scale balance, such as between flower or fruit production and shoot growth, albeit being integrated at higher modular levels (e.g. tree growth; Obeso, 1997). This integration clearly depends on the degree of module autonomy and probably varies among plant species (Trueman and Turnbull, 1994; Obeso *et al.*, 1998). If branches are autonomous, the costs might be detected at lower modular levels but be compensated at higher ones by non-reproductive branches. Alternatively, if branches are only partially autonomous, and their resources for reproduction are easily translocated among modules, the costs of reproduction might only be detectable at the whole plant level, not at the branch level. The only way of integrating costs into different modular levels is by considering the autonomy of these modules. We have previously found that the branches of *Rhamnus alpinus* are only partially autonomous, and their resources can be easily translocated between modules (Bañuelos, 2001). Hence, we hypothesize that the costs of reproduction might not be detected at shoot or branch level, but should be seen at the whole plant level.

The picture becomes even more complex, since costs of reproduction can be either direct or indirect. Direct costs refer to resource allocation at the time of reproduction (Houle, 2001), and constitute the only focus of early life-history studies (Cody, 1966; Williams, 1966). Indirect costs, also called 'delayed' or 'demographic' costs of reproduction (Newell, 1991; Ehrlén and Groenendaal, 2001), may be interpreted as the demographic consequences of resource investment in the reproductive process. These demographic costs are often inferred from eventually reduced, more evenly separated reproductive events, higher mortality rates, lower growth rates and lower vegetative propagation of the individuals that allocate more resources to reproduction, a set of characteristics often found in females (Cipollini and Whigham, 1994; Nicotra, 1999, and references therein).

In this paper, we consider the allocation of resources to reproduction and growth (direct costs), as well as the associated short- and long-term costs (indirect costs), in the dioecious shrub *Rhamnus alpinus*, using a hierarchical, bottom-up approach. Specifically, we addressed the following questions: (1) Do males and females of *R. alpinus* differ in their allocation to reproduction? (2) Is there a trade-off between investment in reproduction and vegetative growth at shoot or branch level? (3) Are there differences between the sexes in lifetime growth patterns? (4) If such differences exist, are they reflected in size distributions and sex ratios?

MATERIAL AND METHODS

Study species

Rhamnus alpinus (L.) (Rhamnaceae) is a dioecious broad-leaved shrub or small tree (<4 m). It occurs in rocky, mainly calcareous slopes in the mountains of southern and southern-central Europe and North Africa (Tutin *et al.*, 1968). Wintering buds open in spring, at the end of April or the beginning of May, and the flowering period extends for 2–3 weeks. Its greenish flowers are clumped in axillary positions on current year stems. Flowers are mainly insect-pollinated, and pollen arrival is required for the formation of fruits (Bañuelos and Obeso, 2003). The fruits are black, fleshy drupes with up to four pyrenes. Fruits ripen in late August and are mainly dispersed by birds. The mean ($\pm$ s.e., $n = 50$) fresh mass of individual fruits is 255 ± 13 mg, dry mass 52 ± 2 mg and mean fruit width 7.4 ± 0.1 mm. In the study area, seeds are heavily exploited by birds (mainly *Fringilla coelebs* and *Pyrrhula pyrrhula*, pre-dispersal seed predators) and rodents (mainly *Apodemus sylvaticus*, post-dispersal seed predator), which may help to explain the local scarcity of seedlings (M.J. Bañuelos, personal observation). Leaves begin to wither in late August, and usually shrubs become completely leafless during the second half of September, denoting the end of the 4-month growing period. Vegetative spread by suckers arising from shallow lateral roots is common at the study sites, producing clonal clumps of shrubs. Assigning trunks to genets was not difficult, since trunks from the same genet typically grow in isolated tufts. Juvenile shrubs were scarce in the study area: shrubs less than 30 cm high, all of them non-reproductive, constituted 12% of total shrubs.

Study sites

We conducted the study at seven sites located between 1000 and 1600 m above sea level in the Cantabrian Range, northwest Spain, within the boundaries of the Somiedo Regional

Park: Putracón (PU), Llamardal (LL), Valle (VL), Penouta (PT), Pena Mochada (PM), Santa Eufemia (SE) and El Texu (TX). All selected populations grew in calcareous open areas, either in pure patches of *R. alpinus* or co-occurring with other woody species such as *Corylus avellana*, *Sorbus aria* or *Crataegus monogyna*. We checked that minimum main trunk width in reproductive shrubs was about 2 cm and then selected these study areas during November 1997, each one including at least 100 reproductive-sized shrubs. In spring 1998, we determined the sex of 700 tagged shrubs. There were 399 males, 254 females, 10 hermaphrodites (these turned out, functionally, to be males and are therefore included in the 399 males above) and 47 non-flowering shrubs. In spring 1999, 406 were males, 273 females and 21 non-flowering. There are no records of *R. alpinus* switching sex, and no individual did so during the present study.

Mean annual rainfall in the area approaches 950 mm and mean annual temperature varies around 8°C, oscillating between temperatures above 30°C in summer and below 0°C in winter.

Shoot level analysis

We examined differences in resource allocation to reproduction and vegetative growth at shoot level between sexes, populations and individual plants during two consecutive reproductive seasons. We tagged 10 randomly chosen reproductive shoots in 25 shrubs per sex (5 shrubs per sex and study site at PU, LL, PM, VL and PT) in May 1998, just before flowering. Hereafter, these shrubs are referred to as control shrubs, and none of the experimental procedures were performed on them. In the first reproductive season, each tagged shoot produced flowers and leaves. Flowering shoots do not produce leaves or flowers the following season, but can ramify and produce new reproductive shoots. Here, the clump of old stem (1998), new shoots (1999) and reproductive structures (1999) is referred to as a 'branch'.

In May 1999, we estimated biomass allocation to flowering at shoot level in males and females using non-destructive sampling. We counted flowers produced per shoot (see above) and then multiplied by the corresponding mass of individual male and female flowers, previously obtained from a sample of 200 random flowers per sex. We determined final dry mass allocation for both sexes at shoot level in August 1999, separating leaves, new stems and sexual structures, to the nearest 0.1 mg, after oven-drying at 60°C.

To examine whether there were short-term delayed costs of reproduction, we selected at random one new shoot per control branch in 1999 and monitored its growth and reproduction. The mass of reproductive structures and vegetative growth was determined after oven drying.

To check for possible trade-offs at this level, we performed correlation analyses to compare reproductive biomass per shoot both with vegetative biomass (direct trade-off) and with the following year reproductive and vegetative biomass (delayed trade-off). We used analyses of variance to test for differences in resource allocation between sexes (fixed factor), localities (random factor) and shrubs (random factor nested in the sex $\times$ locality interaction).

To determine whether reproductive and vegetative shoots differed in their photosynthetic active biomass, we determined the dry mass of leaves in three reproductive and three non-reproductive shoots, randomly chosen from five males and five females sampled at the

PU locality in August 1999, which were different from shrubs used in any other sampling. Using a factorial analysis of variance, we examined differences in reproductive stage (fixed factor) and shrubs (random factor nested into sex) between the sexes, after logarithmic transformation of the data.

Branch level analysis

Shoots are clumped in branches, the next hierarchical level we considered. To analyse resource allocation at the branch level in males and females, we estimated biomass allocation to flowers as described above for the whole branch. Final dry mass allocation at the branch level was determined for both sexes in August 1999, separating new shoots (grown during the same season), old stems (grown during the previous season), leaves and sexual structures. We estimated reproductive allocation (RA) as the ratio between the dry masses of sexual structures to total new biomass (sexual structures + new stems + dry mass of leaves). We calculated the relative growth rate (RGR) per year as:

$$\text{RGR} = \log_e (\text{branch mass}) - \log_e (\text{branch mass} - \text{new stem mass})$$

We compared reproductive biomass per branch with relative growth rate using correlation analysis on log-transformed data. We used factorial analyses of variance to test for differences in the amount of photosynthetic biomass, shoot growth, reproductive structures, relative growth rate and reproductive allocation between sexes (fixed factor), localities (random factor) and shrubs (random factor nested in the sex $\times$ locality interaction).

We used dry mass as an integrative measure of allocation; however, to examine nutrient allocation to different structures we pooled leaves, new stems, old stems, flowers and fruits of 10 shrubs per sex into combined samples and analysed them for nitrogen content (samples collected on 24 August 1999, except flowers, which were collected on 27 May 1999). The analyses were performed by combustion using a standard automated CNH procedure (PE 2400 Series II, CNHS/O).

We also compared branch structure in males and females by recording the number of shoots produced per control branch, and then calculating the percentage of reproductive ones.

There is evidence that seed production in *R. alpinus* is not pollen-limited (Bañuelos, 2001), so increased reproductive effort is not inducible by adding pollen. We have also previously studied the extreme case of limiting resources, created by applying different treatments of defoliation and girdling (Bañuelos, 2001). We found that reduced resources did not limit reproductive output while branches remained connected to the main trunk. During spring 1998, we randomly chose 10 males and 10 females at location PU, which were different from control shrubs. We tagged six reproductive branches per shrub, keeping three of them as controls and assigning the other three to a debudding treatment where all floral buds were removed. Additionally, we tagged three non-reproductive branches per shrub. At the end of the growing season, we collected all tagged branches and compared relative growth rates. Five male shrubs were lost due to a wildfire, so we tested for differences between treatments with an analysis of variance for each sex due to an unbalanced design (sex and treatment as fixed factors, shrub nested into sex as a random factor).

Shrub level analysis

At the shrub level, we determined resource allocation for males and females in 1998 and 1999 by estimating the new shoots produced per plant and year. We counted the number of branches in all control shrubs, and then multiplied by the mean number of shoots per branch for each individual shrub (obtained from 10 randomly selected branches per shrub). We also determined the percentage of reproductive shoots at shrub level from 100 randomly selected shoots per control shrub.

We estimated growth rates at the shrub level by measuring annual ring widths. To do so, we selected a pool of 50 healthy shrubs from three localities (17, 17 and 16 from PU, VL and TX, respectively) during February 1998 before they were sexed. We took one or two cores from the main trunk of each shrub, 15 cm up from the base, using a Pressler bore. We mounted the cores on wooden strips and sanded them. We measured annual shrub-ring widths to the nearest 0.01 mm using a sliding-stage micrometer and the software MEDIR 1.13 (Krusic *et al.*, 1996). During May 1998, we confirmed that the cores corresponded to 32 males and 18 females. After checking for the absence of false rings in the cores, we accurately aged the shrubs and compared the ages of males and females using one-way analysis of variance.

Our observations in the field suggested that there was a minimum reproductive size for this species: shrubs less than 30 cm high and with a main trunk diameter less than 2 cm were all non-reproductive. This corresponded to an approximate age of 10–12 years as measured by ring data. Taking this into account, we compared mean growth rates of males and females before and after the estimated age for reproduction with successive analyses of variance, excluding from these analyses those trees younger than 15 years (4 males and 3 females).

To determine whether climate could have influenced shrub growth, we obtained annual precipitation records for 1972–1994 from the nearest weather station, located in Pola de Somiedo (Asturias), which is 630 m above sea level and 6.5 km as the crow flies from the furthest population. Thereafter, we examined whether ring-width data showed any relationship with rainfall using linear regression analyses on log-transformed data.

Population level analysis

We analysed deviations from a 1:1 sex ratio using *G*-tests, Yates's correction for continuity, finally obtaining Williams' adjusted *G* (Fowler *et al.*, 1998). We could not pool samples from different localities because of heterogeneity among populations, so we applied *G*-tests separately to the data from each locality.

To establish if males and females differed in size distribution, and if bigger (and, therefore, older) shrubs were more likely to reproduce, we recorded the diameter of the trunks at a height of 15 cm from the base, for 350 tagged shrubs, 50 per study site (180 males, 150 females and 20 non-flowering shrubs). We considered the widest trunk within the clump as the main one, and the others as suckers. We calculated the basal area from the diameters and the sum of the basal areas of all suckers plus the main trunk was used as an estimate of shrub size.

If there is differential mortality between the sexes, a sex ratio biased towards the more resistant sex will be more evident in older populations. Hence, we used correlation analysis to reflect the mean size of shrubs (as a proxy for age) and sex ratio as indirect evidence of

differential mortality between the sexes. We used mean trunk diameter of females and the proportion of males in each population to avoid dependence among variables.

RESULTS

Shoot level analysis

Females' reproductive investment per shoot was 9.8-fold higher than that of males ($F_{1,4} = 341.3$, $P < 0.001$; see design of the analysis in Table 3) (Fig. 1). However, there was no evidence of a trade-off at this level, since differences in the values of leaves and stem biomass were not significant ($F_{1,4} = 2.12$ for leaves and $F_{1,4} = 2.31$ for stem; $P > 0.5$ in both cases). Moreover, females' reproductive investment per shoot in 1998 showed no trade-off with reproductive or vegetative investment in the subsequent year ($R^2 = 0.04$, $F_{1,247} = 10.5$, $P < 0.01$ for fruit production; $R^2 < 0.01$, $F_{1,247} = 0.04$, $P > 0.05$ for relative growth rate; $R^2 = 0.1$, $F_{1,23} = 0.35$, $P = 0.5$ for the degree of ramification). All correlations for males were non-significant (results not shown).

Non-reproductive shoots had a higher biomass of leaves than reproductive shoots in both sexes (Table 1), although the difference was greater in females, whose reproductive shoots had 1.83 times more biomass of leaves than non-reproductive shoots; the value for males was 1.28 times. This difference, together with the higher proportion of vegetative shoots in females (see results for branch level), helped to diminish the difference in photosynthetic biomass between branches (Fig. 1).

Branch level analysis

Females invested 1.3 times more total biomass per branch than males in one reproductive season, mainly due to the 9.3-fold higher investment of females in reproductive structures (Fig. 1, Tables 2, 3). Neither allocation to leaves and stems nor relative growth rate differed

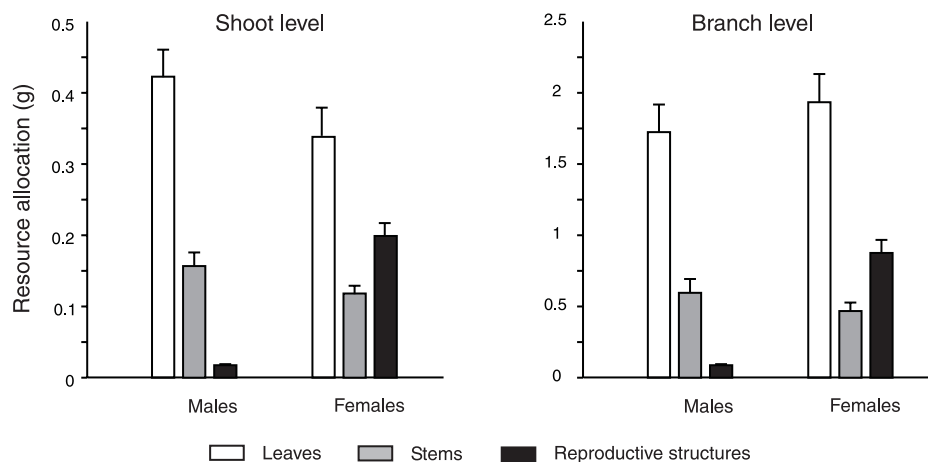


Fig. 1. Dry mass allocation (mean $\pm$ S.E.) of *Rhamnus alpinus* to leaves, shoot growth and reproductive structures at the shoot and branch level ($n = 250$, 25 shrubs per sex and 10 shoots or branches per shrub). Note the scales are different for the two graphs.

Table 1. Results of analysis of variance examining the effect of reproductive stage of the branches (reproductive vs vegetative) on log-transformed dry mass of leaves per branch in *Rhamnus alpinus*

	d.f.	MS	F	P
Sex ^a	1	8.82	2.88	0.12
Reproductive stage ^b	1	9.21	6.11	0.03
Sex × reproductive stage ^b	1	2.09	1.39	0.27
Shrub(sex)	8	3.06	2.89	0.01
Reproductive stage × shrub(sex)	8	1.50	1.42	0.21
Error	40	1.05		

^a Shrub(sex) as error term. ^b Reproductive stage × shrub(sex) as error term.

Table 2. Dry mass (mean ± s.e.) and carbon and nitrogen content in the different components of the branch in *Rhamnus alpinus*: leaves, new shoots, old stems and reproductive structures ($n = 250$, 25 shrubs per sex and 10 branches per shrub)

	Males			Females		
	Mass (g)	C (%)	N (%)	Mass (g)	C (%)	N (%)
Reproductive structures						
flowers	0.09 ± 0.003	46.5	4.11	0.05 ± 0.001	46.2	4.44
fruits				0.9 ± 0.04	48.9	1.51
Leaves	1.7 ± 0.1	44.5	2.53	1.9 ± 0.1	43.3	1.54
New shoots	0.6 ± 0.1	46.3	0.99	0.5 ± 0.03	46.1	0.90
Old stems	0.52 ± 0.05	47.5	0.97	0.75 ± 0.06	46.7	0.79

Table 3. Results of analyses of variance examining the effects of sex, locality and individual shrubs in the investment of dry mass per branch considering leaves, new shoot and reproductive structures separately, and in reproductive allocation (RA) and relative growth rate (RGR) in *Rhamnus alpinus*

Source of variation	d.f.	Leaves		New shoot		Reproductive structures		RGR		RA	
		MS	F	MS	F	MS	F	MS	F	MS	F
Locality ^b	4	6.60	6.75**	3.30	9.47**	0.95	5.99**	4.10	7.20**	0.19	2.79*
Sex ^a	1	1.60	2.36	0.62	2.23	309.2	598.2**	1.60	2.64	7.33	356.3**
Locality × sex ^b	4	0.71	0.73	0.28	0.77	0.51	3.23*	0.62	1.09	0.02	0.29
Shrub (locality × sex)	40	0.98	3.43**	0.35	2.69**	0.15	4.20**	0.56	3.48**	0.068	6.32**
Error	445	0.28		0.13		0.03		0.16		0.011	

Note: Data were log-transformed for all dependent variables, except for reproductive allocation, which were arcsine-transformed (* $P < 0.05$; ** $P < 0.01$). ^a Locality × sex as error term. ^b Shrub(locality × sex) as error term.

significantly between the sexes. Variations between different parts of the branch in terms of percentage of carbon content were relatively minor. Both the percentages of carbon and nitrogen were similar in flowers of males and females; however, the proportion of nitrogen was higher in flowers than fruits. Male leaves contained more nitrogen than those of females (Table 2).

Reproductive and vegetative biomasses at branch level were positively correlated ($R^2 = 0.11$, $F_{1,248} = 29.0$, $P < 0.01$ for shoot growth; $R^2 = 0.11$, $F_{1,248} = 28.6$, $P < 0.01$ for relative growth rate). None of the correlation analyses performed for males was significant. Both sexes showed a similar degree of ramification (3.81 ± 0.11 for males and 3.91 ± 0.10 for females; $n = 250$ branches in both cases); however, males showed a higher percentage of reproductive shoots per branch (97.3 ± 0.5 for males vs 85.4 ± 1.1 for females; $n = 250$ branches for each sex).

Bud removal did not affect relative growth rate in either males or females (Table 4), and the significant shrub effect and shrub $\times$ treatment interaction indicated that patterns of relative growth rate after debudding depended on individual shrubs.

Shrub level analysis

Female reproductive investment at shrub level was 7.3 times higher than that of males in terms of biomass and 2.7 times in terms of nitrogen, taking into account mean reproductive biomass per shoot (see above), mean production of new shoots per year (1729 ± 195 for males, 1564 ± 194 for females; $n = 25$ shrubs per sex) and mean percentage of reproductive shoots ($92.7 \pm 1.7\%$ for males, $74.4 \pm 2.7\%$ for females; $n = 25$ shrubs per sex).

The age of females and males used for measuring annual shrub-ring widths did not differ significantly (29.1 ± 1.6 years for males, 29.4 ± 2.4 years for females; $F_{1,48} = 0.009$, $P = 0.9$). Both sexes grew at similar rates until they were 10 years old (1.2 ± 0.1 mm $\cdot$ year $^{-1}$ for males, 1.2 ± 0.2 mm $\cdot$ year $^{-1}$ for females; $n = 28$ and 14 , respectively; $F_{1,40} = 0.08$, $P = 0.7$) (Fig. 2),

Table 4. Results of analysis of variance examining variation in squared-root transformed relative growth rate between non-reproductive branches, branches maturing fruits and branches with removed floral buds (variation among shrubs is also considered)

Source of variation	d.f.	MS	<i>F</i>	<i>P</i>
Males				
Treatment ^a	2	0.051	0.14	0.87
Shrub	4	2.35	2.32	0.07
Treatment $\times$ shrub	8	0.36	0.36	0.93
Error	30	1.009		
Females				
Treatment ^a	2	0.012	0.011	0.98
Shrub	9	3.23	6.48	<0.01
Treatment $\times$ shrub	18	1.09	2.19	0.01
Error	60	0.49		

^a Treatment $\times$ shrub as error term.

but older shrubs showed clear inter-sexual differences, with male growth rate being higher than that of females ($1.2 \pm 0.1 \text{ mm} \cdot \text{year}^{-1}$ for males, $0.9 \pm 0.1 \text{ mm} \cdot \text{year}^{-1}$ for females; $F_{1,40} = 5.07$, $P = 0.02$).

For the whole period 1972–1994, the relationship between mean annual ring width of females and precipitation in the months previous to the growing season (January–April) was marginally significant ($r = 0.41$, $P = 0.053$, $n = 23$ years; correlation values for males non-significant) (Fig. 3). During 1984–1991, precipitation in January–April averaged 199

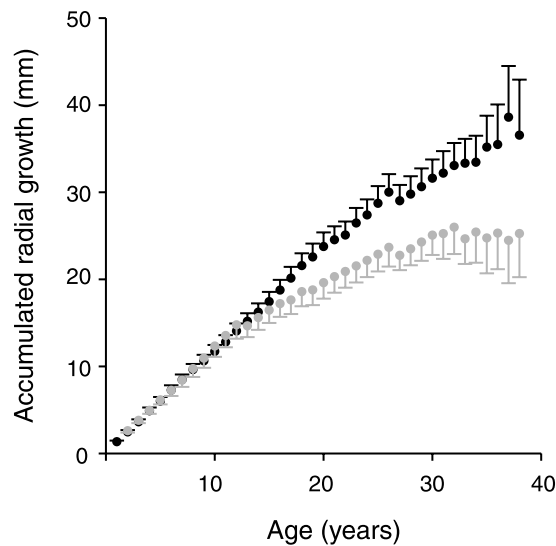


Fig. 2. Accumulated annual ring width (mean $\pm$ 1 s.e.) during the lifetime period for males (black) and females (grey) of *Rhamnus alpinus*. Data non-transformed.

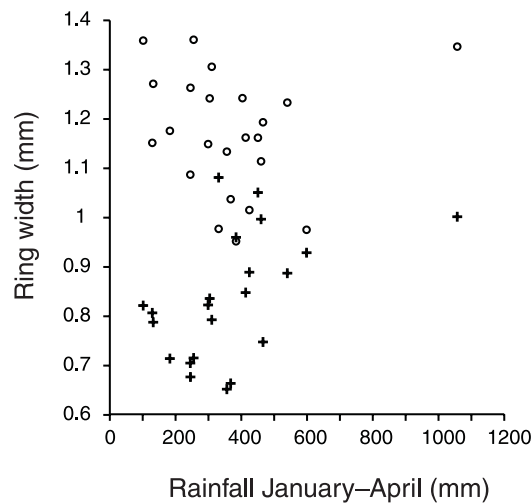


Fig. 3. Ring width (mean $\pm$ 1 s.e.) in male (○) and female (+) shrubs of *Rhamnus alpinus* plotted against mean precipitation (mm) during the months before the growing season (January–April).

mm, and was < 250 mm every year. This is a low precipitation value in the context of the Cantabrian Range, where the mean value for those months during the last 40 years is 525 mm. When only this period was considered in the analysis, the mean ring width of males was greater than that of females ($F_{1,46} = 6.03$, $P = 0.01$; two females were excluded from this analysis since they were too young to cover the whole period).

Population level analysis

Tagged shrubs turned out to be reproductive in 93% and 97% of cases in 1998 and 1999, respectively. Most of the non-reproductive shrubs during 1998 that became reproductive in 1999 were females (73%). Deviations from a 1:1 sex ratio were analysed using G -tests, Yates's correction for continuity, finally obtaining Williams' adjusted G (Fowler *et al.*, 1998). Sex ratio was male-biased in three localities in 1998 and in two localities in 1999 (Table 5). A pooled G -test could not be applied to the data because of heterogeneity among localities ($\Sigma G = 36.2$, $P < 0.01$ in 1998; $\Sigma G = 12.5$, $P < 0.05$ in 1999).

Larger and, therefore, older individuals were more likely to reproduce, since reproductive individuals had a significantly higher basal area ($121 \pm 31 \text{ cm}^2$; $n = 15$ randomly selected) than non-reproductive ones ($42 \pm 9 \text{ cm}^2$; $n = 15$). Individuals that reproduced just one year exhibited an intermediate basal area ($110 \pm 32 \text{ cm}^2$; $n = 15$), but were not significantly different from the other two groups ($F_{2,42} = 4.32$, $P = 0.01$; Tukey test).

Mean basal area was $146 \pm 14 \text{ cm}^2$ for males and $127 \pm 11 \text{ cm}^2$ for females when pooling every trunk of each genet ($n = 126$ shrubs, 18 per sex and population). Mean basal area for the main trunk was $27 \pm 2 \text{ cm}^2$ ($n = 126$) for males and $22 \pm 1 \text{ cm}^2$ ($n = 126$) for females. None of these differences between the sexes was statistically significant (Table 6).

Populations with a larger average main trunk diameter for females had a larger proportion of males (Fig. 4).

DISCUSSION

Resource allocation in *Rhamnus alpinus* was clearly different in males and females. We found evidence that the higher reproductive investment of females entails a 'demographic

Table 5. Number of individuals and sex ratio of *Rhamnus alpinus* in the studied localities (expressed as 1998/1999 when differences between years were found)

Locality	Males	Females	Total reproductive	Total non-reproductive	Sex ratio	Proportion of males	$G (P)$
PU	62	38	100	0	1.63	0.62	5.79*
LL	55	44	99	1	1.25	0.55	1.21
VL	50/57	20/38	70/95	30/5	2.5/1.5	0.71/0.6	13.19**/3.80
PT	58	42	100	0	1.38	0.58	2.57
PM	54	37/38	91/92	9/8	1.45/1.42	0.59	3.18/3.15
SE	56	38	94	6	1.47	0.59	1.72
TX	64	35	99	1	1.82	0.64	8.57**
Total	399/406	254/273	653/679	47/21	1.57/1.48	0.61/0.59	

* $P < 0.05$; ** $P < 0.01$.

Table 6. Results of analyses of variance examining the effects of sex (fixed factor) and locality (random factor) on the total basal area, number of trunks and main trunk basal area in *R. alpinus* (126 males, 126 females)

	d.f.	Main trunk basal area		Total basal area		Number of trunks	
		MS	F	MS	F	MS	F
Locality	6	624	1.57	11645	4.92**	558	7.11**
Sex ^a	1	2345	3.82	6721	2.12	2.29	0.02
Locality × sex	6	614	1.54	3165	1.34	10.5	1.41
Error	238	398		2367		78.5	

Note: Data were squared-root transformed. ** $P < 0.01$.

^a Locality × sex as error term.

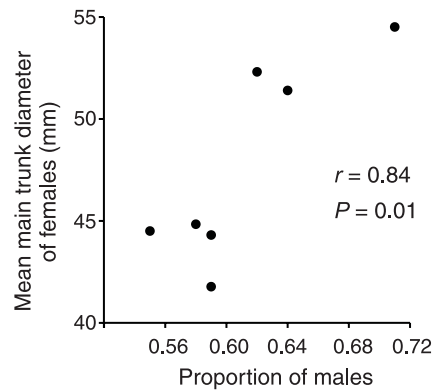


Fig. 4. Relationship between mean main trunk diameter of *Rhamnus alpinus* and the proportion of males at each locality. Data non-transformed. Correlation analysis was performed after logarithmic transformation.

cost', which can be detected at the shrub and population level after a number of reproductive years. These costs were not found at the shoot or branch level, showing that the extent of autonomy and integration of the units within the entire shrub determines our ability to detect the costs of reproduction at lower levels.

Shoot and branch level analysis: no evidence for reproductive costs

In line with what may be considered a general pattern in woody dioecious plants (Wheelwright and Bruneau, 1992; Nicotra, 1999), males allocated more biomass to flowering than females. However, when fruit production was taken into account, females showed a higher overall reproductive investment, without any detrimental effect on shoot growth. The apparent much greater investment in reproductive mass in females at the shoot and branch level receded when the whole shrub was considered, even more so when nitrogen was considered as the currency of allocation.

It is generally accepted that the most appropriate currency to assess reproductive allocation is the one that is limiting in the habitat (Reekie and Bazzaz, 1987; Ashman, 1994).

We assume that nitrogen is generally more limiting than carbon in the study sites, since stony calcareous soils are usually nutrient-poor, whereas photosynthesis is not light-limited in these open areas. In our analyses, carbon and nitrogen did not vary in parallel; therefore, biomass alone cannot be considered a reliable measure of reproductive allocation (Goldman and Willson, 1986). Although carbon content is more constant both in space (reproductive structures, leaves, stem) and time (flowers *vs* fruits), the relative nitrogen content of reproductive structures showed a declining trend throughout the season (from flowers to fruits), as found in previous studies (see McDowell *et al.*, 2000, and references therein). However, the total nitrogen content was higher in fruits than in flowers due to the higher fruit mass. The fact that male leaves had a greater nitrogen content than female leaves might be due to the need for nitrogen allocation to nearby fruits in female shoots. In this sense, McDowell *et al.* (2000) found that foliage near female cones in Douglas fir had a consistently lower nitrogen content than foliage far from female cones.

Neither shoot growth nor relative growth rate was significantly affected by flower or fruit production in the previous year. We failed to detect any significant trade-off, and there was even a weak positive relationship between fruit production and relative growth rate in females. Moreover, fruit production was positively correlated at branch level during both study years. These cases of apparent negative cost of reproduction occur mainly in correlational studies and are usually attributed to a high variance in resource availability. If there is a high variance between shrubs in resource availability, a positive correlation can be found when a trade-off is expected, independently of the relationship between branches (van Noordwijk and de Jong, 1986; see Fig. 5a). In our study, individual shrubs overlapped in their amount of vegetative and reproductive biomass per branch (Fig. 5b), suggesting only small differences among shrubs in resource availability. The positive relationship between reproduction and growth is maintained even within a shrub (Fig. 5b). If branches occupying the best positions grow larger and produce more flowers and fruits than branches in less profitable positions, then a positive correlation between fruit production and growth could be generated within the shrub (Reznick, 1985; Partridge, 1989). Resources may be preferentially allocated to branches in more favourable positions, promoting tree growth towards these positions and optimizing the use of available resources. In contrast, branches in less favourable positions (e.g. in shade) may have both reduced growth and reproductive output (Henriksson, 2001). In the present study, although the whole shrub had to be considered to assess costs derived from reproduction, the branch seems to be the modular unit in relation to resource variation, and the trade-offs might be hidden by this non-controlled resource variation within the shrub.

Experimental reduction of current reproductive effort showed no increased branch relative growth rate, suggesting that growth and reproduction might not compete for resources at the branch level. However, the responses depended on individual shrubs, which might be attributed to differences in nutrient balance between individuals. Most previous studies of the experimental reduction of reproductive effort found significant effects on growth or reproduction either in the current season or in the following season (Fox and Stevens, 1991; Cipollini and Whigham, 1994). In this sense, although we did not test for the effects of the experimental reduction of reproductive effect on subsequent reproduction, observational data showed that fruit production in 1998 had no effect on relative growth rate or on branch ramification during 1999. Therefore, no delayed costs of reproduction were found in the short term at shoot or branch level.

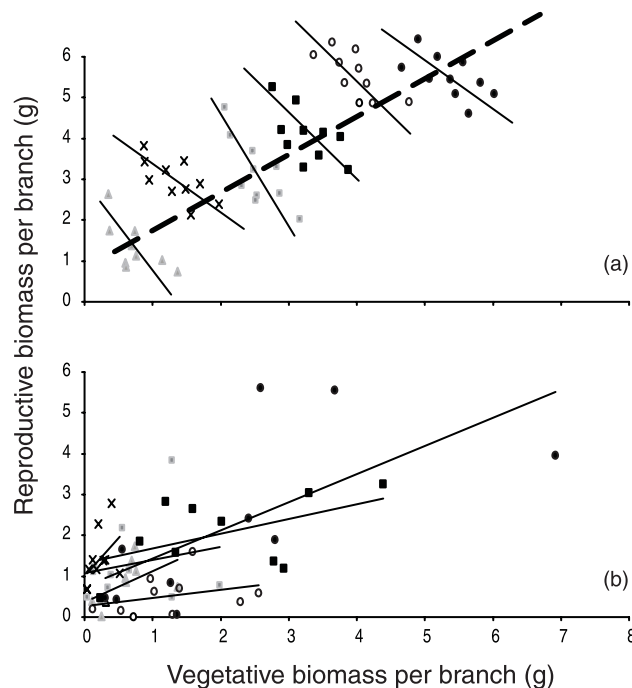


Fig. 5. (a) Theoretical positive spurious relationship between vegetative and reproductive biomass of branches when a trade-off is expected (dotted line), as predicted by van Noordwijk and de Jong (1986). Continuous lines represent the relationship within each shrub; differences between shrubs hide the existing trade-off. (b) Real data for *Rhamnus alpinus*; the positive relationship is maintained even within the shrubs. In both cases, different symbols indicate different shrubs, and each point represents a branch.

Shrub and population level analyses: evidence for demographic costs

The annual growth rates of shrubs were not significantly different for males and females. However, when considering the lifetime of the plant, the curve for females diverted at a certain point, indicating a lower growth rate for females (Fig. 2). Our field observations indicate that most shrubs with a main trunk diameter less than 2 cm are non-reproductive, and there may be a connection between the beginning of reproduction and this change in growth rate.

Main trunk ring-width was clearly greater for males during relatively xeric reproductive seasons. The better performance of the males in these stressful conditions may be due to more conservative water-use patterns by males, which seems to be a general pattern in the few studies considering the effect of water limitation in both sexes (Dawson and Ehleringer, 1993; Retuerto *et al.*, 2000; but see Crawford and Balfour, 1983). Females are filling the fruits during periods of greatest potential drought (July–August) and two processes are occurring at this time. First, an important amount of water is allocated to the fleshy pulp of the fruits. Second, fruits are sinks of photosynthate, which helps to keep the stomata open for gas interchange and subsequent water loss.

A consequence of the inter-sexual differences in growth patterns related to stressful

conditions could be that reproductive costs might be more noticeable during dry years, so there might be a delay in the timing of the appearance of the costs of reproduction, which is more difficult to detect in younger shrubs. On the other hand, the Cantabrian Range has an oceanic climate, according to Köppen criteria (Critchfield, 1983), characterized by a regular precipitation regime, without an acute summer drought. In this environment, unpredictable drier periods will have a stronger effect than in more predictable, less humid areas. Considering that *R. alpinus* shrubs live on gravel and rocky limestone-derived soils that are particularly prone to water stress, differences in growth attributable to water limitation between the sexes could be relatively frequent.

We found a higher number of males in all five populations, the difference being significant in two of them. Male-biased sex ratios might be attributable to: (1) a male-biased embryo sex ratio (Marion and Houle, 1996); (2) higher shrub mortality among females; (3) greater vegetative multiplication by males (Obeso, 1998); and (4) male reproduction starting earlier in life and being more frequent than in females (Nicotra, 1998). We have no information about sex ratio in the embryos or non-reproductive shrubs, but there was no bias due to clonal reproduction because the studied individuals were widely spaced and no shrubs with both male and female individuals were observed. If there is a higher mortality rate among females because of the cumulative effects of the costs of reproduction, older populations of reproductive shrubs should exhibit a male-biased sex ratio, whereas younger populations should not. In this study, populations with a larger mean main trunk diameter of females also showed a larger proportion of males. Since main trunk diameter reflects the age of the shrubs and the number of reproductive episodes, we propose that the observed male biases are a consequence of the higher reproductive costs of females.

CONCLUSIONS

Three main conclusions may be drawn from this study. First, that the trade-offs related to the cost of reproduction are extremely variable at the individual and population level. Second, that studies of the cost of reproduction in woody species should consider not only all levels of hierarchical organization within the plant, but also the degree of integration between them (i.e. branch autonomy vs branch independence). The trade-offs expected from the hypothesis of the cost of reproduction may be hidden within the growth season when the branches are only semi-autonomous for fruit production and growth. And, third, that the lifetime costs of reproduction in dioecious species can be translated into demographic costs of reproduction and subsequent effects on size distributions and sex ratios. To the best of our knowledge, this is the first time that effects of reproductive costs have been shown at the population level.

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REFERENCES

- Allen, G.A. and Antos, J.A. 1993. Sex ratio variation in the dioecious shrub *Oemleria cerasiformis*. *Am. Nat.*, **141**: 537–553.
- Ashman, T.L. 1994. A dynamic perspective on the physiological cost of reproduction in plants. *Am. Nat.*, **144**: 300–316.
- Bailey, R.C. 1992. Why we should stop trying to measure the cost of reproduction correctly. *Oikos*, **65**: 349–352.
- Bañuelos, M.J. 2001. Ecología del crecimiento y de la reproducción en el arbusto dioico *Rhamnus alpinus* L. PhD dissertation, Departamento de Biología de Organismos y Sistemas, Universidad de Oviedo, Asturias, Spain.
- Bañuelos, M.J. and Obeso, J.R. 2003. Maternal provisioning, sibling rivalry and seed mass variability in the dioecious shrub *Rhamnus alpinus*. *Evol. Ecol.*, **17**: 19–31.
- Cipollini, M.L. and Whigham, D.F. 1994. Sexual dimorphism and cost of reproduction in the dioecious shrub *Lindera benzoin* (Lauraceae). *Am. J. Bot.*, **81**: 65–75.
- Cody, M.L. 1966. A general theory of clutch size. *Evolution*, **20**: 174–184.
- Crawford, R.M.M. and Balfour, J. 1983. Female predominant sex ratios and physiological differentiation in arctic willows. *J. Ecol.*, **71**: 149–160.
- Critchfield, H.J. 1983. *General Climatology*, 4th edn. Englewood Cliffs, NJ: Prentice-Hall.
- Dawson, T.E. and Ehleringer, J.R. 1993. Gender-specific physiology, carbon isotope discrimination, and habitat distribution in boxelder, *Acer negundo*. *Ecology*, **74**: 798–815.
- Delph, L.F. 1999. Sexual dimorphism in live history. In *Gender and Sexual Dimorphism in Flowering Plants* (M.A. Geber, T.E. Dawson and L.F. Delph, eds), pp. 149–173. Berlin: Springer-Verlag.
- Delph, L.F. and Meagher, T.R. 1995. Sexual dimorphism masks life history trade-offs in the dioecious plant *Silene latifolia*. *Ecology*, **76**: 775–785.
- Dudash, M.R. and Fenster, C. 1997. Multiyear study of pollen limitation and cost of reproduction in the iteroparous *Silene virginica*. *Ecology*, **78**: 484–493.
- Ehrlén, J. and Groenendaal, J.V. 2001. Storage and the delayed costs of reproduction in the understorey perennial *Lathyrus vernus*. *J. Ecol.*, **89**: 237–246.
- Fowler, J., Cohen, L. and Jarvis, P. 1998. *Practical Statistics for Field Biology*. Chichester: Wiley.
- Fox, J.F. 1995. Shoot demographic responses to manipulation of reproductive effort by bud removal in a willow. *Oikos*, **72**: 283–287.
- Fox, J.F. and Stevens, G.C. 1991. Costs of reproduction in a willow: experimental responses vs. natural variation. *Ecology*, **72**: 1013–1023.
- Goldman, D.A. and Willson, M.F. 1986. Sex allocation in functionally hermaphroditic plants: a review and a critique. *Bot. Rev.*, **52**: 157–194.
- Henriksson, J. 2001. Differential shading of branches or whole trees: survival, growth, and reproduction. *Oecologia*, **126**: 482–486.
- Houle, G. 2001. Reproductive costs are associated with both the male and female functions in *Alnus viridis* ssp. *crispa*. *Ecoscience*, **8**: 220–229.
- Jönsson, K.I. and Tuomi, J. 1994. Costs of reproduction in a historical perspective. *Trends Ecol. Evol.*, **9**: 304–306.
- Krusic, P.R., Homes, R.L. and King, J.C. 1996. MEDIR version 1.13 measurement program. In *The International Tree-Ring Data Bank Program Library, Version 2.0 User's Manual* (H.D. Grissino-Mayer, R.L. Holmes and H.C. Fritts, eds), pp. 18–20. Tucson, AZ: Laboratory of Tree-Ring Research, University of Arizona.
- Lovett-Doust, J. and Lovett-Doust, L. 1988. Modules of production and reproduction in a dioecious clonal shrub, *Rhus typhina*. *Ecology*, **69**: 741–750.
- Marion, C. and Houle, G. 1996. No differential consequences of reproduction according to sex in *Juniperus communis* var. *depressa* (Cupressaceae). *Am. J. Bot.*, **83**: 480–488.

- Marquis, R.J. 1992. A bite is a bite is a bite? Constraints on response to folivory in *Piper arieianum* (Piperaceae). *Ecology*, **73**: 143–152.
- McDowell, S.C.L., McDowell, N.G., Marshall, J.D. and Hultine, K. 2000. Carbon and nitrogen allocation to male and female reproduction in Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *galuca*, Pinaceae). *Am. J. Bot.*, **87**: 539–546.
- Newell, E.A. 1991. Direct and delayed costs of reproduction in *Aesculus californica*. *J. Ecol.*, **79**: 365–378.
- Nicotra, A.B. 1998. Sex ratio variation and spatial distribution of *Siparuna grandiflora*, a tropical dioecious shrub. *Oecologia*, **115**: 102–113.
- Nicotra, A.B. 1999. Reproductive allocation and the long-term costs of reproduction in *Siparuna grandiflora*, a dioecious neotropical shrub. *J. Ecol.*, **87**: 138–149.
- Obeso, J.R. 1997. Cost of reproduction in *Ilex aquifolium*: effects at tree, branch and leaf levels. *J. Ecol.*, **85**: 159–166.
- Obeso, J.R. 1998. Effects of defoliation and girdling on fruit production in *Ilex aquifolium*. *Funct. Ecol.*, **12**: 486–491.
- Obeso, J.R. 2002. The costs of reproduction in plants. *New Phytol.*, **155**: 321–348.
- Obeso, J.R. and Grubb, P.J. 1993. Fruit maturation in the shrub *Ligustrum vulgare* (Oleaceae): lack of defoliation effects. *Oikos*, **68**: 309–316.
- Obeso, J.R., Álvarez-Santullano, M. and Retuerto, R. 1998. Sex-ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (Aquifoliaceae). *Am. J. Bot.*, **85**: 1602–1608.
- Partridge, L. 1989. An experimentalist's approach to the role of costs of reproduction in the evolution of life-histories. In *Toward a More Exact Ecology* (P.J. Grubb and J.B. Whittaker, eds), pp. 231–246. Oxford: Blackwell Scientific.
- Ramsey, M. 1997. No evidence for demographic costs of seed production in the pollen-limited perennial herb *Blandfordia grandiflora* (Liliaceae). *Int. J. Plant Sci.*, **158**: 785–793.
- Reekie, E.G. and Bazzaz, F.A. 1987. Reproductive effort in plants. 2. Does carbon reflect the allocation of other resources? *Am. Nat.*, **129**: 897–906.
- Retuerto, R., Fernández-Lema, B., Rodríguez-Roiloa, S. and Obeso, J.R. 2000. Gender, light and water effects in carbon isotope discrimination, and growth rates in the dioecious tree *Ilex aquifolium*. *Funct. Ecol.*, **14**: 529–537.
- Reznick, D. 1985. Cost of reproduction: an evaluation of the empirical evidence. *Oikos*, **44**: 257–267.
- Reznick, D. 1992. Measuring the costs of reproduction. *Trends Ecol. Evol.*, **7**: 42–49.
- Ruohomäki, K., Haukioja, E., Repka, S. and Lehtilä, K. 1997. Leaf value: effects of damage to individual leaves on growth and reproduction of mountain birch shoots. *Ecology*, **78**: 2105–2117.
- Thomas, S.C. and LaFrankie, J.V. 1993. Sex, size, and interyear variation in flowering among dioecious trees of the Malayan rain forest. *Ecology*, **74**: 1529–1537.
- Trueman, S.J. and Turnbull, C.G.N. 1994. Fruit set, abscission and dry matter accumulation on girdled branches of *Macadamia*. *Ann. Bot.*, **74**: 667–674.
- Tuomi, J., Hakala, T. and Haukioja, E. 1983. Alternative concepts of reproductive effort, cost of reproduction, and selection in life-history evolution. *Am. Zool.*, **23**: 25–34.
- Tutin, T.G., Heywood, V.H., Burges, N.A. et al. 1968. *Flora Europaea*, Vol 2. Cambridge: Cambridge University Press.
- van Noordwijk, A.J. and de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.*, **128**: 137–142.
- Watson, M.A. and Casper, B.B. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. *Annu. Rev. Ecol. Syst.*, **15**: 233–258.
- Wheelwright, N.T. and Bruneau, A. 1992. Population sex ratios and spatial distribution of *Ocotea tenera* (Lauraceae) trees in a tropical forest. *J. Ecol.*, **80**: 425–432.
- Williams, G.C. 1966. Natural selection, the costs of reproduction, and the refinement of Lack's principle. *Am. Nat.*, **100**: 687–690.

