

Sébastien Lavergne · Max Debussche  
John D. Thompson

## Limitations on reproductive success in endemic *Aquilegia viscosa* (Ranunculaceae) relative to its widespread congener *Aquilegia vulgaris*: the interplay of herbivory and pollination

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**Abstract** Plant reproduction can be strongly affected by herbivory and different features of pollination ecology, such as pollinator visitation rates and capacity for self-pollination. The purpose of this study is to compare the relative impact of herbivory and pollination on maternal reproductive success in endemic *Aquilegia viscosa* and its widespread congener *Aquilegia vulgaris*. We conducted herbivore exclusion experiments in two populations of each species in 2 different years and showed that the maternal fertility of *A. viscosa* was significantly more limited by floral predation and pre-dispersal seed predation than its widespread congener. In the absence of herbivory, *A. viscosa* retained significantly lower maternal fertility than *A. vulgaris*. Experimental pollinations in an insect-free glasshouse showed that the two species have an equal seed/ovule ratio both in the absence of pollinators and in the presence of non-limiting outcross pollination. Pollinator visitation rates were significantly higher in populations of *A. vulgaris* than in populations of *A. viscosa*. In addition, path analyses showed that spur length, an important trait for pollinator attraction in *Aquilegia*, and, indirectly sepal and petal width, contribute positively to the seed/ovule ratio in *A. vulgaris*, but not in *A. viscosa*. These results indicate that maternal fertility of endemic *A. viscosa* is strongly reduced by flower and seed predation despite low rates of pollinator visitation, and that pollen or resource limitation in the wild may further reduce maternal fertility. Finally, floral trait variation appears to be decoupled from fertility variation in endemic *A. viscosa*, which possibly constrains the evolution of reproductive traits in this species.

**Keywords** Endemism · Reproductive success · Pollination · Herbivory · *Ranunculaceae*

### Introduction

Why many species have restricted endemic distributions whilst their close relatives are more widely distributed has long fascinated plant ecologists, biogeographers and population geneticists. Such interspecific variation in range size can in many cases be clearly associated with geographical barriers to dispersal, and may in part be due to the biological traits and ecological requirements of particular species (Cowling et al. 1994; Desmet and Cowling 1999; Médail and Verlaque 1997; Hedge and Ellstrand 1999; Lavergne et al. 2003, 2004). A trait that may be of primary importance in regulating the abundance and distribution of a plant species is its capacity for seed production in nature (Byers and Meagher 1997; Eriksson and Jakobsson 1998). Indeed, in a recent comparative study of restricted endemic and widespread species in the western Mediterranean, we have found that restricted endemic species generally have lower maternal fertility than their widespread congeners (Lavergne et al. 2004). However, there are few comparative data on the ecological factors that may limit the reproductive success of rare or endemic plants relative to their more common or widespread relatives (Murray et al. 2002).

Plant reproductive success and fitness show considerable variation within and among natural populations in response to diverse ecological (Herrera et al. 2002) and genetic (Thompson et al. 2004) causes. In animal-pollinated flowering plants, ecological factors can exert a strong influence on reproductive success. First, floral and seed predators and herbivores can dramatically limit seed production (Ayre and Whelan 1989; Escarré et al. 1999), seedling survival and recruitment (Louda 1982), and ultimately population growth (Ehrlén 1996). Second, variation in insect pollinator activity, and thus in

S. Lavergne (✉) · M. Debussche · J. D. Thompson  
Centre d'Ecologie Fonctionnelle et Evolutive,  
CNRS, UMR 5175, 1919 route de Mende,  
34293 Montpellier cedex 5, France  
E-mail: sebastien.lavergne@uvm.edu

*Present address:* S. Lavergne  
Department of Botany and Agricultural Biochemistry,  
University of Vermont, 233 Marsh Life Sciences Building,  
109 Carrigan Drive, Burlington, VT 05405, USA

rates of pollen transfer, can also strongly limit the number and quality of seeds produced (Sih and Baltus 1987; Burd 1994; Agren 1996; Charpentier et al. 2000) although the effects of such pollinator limitation may be highly variable in space and time (Baker et al. 2000).

An important point to recognise here is that pollination and herbivory can have non-additive effects on reproductive success. Factorial experiments manipulating the presence of pollinators and herbivores on *Paeonia broteroi* (Herrera 2000) and *Helleborus foetidus* (Herrera et al. 2002), have shown an interactive effect of these two factors, due to the fact that the negative impacts of herbivory were observed only where pollinators were present. As a result, the strong impact of herbivory on reproductive success may weaken pollinator-mediated selection on reproductive traits (Levri and Real 1998). Herbivory may also strongly affect the attractiveness of plants to pollinators, as reported in *Raphanus raphanistrum* (Strauss et al. 1996). To understand the precise nature of limitations on reproductive success, it is thus essential to jointly evaluate the respective roles of herbivory and pollination (Strauss and Armbruster 1997; Herrera et al. 2002). However, most studies of rare plant species have focused on either herbivory (Bevill et al. 1999; Lord and Kelly 1999; Fletcher et al. 2001; Simon et al. 2001) or pollination (Robertson et al. 1998; Kéry et al. 2000) and few studies have compared the relative impact of these two factors on maternal reproductive success of closely related rare and widespread species (Gaston and Kunin 1997; Kunin 1997).

The objective of this study is to quantify the extent to which herbivory and pollination limit maternal reproductive success in the narrow endemic *Aquilegia viscosa* Gouan (Ranunculaceae) relative to its widespread congener *Aquilegia vulgaris* L., in southern France. First, we conducted a herbivore exclusion experiment in two natural populations of each species over 2 years in order to assess the impacts of flower parasitism and pre-dispersal seed predation on maternal fertility in the two species. In parallel, we compared seed production by intact (un-predated) flowers of each species in natural conditions. Second, we assessed the capacity of each species to produce seeds in the absence of pollinators by comparing fruit and seed set in unmanipulated and outcrossed flowers in an insect-free glasshouse. Third, we quantified pollinator visitation rates in the same two natural populations of each species. Fourth, we quantified any correlated variation in floral trait and maternal fertility in each species to assess the potential for selection by pollinators on floral traits.

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## Materials and methods

### Study species and sites

We studied two species of *Aquilegia* with contrasting distribution patterns. *A. viscosa* is endemic to the

Languedoc-Roussillon region of southern France and north-eastern Spain, where it occurs on limestone cliffs and screes from 500 to 1,500 m elevation, and flowers from June to early July. This species is protected by law in France and Spain (Chauvet 1989; Saez et al. 1998). In contrast, *A. vulgaris* is widely distributed throughout Eurasia and occurs locally in North Africa. This species occurs in larger and denser populations, in meadows, along water courses and in open woodlands up to 1,200 m elevation, and flowers from May to July. Both species are polycarpic herbaceous perennials, are self-compatible and highly inter-fertile (S. Lavergne, unpublished data). Both species bear nodding and long spurred flowers, whose colour ranges from pale blue to violet. The fruit is composed of four to five free and erect carpels.

We studied two populations of each species: “Tesson” (isolated from other sites by ~10 km) and “Séranne” (one population in a single massif) for *A. viscosa* (~50 and 70 flowering individuals, respectively), and “Ganges” and “Bez” for *A. vulgaris* (~120 and 150 flowering individuals respectively). The populations of *A. vulgaris* are part of a network of many populations along the banks of watercourses and in moist open areas in the region. The four populations are located in southern France, ~50 km north of Montpellier, in a roughly 15×15 km area (centred on 43°55'N, 3°35'E) with same Mediterranean meso-climate.

### Herbivore exclusion

We conducted herbivore exclusion experiments in each population from April to July 2000 and 2001. In all sites, the guild of herbivores observed on the two *Aquilegia* species included insects feeding on flowers and leaves, several *Diptera* and *Lepidoptera* which lay eggs in floral buds, resulting in their abortion or in abnormal flower development, and *Curculionidae* feeding on flower buds, developed flowers and seeds prior to fruit maturation. Herbivory thus has direct effects on two components of female fertility: fruit/flower ratio and the number of viable seeds/carpel. Such herbivory could also reduce the attractiveness of flowers to pollinators. Some herbivory also occurs on the vegetative parts of the plants, which may also impact on female fertility, although such herbivory probably has a much lower impact on maternal fertility than direct parasitism and predation of flowers and developing seeds in the study species.

In each year of study, eight pairs of reproductive individuals of roughly the same vegetative size and flower number were selected at random in each population. For each pair, one individual was sprayed every 10 days with a systemic carbamate insecticide containing methomyl (Lamate). The other individual was used as a control and was sprayed at the same time with an equal quantity of water. On each studied individual, we

counted the flower number at the first visit (including flower buds larger than 1 cm). During the rest of the experiment, each flower producing a normally developed fruit was recorded, to calculate the fruit/flower ratio. At the end of fruit maturation, three to five ripe fruits were randomly harvested on each individual to count the seed number per carpel. For each individual, total female fertility was quantified as the number of initiated fruits  $\times$  mean seed number per fruit. The individuals studied during the 2nd year were chosen independently from those studied during the 1st year. The insecticide used here has already been shown to have no significant effect on plant growth and reproduction (Escarré et al. 1999). Hence, the difference in fertility between protected and control plants was not due to a direct effect of insecticide on performance.

### Morphology and fertility of intact flowers

During the 2001 flowering season, 30–50 individuals in each studied population (including the individuals protected with insecticide) were used to study the natural variation in reproductive success (fruit/flower and seed/ovule ratios) and to quantify the relationships between floral display and reproductive success. On each individual, stem height was measured, flower number was counted, and one to five intact and normally developed flowers were selected at random. For a total of 222 flowers, we noted their position on the inflorescence and measured flower width (between sepal tips), corolla diameter, spur length, stigma–anther distance and pistil length (to 0.1 mm) using digital calipers. During subsequent visits, if the same flowers produced a normally developed fruit and remained intact, ripe and closed fruits were harvested from the same flowers (one or two fruits per individual). Since viable seeds can be easily distinguished from unfertilised ovules, seeds and unfertilised ovules were counted in each carpel to calculate the seed/ovule ratio. On the subset of individuals protected with insecticide in the herbivory experiment, each flower was labelled and the normal development of fruit recorded in order to estimate the fruit/flower ratio. Thus, fruit/flower ratio was estimated on a total of 31 individuals and seed/ovule ratio for a total of 169 fruits.

On 15 individuals in each population of each species, we collected one flower bud to estimate the pollen/ovule ratio. The number of pollen grains produced per anther was estimated in five anthers per bud, and mean pollen grain number produced per anther was multiplied by the total number of anthers counted in each bud (40–50 anthers). The five anthers were left to dehisce in an Eppendorf tube and pollen was directly counted in a glycerine and saccharose solution (following Affre et al. 1995) on a Mallassez cell with an Olympus light microscope. We counted the number of ovules per flower in the same bud used for the pollen count under a dissecting microscope.

### Pollinator activity

In 2001, we observed pollinator activity during peak flowering in each population in order to identify pollinators and to quantify their visitation frequency. Periodic observations were conducted over 1–2 h in sunny and non-windy conditions from 10 a.m. to 2 p.m. to give a total of 7–10 h of observations per population. We recorded the number of pollinators visiting at least one flower in an observed patch of plants. We could not follow the foraging behaviour of each pollinator between flowers and plants because of the steep slope and rocky nature of *A. viscosa* habitats.

### Experimental pollinations

In 2000, we performed experimental pollinations in an insect-free glasshouse located in the CEFÉ-CNRS experimental gardens in Montpellier, using plants transplanted in 1999 from the Ganges population of *A. vulgaris* (ten individuals) and from the Tessone population of *A. viscosa* (nine individuals). For each plant, flowers were randomly assigned to each of three treatments: spontaneous self-fertilisation (un-manipulated flower), hand self-pollination (using pollen from a different flower on the same individual), and hand cross-pollination (emasculated flowers pollinated with pollen from a different individual). Fruits were collected just before dehiscence in order to count viable seeds and unfertilised ovules in each carpel.

### Statistical analyses

The effects of herbivory on maternal fertility were analysed separately for each species and each year of study. We used PROC GENMOD (SAS 1999) to fit generalised linear models of variance on fruit/flower ratio, seed number per carpel, and individual fecundity. The fruit/flower ratio was analysed as a binomial variable and using a logit link function, whereas the seed number per carpel and the individual fecundity were analysed with a Poisson error and a logarithm link function. Population, insecticide treatment and their interaction were specified as fixed effects in the models. We also performed contrast analyses using the CONTRAST option to test for significant differences in fruit/flower ratio, seed number per carpel, and total seed output between protected and control individuals in each population and year.

To analyse variation in fruit/flower and seed/ovule ratios under natural conditions and in experimental pollinations, we performed generalised linear models with a binomial error and a logit link function using PROC GENMOD (SAS 1999). For data obtained under natural conditions, fruit/flower and seed/ovule ratios were analysed with the following effects: species, population nested within species, flower position, and flower position  $\times$  species. For the results of experimental pollinations, we specified the following effects: species,

treatment and species  $\times$  treatment. We also performed contrast analyses in PROC GENMOD using the CONTRAST option to test for significant differences in fruit/flower and seed/ovule ratios (1) between populations and between species under natural conditions, and (2) between different pollination treatments within and between species in glasshouse experiments.

We analysed the relation between floral traits and seed/ovule ratio by performing a path analysis. Seed/ovule ratio was normalised by arcsine square root transformation. For each species, the effects of each floral trait on seed/ovule ratio was estimated as the standardised partial regression coefficient, fitted by a multiple regression model, using the STB option in PROC REG (SAS 1999). Relationships between floral traits were quantified with a Pearson product-moment correlation in PROC CORR (SAS 1999).

To analyse the variation in frequency of pollinator visits, pollen/ovule ratio, flower number, flower width at sepal tips, corolla diameter, spur length, stigma-anther distance and pistil length between species and populations of both species, we performed non parametric analyses. We performed Kruskal-Wallis ANOVA and Dunn's pairwise comparisons to test for differences between species and populations using Statistix 2000 (Analytical Software, Tallahassee, Fla.). We also calculated the coefficient of variation of each reproductive trait within each of the study populations.

## Results

### Herbivore exclusion

During the 2 years of study, no mortality occurred on the studied individuals. In *A. vulgaris*, neither insecticide treatment nor the interaction between insecticide treatment and population had significant effects on fruit/flower ratio, seed number per carpel and fecundity,

except for an effect on fruit/flower ratio in 2000 (Table 1, Fig. 1), due to protected individuals having a significantly higher fruit/flower ratio than control individuals in the Ganges population (Fig. 1a). In *A. viscosa*, protected plants consistently had a higher maternal fertility than plants exposed to herbivory in both sites in both years, except in the Séranne population in 2001 (Fig. 1c). In this species, insecticide treatment had a significant effect on fruit/flower ratio and fecundity during both years of study, except for seed number per carpel in 2001 (Table 1). The effect of insecticide on fruit/flower ratio in 2000 and on seed number per carpel in 2001 differed significantly between populations of *A. viscosa* (Table 1, Fig. 1a, b).

### Morphology and fertility of intact flowers

Fruit/flower ratio in intact flowers varied significantly ( $F_{2,27} = 7.75$ ,  $P < 0.01$ ) between populations within species (Fig 2a) but not among species ( $F_{1,27} = 3.13$ ,  $P > 0.05$ ) or in relation to flower position ( $F_{2,27} = 0.85$ ,  $P > 0.05$ ), and there was no flower position by species interaction effect ( $F_{2,27} = 0.56$ ,  $P > 0.05$ ). Seed/ovule ratio in intact flowers varied significantly between species ( $F_{1,161} = 6.54$ ,  $P < 0.05$ ), populations within species ( $F_{2,161} = 3.13$ ,  $P < 0.05$ ), and flower position ( $F_{2,161} = 5.02$ ,  $P < 0.01$ ), with a significant interaction between flower position and species ( $F_{2,161} = 5.50$ ,  $P < 0.01$ ). *A. vulgaris* had a significantly higher mean seed/ovule ratio than *A. viscosa* in the absence of herbivory (Fig. 2b). The significant interaction between species and flower position was due to a decline in seed/ovule ratio with flower position in *A. vulgaris* but not in *A. viscosa*. Seed/ovule ratio differed significantly between populations in *A. vulgaris* but not in *A. viscosa* (Fig. 2b).

Most reproductive traits varied significantly between species (Table 2). Individuals of *A. viscosa* were smaller, and displayed significantly fewer flowers, with lower

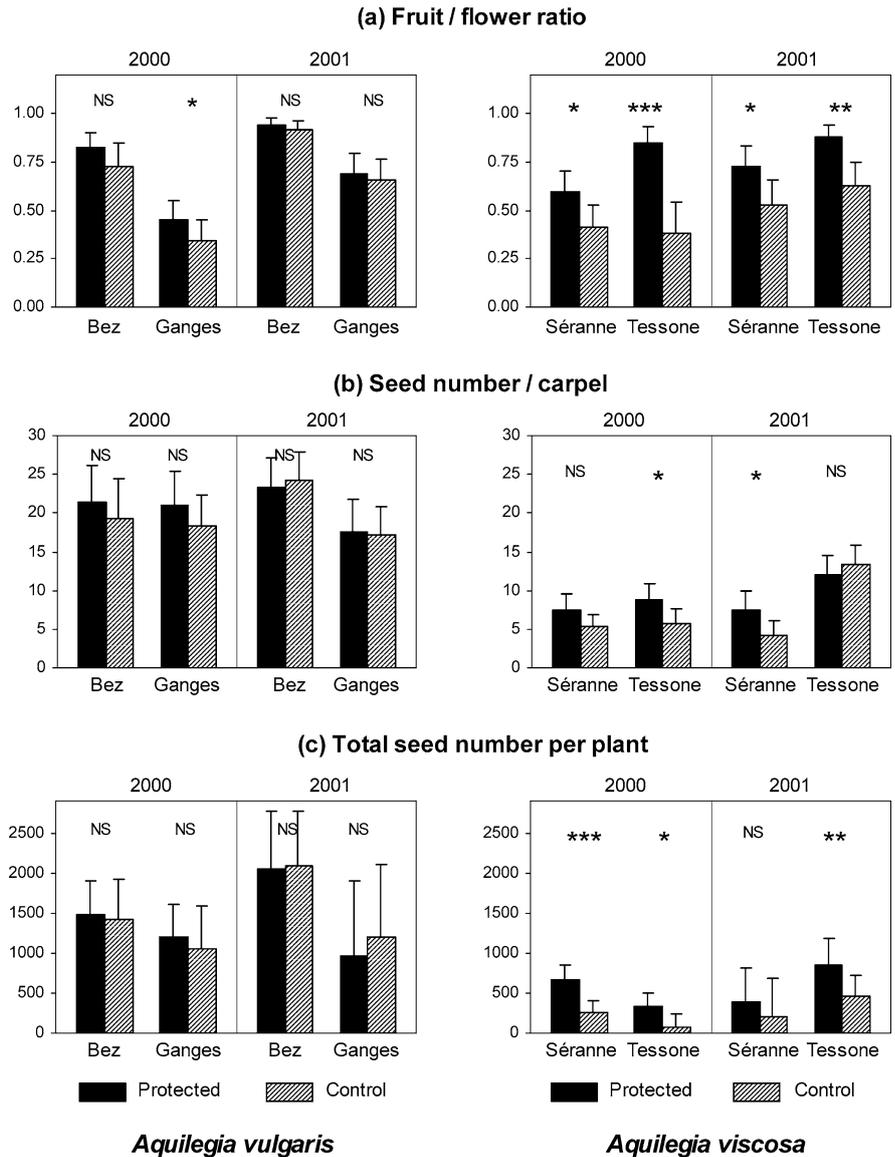
**Table 1** *F*-Values and degrees of freedom (from generalised linear models) associated with variation in the components of maternal fertility among populations and in relation to insecticide treatment during 2 years of study on two species of *Aquilegia*

	<i>Aquilegia vulgaris</i>		<i>Aquilegia viscosa</i>	
	2000	2001	2000	2001
Fruit/flower ratio				
Population	41.81 <sub>1,28</sub> ***	28.26 <sub>1,28</sub> ***	3.68 <sub>1,20</sub> NS <sup>a</sup>	1.47 <sub>1,26</sub> NS
Insecticide	3.23 <sub>1,28</sub> NS	0.31 <sub>1,28</sub> NS	23.29 <sub>1,20</sub> ***	22.00 <sub>1,26</sub> ***
Population $\times$ Insecticide	6.08 <sub>1,28</sub> *	0.03 <sub>1,28</sub> NS	5.25 <sub>1,20</sub> *	3.48 <sub>1,26</sub> NS
Seed number/carpel				
Population	0.10 <sub>1,53</sub> NS	12.87 <sub>1,71</sub> ***	0.78 <sub>1,89</sub> NS	40.70 <sub>1,53</sub> ***
Insecticide	1.24 <sub>1,53</sub> NS	0.01 <sub>1,71</sub> NS	8.88 <sub>1,89</sub> **	3.17 <sub>1,53</sub> NS
Population $\times$ Insecticide	0.02 <sub>1,53</sub> NS	0.11 <sub>1,71</sub> NS	0.17 <sub>1,89</sub> NS	6.62 <sub>1,53</sub> *
Total seed production				
Population	4.64 <sub>1,28</sub> *	17.82 <sub>1,28</sub> ***	13.70 <sub>1,20</sub> ***	7.87 <sub>1,26</sub> **
Insecticide	3.33 <sub>1,28</sub> NS	0.20 <sub>1,28</sub> NS	15.05 <sub>1,20</sub> ***	4.78 <sub>1,26</sub> *
Population $\times$ Insecticide	0.34 <sub>1,28</sub> NS	0.14 <sub>1,28</sub> NS	0.23 <sub>1,20</sub> NS	0.29 <sub>1,26</sub> NS

<sup>a</sup>Not significant

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

**Fig. 1** Mean values ( $\pm$ SE) of **a** fruit/flower ratio, **b** seed number per carpel and **c** total seed number per plant on control and protected individuals in two populations of *Aquilegia vulgaris* and *Aquilegia viscosa*. Mean and standard errors were calculated by back transformation of least square estimates generated by generalised linear models. Significance levels: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; NS non significant



pollen/ovule ratio, smaller flower width at sepal tips, smaller corollas, shorter spurs, shorter pistil and less stigma–anther separation (Table 2). Reproductive trait variation (the coefficient of variation among plants) was similar for each species (Table 2). In *A. vulgaris*, path analyses showed that (1) spur length and stigma–anther distance were significantly positively correlated with seed/ovule ratio, and that (2) flower width and pistil length were significantly correlated with spur length and stigma–anther separation and thus showed indirect positive correlations with seed/ovule ratio (Fig. 3a). In *A. viscosa*, no floral traits had significant direct or indirect contribution to seed/ovule ratio (Fig. 3b).

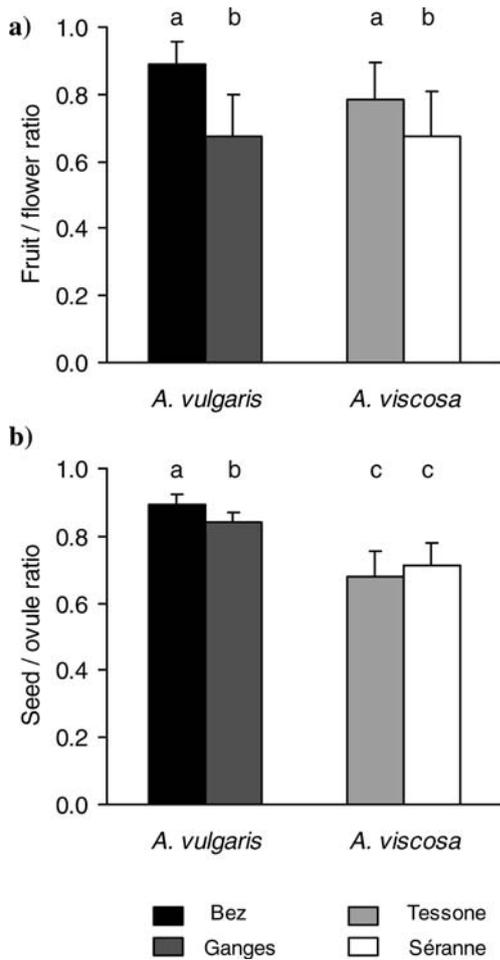
#### Pollinator activity

Species foraging inside the flowers, i.e. potential pollinators, were almost exclusively generalist

bumblebees (*Bombus* sp.). The frequency of pollinator visits was significantly higher (Kruskal-Wallis  $F_{1,20}=61.4$ ;  $P < 0.001$ ) in populations of *A. vulgaris*, which received a mean of  $5.8 (\pm 0.93)$  and  $3.9 (\pm 1.37)$  visits per hour in the Bez and Ganges populations, respectively, than in *A. viscosa*, which received only  $0.35 (\pm 0.24)$  visits per hour in the Séranne population and no visits during our observation periods in the Tessone population.

#### Experimental pollinations

In the glasshouse, the fruit/flower ratio showed no consistent variation between species and pollination treatments. However, the seed/ovule ratio differed significantly between species and pollination treatments ( $F_{1,15}=9.45$ ;  $P < 0.01$  and  $F_{2,15}=42.37$ ;  $P < 0.001$ ), and



**Fig. 2** Mean values ( $\pm$ SE) of **a** fruit/flower ratio and **b** seed/ovule ratios of intact flowers in two populations of *A. vulgaris* and *A. viscosa* in 2001. Mean values and standard errors were calculated by anti-logit transformation of least square estimates generated by generalised linear models. Bars with the same code letter indicate that mean values were not significantly different following contrast analyses

the effect of different pollination treatments also varied between the two species, hence the significant species  $\times$  pollination treatment interaction ( $F_{2,15} = 4.46$ ;  $P < 0.05$ ).

**Table 2** Mean values (with coefficients of variation) of reproductive traits in *A. vulgaris* and *A. viscosa* in the four study populations.  $F$ -values were obtained with Kruskal-Wallis ANOVA. Letter codes indicate mean values that are significantly different among populations ( $P < 0.05$ ) after Dunn's pair-wise comparison tests

	Between species ( $F$ -value)	<i>A. vulgaris</i>		<i>A. viscosa</i>	
		Bez	Ganges	Tessone	Séranne
Stem height (cm)	168.1***	78.0 (18.5) a	84.9 (12.9) a	28.1 (14.8) b	26.5 (12.9) b
Flower number	74.1***	15.8 (58.4) a	14.3 (39.3) a	6.16 (47.8) b	9.11 (46.1) c
Flower width (mm)	211.1***	64.5 (8.27) a	57.5 (9.13) b	47.0 (7.10) c	48.1 (12.8) c
Corolla diameter (mm)	330.5***	28.4 (6.86) a	27.7 (7.44) a	22.3 (9.99) b	20.7 (10.9) b
Spur length (mm)	19.1***	29.9 (4.74) a	28.4 (5.76) b	25.0 (0.53) c	25.2 (9.29) c
Stigma-anther distance (mm)	4.1*	1.82 (21.4) a	1.64 (19.6) b	1.61 (30.2) b	1.57 (26.9) b
Pistil length (mm)	110.4***	20.0 (6.93) a	19.1 (9.85) a	16.9 (9.78) b	16.6 (8.76) b
Pollen/ovule ratio	10.4**	12 651 (23.5) a	12 778 (23.16) a	10 701 (23.4) b	9 940 (25.6) b

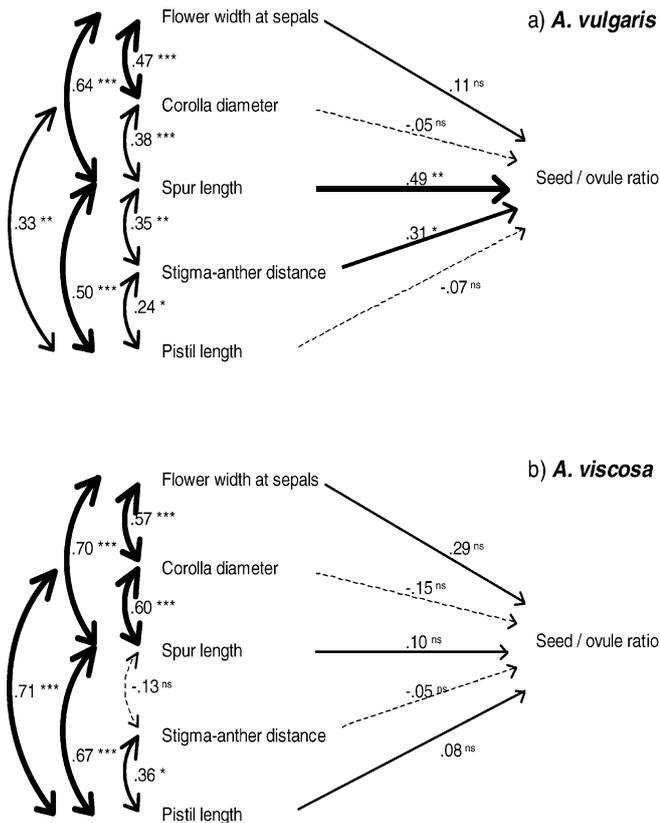
\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

Contrast analyses showed that in both species, unmanipulated flowers had a lower seed/ovule ratio than either selfed or crossed flowers (Fig. 4). In *A. vulgaris*, self-pollination yielded a higher seed/ovule ratio than cross-pollination, whereas in *A. viscosa*, seed/ovule ratio did not differ between self and cross-pollination treatments (Fig. 4). Seed/ovule ratio in the absence of pollinators, i.e. resulting from spontaneous selfing was not significantly different between *A. vulgaris* and *A. viscosa* (Fig. 4). Hence, the capacity to set seed in the absence of pollinators, or the index of auto-fertility (seed/ovule ratio on spontaneous selfing relative to outcrossing) was not different between the two species.

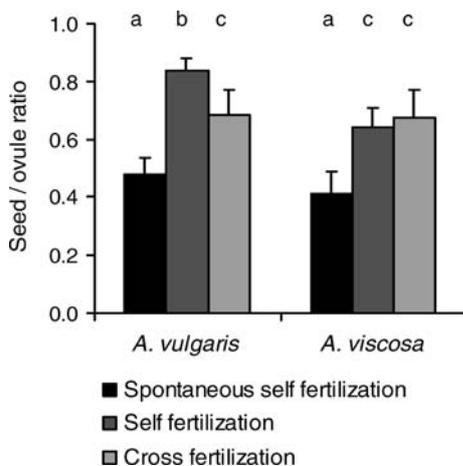
## Discussion

Plant reproductive success is strongly influenced by pollinator activity and the presence of herbivores. In this study, we demonstrate a much stronger impact of herbivores on the maternal fertility of an endemic plant species relative to a widespread congener. This work was only conducted on a single pair of species, hence the general significance of the results should be interpreted with some caution. However, recent works have shown that endemic species can show consistently reduced maternal fertility relative to more widespread congeners (Murray et al. 2002; Lavergne et al. 2004). Our results thus provide insights into the demographic factors that may reduce colonisation capacity and local abundance of endemic species, and the ecological factors critical for their persistence.

A primary result of our study is that the maternal fertility of the endemic *A. viscosa* is strikingly more negatively affected by herbivory, in the form of floral and seed predation, than is the fertility of its widespread congener *A. vulgaris*. Insecticide treatment generally had a significant beneficial effect on maternal fertility in the restricted endemic *A. viscosa* but not in its widespread congener *A. vulgaris*. The two *Aquilegia* species occur in the same study area and have a similar guild of herbivores. The differences in the impact of herbivory on maternal fertility observed in our study may be due to



**Fig. 3** Path diagrams showing the relationships between floral traits and seed/ovule ratio in *A. vulgaris* (a) and *A. viscosa* (b). Straight arrows indicate partial correlation of floral traits with seed/ovule ratio, and curved arrows depict product-moment correlations between floral traits. Arrow width indicates strength of correlation. Solid and dashed arrows indicate that the relationships are positive and negative, respectively. Significance levels: \* $P < 0.05$ ; \*\* $P < 0.01$ . NS non significant



**Fig. 4** Mean values ( $\pm$ SE) of seed/ovule ratio obtained after spontaneous self-pollination, hand self-pollination and outcross-pollination on *A. vulgaris* and *A. viscosa*. Mean values and standard errors were calculated by anti-logit transformation of least square estimates generated by generalised linear models. Bars with the same code letters indicate pollination treatments that did not produce significantly different seed/ovule ratios

either a higher relative preference of insects for the rare *A. viscosa* compared with widespread *A. vulgaris* (see also Landa and Rabinowitz 1983; Scheidel and Bruehlheide 1999), or a higher density of flower parasites and seed predators in the habitat of the rare species (Kéry et al. 2001). Further experimental work, using plants transplanted among sites, will be needed to distinguish between these two alternatives.

In intact flowers, seed/ovule ratio remained significantly higher in *A. vulgaris* than in *A. viscosa*. However, both species show a similar significant decline in maternal fertility when they undergo autonomous self-pollination in the absence of insect pollinators relative to hand-out-crossing in the glasshouse. Hence, the low pollinator visitation rates observed in *A. viscosa* populations may also contribute to reducing the reproductive success of this species. The differences in pollinator visitation rates may be caused by poor attractiveness of *A. viscosa* populations, which contain fewer and smaller flowering individuals (e.g. Matsumura and Whashitani 2000; Thompson 2001) with less attractive flowers (smaller corolla and shorter nectar spur), or generally lower pollinator abundance in sites where this species occurs (Solbrig and Rollins 1977). Moreover, *A. viscosa* occurs in scree slopes and rocky habitats where resource availability may be lower than in habitats of *A. vulgaris*. Hence, resource limitation may also contribute to maternal fertility differences between the two species in natural populations.

An important feature of these results is that in endemic *A. viscosa*, herbivory reduces maternal fertility even when pollinator visitation is extremely low. This result differs from those reported for other species (Herrera 2000; Herrera et al. 2002) where the impact of herbivory on fertility and fitness is minimal in situations where pollinators are absent. This difference may be due to the fact that, although *A. viscosa* has very low rates of pollinator servicing, it is capable of significant spontaneous selfing in the absence of pollinators. As a result, even in the absence of pollinators, maternal fertility is still strongly reduced by herbivory. It is possible that the high rates of floral herbivory observed in this species actually reduce the attractiveness of individuals to insect pollinators, and thus indirectly contribute to reduced maternal fertility by altering attractiveness (Strauss et al. 1996).

Given the extremely low pollinator visitation rates in populations of *A. viscosa*, one would predict the evolution towards increased self-fertility in order to facilitate reproductive assurance, as shown elsewhere in rare and endemic species (Solbrig and Rollins 1977; Wyatt 1986; Karron 1997). However, in our experimental pollinations, *A. vulgaris* and *A. viscosa* showed no significant difference in seed/ovule ratio after autonomous selfing, indicating that the statistical difference in stigma-anther separation have no functional significance in terms of the ability of the species to self-pollinate in the absence of pollinators. Stigma-anther separation is well known to influence the capacity for automatic selfing in many

species, including *Aquilegia* (Eckert and Schaefer 1998). The lack of evolution of floral traits to facilitate reproductive assurance in *A. viscosa* may result from the lack of any correlation between reproductive trait variation and female fertility in the endemic *A. viscosa* and/or the possibility that contemporary pollination regimes are a poor gauge of historical pollinator selection pressures (Herrera et al. 2001).

Our results are pertinent not only to the strong current interest in the relative role of ecological factors that may limit plant fitness in rare and common species, but also to the evaluation of the causes of rarity and the development of rare species conservation guidelines. Although a number of studies have compared patterns of biological attributes between fairly large samples of rare and common species (e.g. Hedge and Ellstrand 1999), there remains a paucity of comparative data on the impact of ecological factors on the reproductive success of closely related rare and widespread species (Murray et al. 2002). Our study provides a clear demonstration of how the negative effects of herbivory on maternal fertility are specific to an endemic species. Globally, herbivores reduced maternal fertility of endemic *A. viscosa* by up to 56%, and this reduction is exacerbated by a lower seed/ovule ratio in intact flowers, either due to very low pollen transfer, as a result of low pollinator visitation rates in this species, or some form of resource limitation in habitats of this species. Experimental tests of the relative roles of resource and pollinator limitation would thus be worthwhile. Whatever the cause, such reduced fertility may strongly reduce local populations growth rates and the ability of this species to colonise new sites (Silvertown et al. 1993). Populations of endemic *A. viscosa* would thus clearly benefit from protection from natural enemies (see also Bevill et al. 1999). However, the ways and means of such protection may create a conflict of interest between insect and plant conservation, as illustrated in *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli* (Kéry et al. 2001).

Finally, herbivory may reduce the potential for natural selection on floral traits associated with plant-pollinator interactions if its effects on seed set mask any pollinator-mediated selection (Herrera 2000; Herrera et al. 2002). In this study, we detected very different patterns of relationships among floral traits and maternal fertility in natural populations of the two *Aquilegia* species. In *A. vulgaris*, we detected a positive correlation between nectar spur length and seed/ovule ratio, suggesting that selection may act on this trait in this species to increase pollinator attraction, as suggested in other *Aquilegia* species (Hodges and Arnold 1994; Brunet and Eckert 1998). In contrast, despite similar amounts of floral trait variation in *A. viscosa*, we detected no association with variation in maternal fertility. It is thus possible that the high levels of herbivory, which greatly reduce maternal fertility in this species, in association with low pollinator servicing, may limit the response to any pollinator-mediated selection in *A. viscosa*.

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