

Geographic and within-population variation in the globeflower–globeflower fly interaction: the costs and benefits of rearing pollinators' larvae

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Received: 26 September 2005 / Accepted: 26 September 2006 / Published online: 18 October 2006
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Abstract Interspecific interactions can vary within and among populations and geographic locations, and this variation can influence the nature of the interaction (e.g. mutualistic vs. antagonistic) and its evolutionary stability. Globeflowers are exclusively pollinated by flies, whose larvae feed only on their seeds. Here we document geographic variability in costs and benefits in globeflowers in sustaining their pollinating flies throughout the range of this arctic-alpine European plant over several years. A total of 1,710 flower heads from 38 populations were analysed for their carpel, egg and seed contents. Individual and population analyses control for the confounding influences of variation in both: (1) population traits, such as fly density and egg distribution among flower heads; and (2) individuals traits, such as carpel and egg numbers per flower head. Despite considerable variation in ecological conditions and pollinator densities across populations, large proportions (range 33–58%) of seeds were released after predation, with a benefit-to-cost ratio of 3, indicating

that the mutualism is stable over the whole globeflower geographical range. The stability of the mutualistic interaction relies on density-dependent competition among larvae co-developing in a flower head. This competition is revealed by a sharp decrease in the number of seeds eaten per larva with increasing larval number, and is intensified by non-uniform egg distribution among globeflowers within a population. Carpel number is highly variable across globeflowers (range 10–69), and flies lay more eggs in large flowers. Most plants within a population contribute to the rearing of pollinators, but the costs are greater for some than for others. Large globeflowers lose more seed to pollinator larvae, but also release more seed than smaller plants. The apparent alignment of interests between fly and plants (positive relationship between numbers of seed released and destroyed) is shown to hide a conflict of interest found when flower size is controlled for.

Keywords Pollination mutualism · Seed predators · Egg aggregation · Density-dependent competition · Flower size

Communicated by Jacqui Shykoff.

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Introduction

Some plants are highly specialized for their pollination by insects whose larvae feed on developing seeds. In these systems, there is potential for a conflict of interest between interacting species, as an increase in insect fitness (i.e. more eggs laid and more seeds destroyed) is costly for plant seed production, and a negative correlation between viable and destroyed seeds is expected. The classic and best studied examples of extreme obligate mutualism between a plant and a pollinating seed

predator include the fig–fig wasp, the yucca–yucca moth, the senita–senita cactus, and the globeflower–globeflower fly interactions (Addicott 1986; Pellmyr 1989; Anstett et al. 1997; Jaeger and Després 1998; Holland and Fleming 1999). Despite apparent similarity in these mutualisms, the nature of the interaction differs between fig–fig wasps and other systems. A fig-wasp's life is entirely devoted to the transportation of pollen from its natal fig to another fig, the laying of its eggs, and its death in the fig. The only way for a fig to export its pollen is to rear its pollinator's progeny. Male fig success, therefore, entirely depends on pollinator progeny developmental success, and the resource allocated by a fig to the rearing of its pollinator larvae is approximately half its seeds, as expected by sex allocation theory (Charnov et al. 1976). By contrast, in other systems, pollinating insects are free to move from an individual plant to another, and transport pollen from several individuals throughout their lifespan. There is no direct individual benefit for a plant to rear pollinator larvae in terms of pollen export, as pollinators reared by other plants may also transport its pollen. One may expect these systems to be highly susceptible to invasion by cheaters (Axelrod and Hamilton 1981; Herre et al. 1999; Yu 2001), plants that prevent oviposition and/or kill developing larvae (Bao and Addicott 1998). The European globeflower *Trollius europaeus* L. (Ranunculaceae) is a perennial arctic-alpine herb pollinated by *Chiastocheta* flies, whose larvae are specific seed predators of globeflowers (Pellmyr 1989; Jaeger and Després 1998). Each individual plant typically produces, every second year, a single flower composed of several carpels, each containing about 12 ovules. Both male and female flies contribute to passive pollination when visiting flowers to feed on nectar and search for sexual partners (Després 2003), so flowers with many eggs are not necessarily better pollinated than flowers with few or no eggs (Jaeger et al. 2000). Egg hatching success is close to one, and larval mortality is low, so that the number of eggs laid on a flower head is a good indication of the number of larvae co-developing in the flower (Jaeger et al. 2001; Pompanon et al. 2006). Larvae can freely move from one carpel to another, and there is usually only one flower per plant, so selective fruit abortion is not an option for the globeflower (Bull and Rice 1991; Pellmyr and Huth 1994). After larval development, the last instar falls to the soil and overwinters, to emerge as a short-lived adult pollinator the following spring. As a globeflower typically flowers only every second year (Å. M. Hemborg and L. Després, unpublished data), an individual plant never benefits from pollination services by the individual fly it reared as a larva. The difference

between gross benefits (the number of ovules fertilized by flies) and costs (the number of seeds eaten by larvae) determines the net benefit of the interaction for the globeflower. The gross benefit is likely to vary with fly density in the population, because when fly density is high, pollination efficiency is high, while the cost is likely to vary with number of eggs laid on a particular globeflower and the size of this flower (carpel number). The magnitude of variation between benefits and costs is therefore likely to vary with fly density across populations, egg distribution across globeflowers within a population, and individual flower size and egg content.

In this paper, we evaluate costs and benefits of rearing pollinator flies for the European globeflower throughout its ecological range. We answer the following questions:

1. How much does it cost a globeflower population to rear pollinator larvae, and how does this cost vary across populations with variable fly densities and egg distribution among globeflowers?
2. How variable are costs and benefits among individuals within a population, and what is the effect of flower size on the individual cost/benefit outcome?

Materials and methods

A total of 26 globeflower populations were studied at various elevations, six in Swedish Lapland (range 400–670 m a.s.l.) and 20 in the French Alps (range 800–2,500 m a.s.l.) including six populations sampled for 3 consecutive years (1995, 1996, 1997). Study sites represent a wide range of ecological conditions (Jaeger and Després 1998; Hemborg and Després 1999). This resulted in a total of 38 records, each representing a group of globeflowers sharing their pollinators, hereafter called a “population”. An average of 45 flower heads (range 9–119) were sampled per population, resulting in a total of 1,710 globeflowers analysed. For each flower, we counted the number of eggs and the number of carpels. Egg distribution among flowers within a population was estimated as the coefficient of dispersion ($CD = V/M$), where M is the mean number of eggs per flower and V its variance. If $CD = 1$, eggs are randomly distributed in the population (Poisson distribution) and if $CD > 1$, eggs are aggregated, i.e. a few flowers have more eggs than others. We estimated the proportion of fertilized ovules (gross seed production) by counting the number of undeveloped ovules and developing seeds per carpel in five undamaged carpels (Jaeger and Després 1998). Undeveloped ovules were counted on only 1,524 flowers in 36 populations, because some flowers were too damaged and no intact

carpels were available. In each studied population, relative *Chiastocheta* density was estimated as the mean number of eggs per flower.

To estimate seed loss due to a pollinator's larval predation, we selected a total of 308 other flower heads with a number of *Chiastocheta* eggs ranging from 1 to thirty-five, 2 weeks after the end of flowering in three populations (Jaeger et al. 2001). The number of carpels was counted, and flowers were bagged to prevent seed release. Flower heads were collected after completed larval development (4–5 weeks after the end of flowering), and the number of seed destroyed by larvae was estimated by the difference between the number of seeds remaining intact after predation and the estimated number of seeds initiated. *Chiastocheta* larvae are the only predator of globeflower seeds. Although up to six *Chiastocheta* spp. co-exist in alpine globeflower populations (Després and Jaeger 1999), seed consumption per larva was shown to be similar for all species (Pompanon et al. 2006), so that we did not distinguish between *Chiastocheta* spp. in the present study. The proportion of seed eaten per flower head was plotted against individual egg density per carpel and the best fitting model was selected. We then applied this model to each of the 1,710 flowers sampled in the 38 populations to estimate individual and population costs. The proportion of seed released after predation (net seed production) equals the proportion of seed initiated multiplied by one minus the proportion of seed eaten.

Data analysis

Data were first checked for normality (Kolmogorov–Smirnov test of normality) and homogeneity of variances (Levene test). Seed proportions were arcsine square root transformed prior to analyses. Two-way ANOVAs (GLM procedure for unbalanced experimental design) were performed on carpel and egg numbers, and on the absolute number and on the proportion of seed initiated, eaten, and released after predation. All tested effects (population and year) were considered as fixed effects, and mean squares adjusted for unequal sample sizes were used in the ANOVAs. To examine whether fly density in a population had an effect on the slope between pollination efficiency and egg number, we performed an analysis of covariance (ANCOVA) testing for the effect of population, egg density, and interaction on the number of seeds initiated: a significant interaction indicates that the slopes are different within each population. To relate this difference to variation in fly densities, we performed a linear regression of the slopes against fly density across

populations. Linear and non-linear regressions predicting the proportion of seed eaten against egg density per carpel were performed and the best fitting model [residual sum of squares (SS_R) minimum] was selected. Multiple linear regressions predicting the absolute number and proportion of seed initiated and of seed released after predation against egg density per carpel and carpel number were performed in each population, and across populations. We analysed distributions of costs among populations and among globeflowers within a population using Kolmogorov–Smirnov normality test. Spearman's correlations were performed for all pairwise combinations of variables.

Results

All study populations contained *Chiastocheta* flies, with densities ranging from 0.5 to 17 eggs per flower head (Table 1). There were significant differences in carpel and egg numbers per flower across populations (carpel $F_{25,1672} = 15.12$, $P < 0.001$, egg $F_{25,1672} = 23.28$, $P < 0.001$), years (carpel $F_{2,1672} = 22.2$, egg $F_{2,1672} = 77.18$, $P < 0.001$) and population by year interaction (carpel $F_{10,1672} = 5.11$, egg $F_{10,1672} = 21.53$, $P < 0.001$), reflecting highly variable ecological environments. Mean carpel number per flower varied across populations and years ($n = 38$ observations) from 25 to 42 (Table 1).

At the individual level, 92% of the 1,710 analysed flowers contained at least one egg (range 0–63) and carpel number was highly variable (range 10–69). Unparasitized flowers were significantly smaller than parasitized flowers (30.3 vs. 32.85; $F_{1,1708} = 8.94$, $P = 0.003$). The proportion of unparasitized flowers ranged from 0 to 75% across populations and this proportion decreased with increasing fly densities ($r_s = -0.876$, $P < 0.001$). Within each population, eggs were not randomly distributed among flowers as shown by a coefficient of dispersion higher than 1 in all populations (range 1.07–7.39, significantly higher than one in 35 out of 38 populations, Table 1). Large flowers tended to be more heavily infected than small flowers, as shown by a positive correlation between the number of eggs and the number of carpels per flower, significant in 24 out of 38 populations (Table 2). Although large flowers attracted more eggs than small flowers, they were not better pollinated (no effect of carpel number on gross seed production in 29 out of 36 populations; when significant, either positive or negative, see Table 2). Furthermore, carpel number was not correlated with egg density per carpel, i.e. large flowers do not concentrate more eggs than small flowers

Table 1 Characteristics of the 38 globeflower populations analysed. The number of sampled flower heads and mean contents per flower head are given. SDs are given in parentheses for directly measured data and for the total mean. *CD* Coefficient of deviation, % proportion, *n.s.* non-significant ($P > 0.05$)

Population name	Flower heads (<i>n</i>)	Carpel no.	Egg no.	CD	% Seeds initiated	No. seed initiated	No. seed eaten	No. seed released	% Ovule released	% Seeds eaten	% Seeds released	Benefit /cost ^a
Chables	40	38 (10)	6.00 (5.7)	5.42	0.86 (0.14)	408 (144)	157	250	0.54	0.38	0.62	2.6
Chamoss	39	28 (9)	4.77 (5.9)	7.39	0.69 (0.21)	206 (89)	74	132	0.44	0.35	0.65	2.8
Che95	45	33 (9)	4.29 (4.4)	4.50	0.89 (0.10)	297 (101)	105	193	0.58	0.34	0.66	2.8
Che96	50	40 (8)	4.08 (3.1)	2.37	0.76 (0.16)	365 (130)	122	243	0.5	0.33	0.67	3.0
Che97	49	29 (9)	4.06 (2.1)	1.07 n.s.	0.79 (0.11)	263 (94)	102	161	0.48	0.39	0.61	2.6
Cot95	38	32 (9)	2.97 (2.6)	2.30	0.84 (0.12)	269 (113)	90	179	0.56	0.33	0.67	3.0
Cot96	50	38 (9)	3.04 (2.2)	1.54	0.75 (0.14)	358 (104)	111	247	0.51	0.31	0.69	3.2
Cot97	49	32 (7)	2.63 (2.3)	1.96	0.74 (0.12)	271 (95)	87	184	0.51	0.31	0.69	3.1
Cro95	50	27 (7)	6.92 (4.4)	2.84	0.93 (0.05)	274 (95)	123	151	0.51	0.45	0.55	2.2
Cro96	49	28 (7)	5.20 (4.0)	3.09	0.74 (0.19)	248 (248)	100	148	0.45	0.39	0.61	2.5
Cro97	73	29 (9)	5.10 (4.3)	3.60	0.73 (0.16)	264 (109)	102	162	0.46	0.37	0.63	2.6
Etelley	46	34 (7)	3.20 (3.3)	3.53	0.67 (0.15)	272 (92)	89	183	0.45	0.32	0.68	3.1
Fardelay	78	42 (9)	5.27 (4.9)	4.52	0.84 (0.14)	386 (123)	133	253	0.53	0.35	0.65	2.9
Gal95	49	31 (7)	16.96 (9.1)	4.90	0.80 (0.12)	299 (79)	167	133	0.36	0.55	0.45	1.8
Gal96	50	29 (8)	4.74 (3.9)	3.24	0.61 (0.17)	183 (64)	66	117	0.39	0.37	0.63	2.8
Gal97	30	24 (8)	8.67 (6.5)	4.86	0.70 (0.16)	194 (96)	93	100	0.37	0.47	0.53	2.1
JouxPlan	92	37 (7)	12.83 (7.1)	3.99	0.89 (0.10)	411 (110)	202	209	0.46	0.49	0.51	2.0
Lac95	46	36 (10)	7.41 (5.9)	4.81	0.68 (0.17)	302 (116)	123	179	0.41	0.4	0.6	2.5
Lac96	49	40 (11)	4.00 (3.7)	3.55	0.56 (0.16)	260 (96)	89	172	0.37	0.33	0.67	2.9
Lac97	40	35 (10)	8.58 (4.9)	2.79	0.77 (0.16)	289 (146)	128	161	0.43	0.45	0.55	2.3
Lau95	39	29 (6)	7.13 (5.9)	4.87	0.86 (0.12)	262 (75)	115	147	0.49	0.43	0.57	2.3
Passy	119	35 (9)	3.82 (3.3)	2.91	0.73 (0.20)	276 (113)	93	183	0.49	0.32	0.68	3.0
Pelly	38	35 (7)	5.71 (4.6)	3.68	0.87 (0.12)	373 (105)	144	228	0.54	0.38	0.62	2.6
PrazFarou	76	36 (10)	5.12 (4.1)	3.33	0.79 (0.16)	328 (139)	124	204	0.5	0.37	0.63	2.7
Rhi1	12	29 (9)	2.25 (2.3)	2.35	0.57 (0.19)	190 (95)	49	141	0.43	0.26	0.74	3.9
Rhi2	20	32 (10)	2.70 (2.0)	1.52	0.53 (0.21)	209 (117)	65	144	0.37	0.3	0.7	3.2
Rhi3	9	24 (5)	2.33 (1.9)	1.50 n.s.	0.49 (0.13)	139 (54)	44	95	0.33	0.32	0.68	3.2
Sales	40	29 (7)	1.78 (1.9)	2.04	–	178 (83)	45	133	–	0.23	0.77	4.0
Salmoiry	38	30 (9)	3.42 (2.7)	2.10	0.79 (0.14)	255 (92)	92	163	0.5	0.35	0.65	2.8
Som95	60	25 (5)	16.0 (11.2)	7.80	0.94 (0.06)	266 (74)	151	115	0.4	0.56	0.44	1.8
Som96	49	28 (7)	4.41 (3.0)	2.09	0.75 (0.17)	259 (71)	101	158	0.45	0.39	0.61	2.6
Som97	50	27 (6)	5.36 (3.1)	1.76	0.80 (0.13)	262 (91)	110	152	0.46	0.42	0.58	2.4
Tete	40	28 (5)	5.00 (1.0)	2.05	0.53 (0.19)	162 (66)	15	147	0.49	0.08	0.92	11.0
Vagnys	37	34 (8)	4.89 (3.6)	2.60	0.82 (0.20)	331 (105)	126	206	0.51	0.37	0.63	2.6
Vallon	20	27 (5)	2.15 (2.3)	2.46	–	222 (68)	61	161	–	0.26	0.74	3.6
tre1	22	31 (8)	2.09 (1.84)	2.09	0.68 (0.16)	236 (83)	63	174	0.5	0.25	0.75	3.7
tre2	15	34 (8)	1.60 (1.8)	1.6	0.63 (0.08)	228 (67)	54	174	0.48	0.23	0.77	4.2
tre3	14	27 (6)	2.71 (1.9)	2.71	0.67 (0.12)	213 (61)	71	142	0.44	0.33	0.67	3.0
Total	1710	31.8 (4.6)	5.12 (3.6)	3.2 (1.5)	0.74 (0.11)	268.7 (66)	99.6 (37)	169.1 (39)	0.46 (0.06)	0.36 (0.09)	0.64 (0.09)	3 (1.4)

^a Number of seeds initiated/number of seeds eaten

(Table 2). The proportion of seed initiated neither increased significantly with egg number in most populations, nor did the absolute number of seeds initiated when corrected for carpel number (Table 2), i.e. flowers with more eggs were generally not better pollinated than flowers with few eggs. However, the slopes of the relationship between the number of seed initiated and the number of eggs differed across populations (ANCOVA, population $F_{37,1709} = 7.13$, $P < 0.001$; egg density $F_{1,1709} = 14.23$, $P < 0.001$; population by egg density interaction $F_{37,1709} = 1.65$, $P = 0.008$). In low fly density populations, the slope was more positive than in high fly density populations (Fig. 1), indicating that globeflowers in low fly density populations benefited more in terms of pollination from visits by ovipositing females than in high fly density populations. Across populations, the mean proportion of seed initiated increased with mean egg number per flower head (Table 3): populations with high fly densities were better pollinated than populations with low fly densities.

Predation costs

In the predation study ($n = 308$) we found no effect of flower size on the proportion of seed eaten (linear regression $F_{1,306} = 0.08$, $P = 0.7$). Therefore, the proportion of seed eaten per flower is best predicted by egg density per carpel rather than by the absolute number of eggs in that flower (DeAngelis and Holland 2006). As *Chiastocheta* larvae are the only predators of globeflower seeds, seed destruction in the absence of larva is zero. A classic assumption in modelling the probability that a seed will be eaten is that larvae move at random

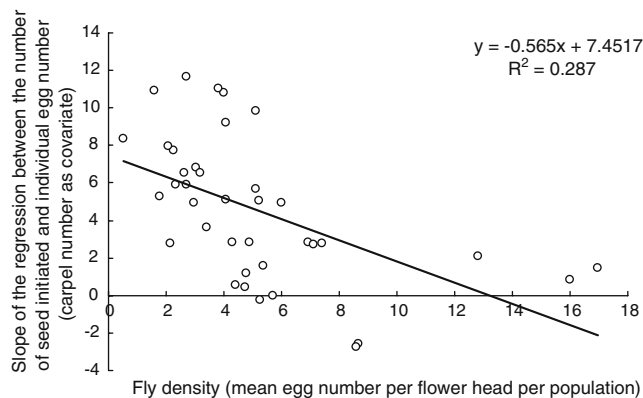


Fig. 1 Linear regression of the slopes of the relationship between the individual number of seeds initiated and individual egg number (with carpel number as covariate) within each population ($n = 38$) against fly density per population estimated as the mean egg number per flower. The negative slope is significant ($P = 0.001$, $r^2 = 0.27$): individual plants benefit more from pollination by ovipositing females in low fly density populations than in high fly density populations

Table 2 Summary of Spearman's correlation analyses within globeflower populations ($n = 36$ or 38) showing the number of populations for which the correlations were significantly positive (+), negative (−) ($P < 0.05$), or n.s.

	Carpel number			Egg number		
	+	n.s.	−	+	n.s.	−
Egg number	24	14	0	−	−	−
Egg density (egg no./carpel no.)	3	31	4	−	−	−
Proportion of seed initiated	2	29	5	5	31	0
Number of seeds initiated	38	0	0	22	16	0
Number of seeds eaten	31	7	0	38	0	0
Number of seeds released	37	1	0	0	36	2
Proportion of seed released	1	33	2	0	10	26

and eat any encountered seed; given Poisson-distributed visits with a mean of ax (where x is egg density per carpel, and a is a constant), the probability for a seed to be eaten is $1 - e^{-ax}$ (e.g. one minus the probability of no visit; Morris et al. 2003). We call this model the “random search model”. The best fitting model predicting the proportion of seed eaten per flower as a function of egg density per carpel ($y = 0.66x^{0.26}$, $SS_R = 10.39$, Fig. 2a) is superior to the linear model ($y = 0.33 + 0.41x$, $SS_R = 12.79$), and much superior to the random search model ($y = 1 - e^{-2.43x}$, $SS_R = 14.89$). This model (“individual cost model”) was then applied to each of the 1,710 flower heads sampled in the studied populations. Costs varied across years ($F_{2,1708} = 59.84$, $P < 0.001$) and populations ($F_{25,1684} = 25.84$, $P < 0.001$). The proportion of seed lost to pollinator larvae ranged from 0 to 87% among individuals within populations, and from 8 to 56% across populations, ca. an average of 36% of the initiated seeds destroyed (Table 1; Fig. 3). Ninety-five percent of all studied populations lost between 25 and 45% of initiated seeds to pollinator larvae, and these costs were normally distributed across populations (Kolmogorov–Smirnov test, $P > 0.05$); by contrast, costs were normally distributed among globeflowers within a population in only 13 populations among 38: in these populations, almost all individuals had at least one egg. In all the 25 remaining populations, where up to 75% of the individuals sampled had no eggs, costs were not normally distributed among individuals: some pay less than others. Mean predation costs per population were plotted against mean egg density per population ($n = 38$). The best fitting model ($y = 0.69x^{0.34}$, $SS_R = 0.02$) takes into account egg aggregation on flowers within each population (Després and Jaeger 1999) to predict the change in population costs with increasing fly density (“population cost model”, Fig. 2b). The population data always lie below the individual cost model, which

Table 3 Simple and multiple regressions predicting the number of seeds initiated and released against egg number in 38 populations. *n* Number of flower heads sampled

Covariate	Seeds initiated						Seeds released						
	None			Carpel			None			Carpel			
Population	<i>n</i>	Slope	SE	<i>P</i>	Slope	SE	<i>P</i>	Slope	SE	<i>P</i>	Slope	SE	<i>P</i>
Chables	40	17.04	3.02	<0.0001	4.92	2.94	0.102	4.11	2.14	0.06	-4.44	2.08	0.040
Chamoss	39	2.96	2.41	0.229	1.16	1.93	0.552	-2.11	1.76	0.24	-3.36	1.46	0.027
Che95	45	11.22	3.08	0.001	2.83	1.90	0.145	0.58	2.20	0.80	-5.21	1.47	0.001
Che96	50	11.69	5.80	0.050	9.22	4.50	0.046	-3.25	4.34	0.46	-5.25	3.15	0.103
Che97	49	21.01	5.82	0.001	5.11	4.87	0.300	7.60	3.89	0.06	-3.88	2.98	0.199
Cot95	38	12.13	6.91	0.088	4.93	5.20	0.350	0.39	4.93	0.94	-5.29	3.30	0.118
Cot96	50	4.25	6.90	0.541	6.82	5.00	0.179	-11.31	5.20	0.03	-9.37	3.78	0.017
Cot97	49	15.89	5.63	0.007	6.54	4.52	0.155	1.86	3.98	0.64	-4.64	3.24	0.159
Cro95	50	10.44	2.71	<0.001	2.85	2.05	0.172	1.72	1.62	0.29	-3.20	1.08	0.005
Cro96	49	14.14	3.34	<0.001	5.05	3.07	0.107	3.20	2.06	0.13	-2.45	1.88	0.199
Cro97	73	14.13	2.53	<0.0001	5.66	1.94	0.005	2.48	1.76	0.16	-3.83	1.23	0.003
Etelley	46	8.38	3.92	0.038	6.52	3.32	0.056	-1.63	2.86	0.57	-3.08	2.33	0.194
Fardelay	78	-0.41	2.90	0.889	-0.23	2.06	0.913	-7.55	2.28	<0.01	-7.41	1.57	<0.0001
Gal95	49	3.99	1.13	0.001	1.47	0.88	0.103	-0.20	0.62	0.75	-1.80	0.39	<0.0001
Gal96	50	2.17	2.33	0.355	0.45	1.85	0.809	-3.98	1.78	0.03	-5.26	1.44	0.001
Gal97	30	6.41	2.51	0.017	-2.59	1.75	0.150	0.94	1.43	0.52	-4.45	0.86	<0.0001
JouxPlan	92	7.63	1.40	<0.0001	2.07	1.26	0.103	0.48	0.78	0.54	-2.95	0.64	<0.0001
Lac95	46	10.57	2.45	<0.0001	2.79	2.47	0.265	1.05	1.87	0.58	-5.44	1.76	0.004
Lac96	49	15.59	2.92	<0.0001	10.83	3.33	0.002	4.36	2.09	0.04	0.18	2.28	0.936
Lac97	40	8.36	4.65	0.080	-2.77	2.72	0.314	1.50	2.84	0.60	-5.46	1.55	0.001
Lau95	39	7.02	1.75	<0.001	2.72	1.76	0.131	0.18	1.13	0.88	-3.09	1.02	0.005
Passy	119	11.00	2.97	<0.001	11.04	2.55	<0.0001	-1.54	2.08	0.46	-1.51	1.73	0.385
Pelly	38	8.59	3.52	0.020	-0.02	2.44	0.994	-1.43	2.53	0.57	-7.53	1.80	<0.001
PrazFarou	76	15.43	3.49	<0.0001	9.84	2.49	<0.001	3.21	2.32	0.17	-0.47	1.67	0.779
Rhi1	12	14.63	2.17	0.257	7.72	7.51	0.331	-3.21	10.60	0.77	-9.59	5.66	0.124
Rhi2	20	17.11	2.98	0.204	5.92	8.19	0.480	1.33	9.72	0.89	-7.24	5.87	0.234
Rhi3	9	6.61	0.58	0.552	5.93	7.30	0.448	-4.10	8.50	0.64	-4.67	5.38	0.419
Sales	40	14.43	6.70	0.038	5.27	4.90	0.289	-1.57	5.38	0.77	-8.92	3.93	0.029
Salmoiry	38	9.14	5.51	0.106	3.62	3.75	0.341	0.27	3.77	0.94	-3.67	2.40	0.135
Som95	60	2.42	0.81	0.004	0.83	0.62	0.187	-1.11	0.43	0.01	-2.03	0.31	<0.0001
Som96	49	0.55	3.42	0.872	0.53	2.71	0.846	-5.39	2.30	0.02	-5.40	1.71	0.003
Som97	50	4.19	4.27	0.330	1.58	2.95	0.594	-2.52	2.78	0.37	-4.32	1.78	0.020
Tete	40	11.83	10.46	0.265	8.36	9.92	0.405	-12.34	9.86	0.22	-15.68	9.32	0.101
Vagnys	37	9.25	4.72	0.058	2.85	4.76	0.554	-0.34	3.21	0.92	-5.01	3.17	0.123
Vallon	20	12.25	6.37	0.070	2.80	4.72	0.561	-3.98	5.26	0.46	-11.52	4.08	0.012
tre1	22	18.40	9.14	0.058	7.96	9.38	0.407	-3.29	8.35	0.70	-12.50	8.65	0.165
tre2	15	15.98	9.24	0.108	10.95	7.85	0.188	-3.44	7.97	0.67	-7.34	7.15	0.324
tre3	14	11.51	8.51	0.201	11.65	4.25	0.019	-3.16	7.39	0.68	-3.05	4.30	0.493

means that egg aggregation among globeflowers results in decreasing global predation costs as compared to predicted costs if eggs were uniformly distributed (Fig. 2b).

Net benefit: female fitness

At the individual level, the proportion of seed released after predation decreased with increasing egg number in most populations, while carpel number had no effect on this proportion (Table 2). By contrast, the absolute numbers of seeds initiated, eaten, and released after predation increased with increasing carpel number (Table 2). This resulted in positive correlations

between the number of seeds eaten and released (significant in 31 out of 38 populations). When carpel number was controlled for by using a multiple regression analysis, the slope of the number of seeds eaten versus seeds released was negative in all populations (significant in 24 out of 38 populations), and the number of seeds released decreased with increasing egg number (Table 3). At the population level, despite a considerable variation in fly densities, the proportion of fertilized ovules released after larval predation was strikingly similar across populations (mean ± SD = 0.46 ± 0.06, *n* = 36; Table 1), with no significant effect of egg number variation across populations on net seed production (Table 4). Populations with low fly

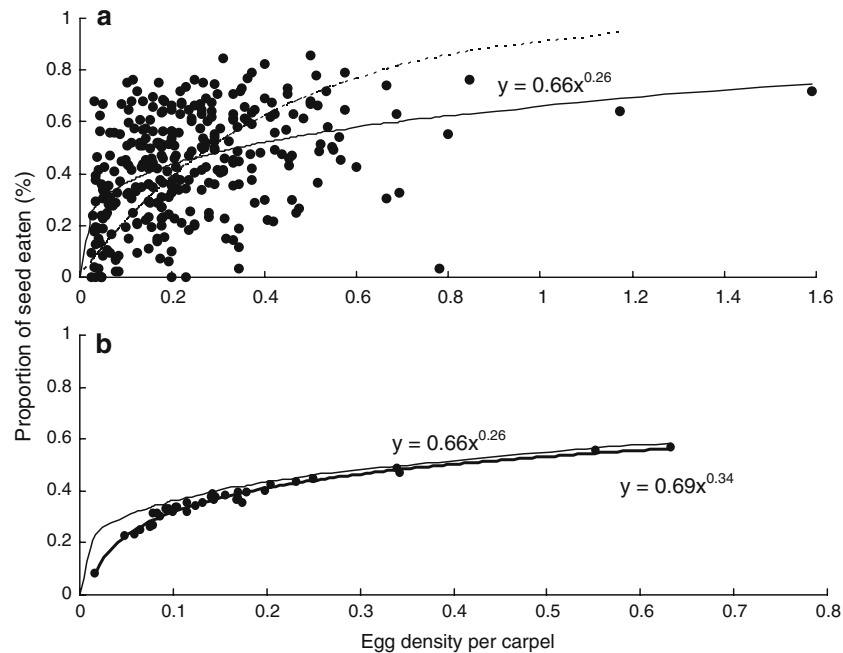


Fig. 2 **a** Proportion of seed eaten per flower head as a function of individual egg load ($n = 308$). The best fitting model: individual cost model ($y = 0.66x^{0.26}$, $SS_R = 10.39$; *solid line*) is superior to the linear model (not shown), and much superior to the random search model ($y = 1 - e^{-2.43x}$, $SS_R = 14.89$; *dashed line*). It shows strong competition among larvae at high densities. **b** Mean proportion of seed eaten per population as a function of mean egg

density per carpel per population ($n = 38$). The best fitting model: population cost model ($y = 0.69x^{0.34}$, $SS_R = 0.02$; *thick line*) is below the individual cost model (*thin line*), which means that egg aggregation among globeflowers within populations results in decreasing predation costs as compared to costs in populations with uniformly distributed eggs

densities produced fewer seeds, because they were less efficiently pollinated, as shown by a significant positive effect of egg number on the proportion of seed initiated, but they were also less infected, resulting in a similar proportion and absolute number of seeds released after predation (Figs. 4, 5). The benefit-to-cost ratio (number of seeds initiated over number of seeds eaten) was about 3, ranging from 1.8 to 11 (Table 1).

Discussion

Pollination efficiency

Gross seed set increased with increasing fly densities across populations, i.e. individual plants benefit from being in a population with many flies; however, within populations, seed set generally did not increase with increasing individual egg load, or only marginally increased in low fly density populations. This indicates that pollination and oviposition are not as closely coupled as in other pollinating seed-eating mutualisms which have been described, such as the fig–fig wasp, the yucca–yucca moth, and the senita cactus–senita moth mutualisms where pollination takes place only during oviposition (Janzen 1979; Holland and Fleming 1999).

Indeed, both male and female *Chiastocheta* were shown to efficiently transfer pollen from a globeflower to another, independently from egg-laying attempts (Després 2003). However, when pollen transfer is very limiting, in low fly density populations, every visit contributes to increase seed set, including visits by ovipositing females. Pollination ranged from 49 to 93% of seed initiated across populations, which is comparable to the pollination efficiency observed in several fig species (46–95%, Herre and West 1997), where pollination is active but performed only by ovipositing females.

Predation costs

Theoretical studies on the population dynamics of seed predators are usually based on the assumption that insects eat seeds randomly (Holland and De Angelis 2001; Morris et al. 2003). Our empirical data do not support a random search model as the best predictor of the proportion of seed eaten as a function of egg density. The best fitting model generated values superior to those of the random search model for low larval density and inferior to those for high larval density. This indicates that larvae do not move randomly from a seed to another but through specific pathways increasing search efficiency at low density (Pellmyr

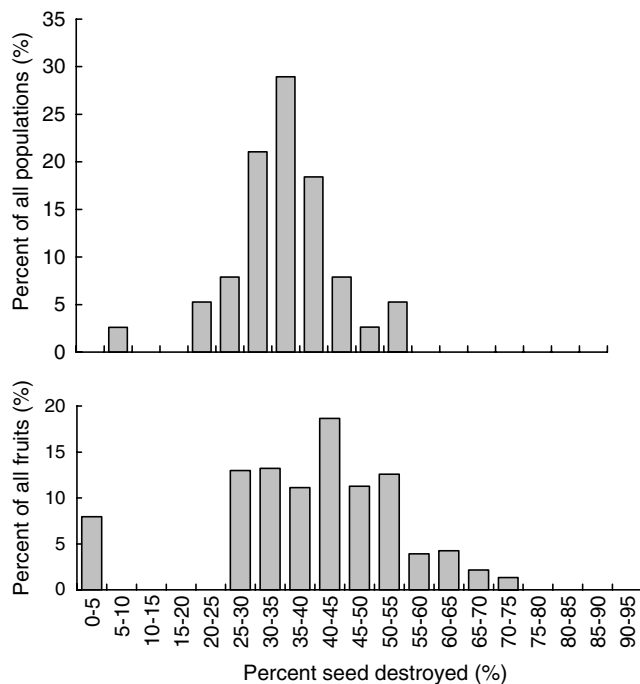


Fig. 3 Distribution of the proportion of seed lost to pollinator larvae for records of 38 globeflower populations (different populations and/or different years), and for all globeflower individuals analysed ($n = 1,710$) among the 38 study populations. Costs are grouped in intervals of 5%. Costs are normally distributed among populations, but not among individuals, because some individuals have no eggs and therefore no costs; such a departure from normality is verified in all populations with more than one unparasitized globeflower (Kolmogorov–Smirnov tests)

1989), but that at high larval density, larval competition prevents efficient seed predation (Jaeger et al. 2001). The population cost model generates values inferior to those of the individual cost model, i.e. egg aggregation on flowers within population decreases costs predicted by the individual cost model. *Chiastocheta* egg aggregation among globeflowers within populations occurs at various latitudes (Johannesen and Loeschke 1996; Després and Jaeger 1999). Egg aggregation is species dependent, i.e. five out of six *Chiastocheta* sp. do aggregate their eggs, while the first species to lay (*C. rotundiventris*) uniformly distributes eggs, avoiding laying more than one egg per flower head (Després and Jaeger 1999); the differences in egg aggregation observed across populations may reflect different species composition of the community of pollinators. This study shows that non-uniform egg distribution among globeflowers benefits the whole population (lower global predation cost due to increased larval competition) but is costly for the few individual plants attracting higher egg densities. These individuals are not larger globeflowers as carpel number is not correlated with egg density per carpel: although larger plants attract more

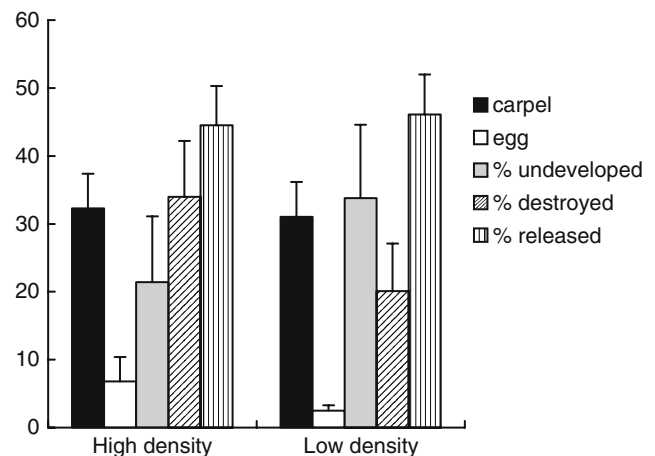


Fig. 4 Mean (\pm SE) carpel and egg numbers and proportion of ovules undeveloped, destroyed and released, in globeflower populations with high and low *Chiastocheta* densities. Fly density per population was estimated as the mean number of eggs per flower head [four or more eggs per flower head ($n = 23$) (High density); less than four eggs per flower head ($n = 15$) (Low density)]. Effect of *Chiastocheta* density on the proportion of: undeveloped ovules $H_{1,36} = 9.9$, $P < 0.001$ (% undeveloped); destroyed ovules $H_{1,36} = 17.9$, $P < 0.001$ (% destroyed); released seeds $H_{1,36} = 0.55$, $P = 0.459$ (% released) (Kruskal–Wallis non-parametric tests)

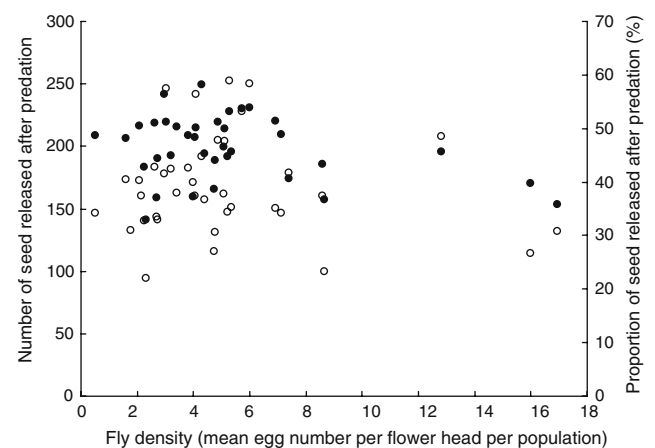


Fig. 5 Mean number (white circles) and proportion (black circles) of seed released per population ($n = 38$ and $n = 36$, respectively) as a function of fly density (mean number of eggs per flower head). The linear regressions are non-significant (not shown)

eggs, they are not disproportionately more infected than smaller plants. This suggests that ovipositing females have a precise evaluation of the size of the resource and of the number of eggs already laid. This evaluation can be visual, olfactory, or tactile, through pheromone deposition by previous ovipositing females (Huth and Pellmyr 1999) or through egg associated compounds (de Jong and Stadler 2001). Whether the female decides to oviposit or not, evaluation of flower size and egg content involves penetrating the closed

Table 4 Multiple regressions predicting absolute number and proportion of seed produced before (*Gross benefit*) and after predation (*Net benefit*) against carpel and egg numbers across populations. *n* Number of populations analysed

Gross benefit	Proportion of seed initiated (<i>n</i> = 36)				Number of seeds initiated (<i>n</i> = 38)			
	Parameter estimate	SE	<i>t</i> -value	<i>P</i> -value	Parameter estimate	SE	<i>t</i> -value	<i>P</i> -value
Intercept	0.4847	0.1205	4.02	0.0003	−124.09	41.70	−2.98	0.0053
Carpel	0.0050	0.0036	1.40	0.1722	11.154	1.2633	8.83	<0.0001
Egg	0.0181	0.0046	3.90	0.0004	7.4753	1.6220	4.61	<0.0001
Net benefit	Proportion of seed released (<i>n</i> = 36)				Number of seeds released (<i>n</i> = 38)			
	Parameter estimate	SE	<i>t</i> -value	<i>P</i> -value	Parameter estimate	SE	<i>t</i> -value	<i>P</i> -value
Intercept	0.3302	0.0669	4.93	<0.0001	−64.405	23.476	−2.74	0.009
Carpel	0.0049	0.0020	2.49	0.0178	7.4968	0.7110	10.54	<0.0001
Egg	−0.0048	0.0025	−1.87	0.0704	−0.9362	0.9130	−1.03	0.3122

corolla and coming into close contact with stigmas and pollen, thereby favouring pollen transfer. Therefore, variation in carpel number among globeflowers within a population may be a way to manipulate fly behaviour by forcing females to visit many flowers.

The mean proportion of seed eaten, 36% (range 8–56% across populations) is comparable to that observed in other pollinating seed parasite mutualisms (18–60% across several fig species, 19–29% in senita cactus and 1–45% in several *Yucca* sp., reviewed in Bronstein 2001). However, the distribution of costs among individuals differs in these various mutualistic interactions. Most globeflowers (92%) bred at least one larva, whereas most senita cactus and yucca fruits contain no pollinator larvae due to egg/larval mortality and/or failed oviposition (Holland and Flemming 1999). In dioecious *Ficus* sp., half the individuals (the female trees) contain no pollinator larvae, while in monoecious *Ficus* sp. all individuals contribute to the pollinator population. The average benefit-to-cost ratio for *T. europaeus* interacting with *Chiastocheta* was 3, comparable to that observed in yucca and senita cactus interacting with their specific pollinating seed predators (2–5, Fleming and Holland 1998). The population benefiting the most from the interaction was that with the lowest fly density, with a benefit-to-cost ratio of 11.

Is there a conflict of interest between the plant and the fly?

As both the plant and the fly rely on seeds for their reproduction, there is potential for a conflict of interest between the partners, each being selected to maximize its fitness by monopolizing the common resource. The expectation is therefore to observe a negative relationship between the number of seeds released by the plant

and the number of seeds eaten by the larvae. However, simple correlations across the 38 populations showed a positive relationship between the number of seeds released (plant fitness) and the number of seeds eaten (fly fitness); such a positive relationship has also been observed across 17 *Ficus* sp. (Herre and West 1997). This suggests that the plant and the pollinator have aligned interests: plants that provide more food to pollinators are also those that produce more viable seeds. However, when the size of the available common resource pool was controlled for statistically, the negative trade-off between eaten and released seeds was ubiquitous (see also Herre and West 1997), as expected in a conflictual relationship.

How are costs distributed across globeflowers?

All individuals do not share equally the cost of rearing pollinator larvae: some, generally large flowers, attract more eggs than others. However, despite large flowers being more heavily infected, and losing a higher absolute number of seeds to larvae, they still released the same proportion of seed after predation as did small flower heads (no effect of carpel number on net proportion of seed produced). Interestingly, individuals with no eggs had significantly fewer carpels than average. These individuals which benefit from pollination while losing fewer seeds to predation can hardly be viewed as cheaters; first, their probability of escaping from oviposition is directly linked to fly density in the population; second, their low investment in carpels reflects more the quality of their microenvironment and/or past reproductive history than a genetically different strategy. Indeed, carpel number is largely environmentally determined, as showed by the significant effect of year on carpel number, and by experimental nutrient supply: transplanted plants given a

nutrient supply 8 times higher than that in natural populations increased flower size by 33% (Å. M. Hemborg and L. Després, unpublished data). Carpel number is thus a plastic character. As globeflowers are long-lived plants, their flower size is likely to vary across years depending on their age and past reproductive history, so that the global cost of rearing pollinators' larvae throughout a plant's lifetime may not differ strongly across individuals.

Mechanisms of stabilization of the interaction

Despite the large fluctuations in fly density observed over time and space, larval predation costs and net seed production remain strikingly constant across populations. The lack of substantial variation in costs and net seed set among populations has been observed in other obligate mutualisms, and supports theoretical predictions for specialized and obligate interactions that selection pressures should be stronger than in facultative mutualisms on traits controlling partner over-exploitation. In yucca and senita cactus both larval/egg intrinsic low survival and high probability of fruit abortion were shown to be important factors limiting seed predation and controlling for seed predator population size (Addicott 1986; Pellmyr and Huth 1994; Holland and Fleming 1999). In the case of the globeflower–globeflower fly interaction, egg/larval survival is high and there is no fruit abortion. The stability of the interaction comes from fly population regulation by density-dependent competition, as revealed by negative correlations between fly fitness and fly density: larval mass decreases with increasing number of larvae co-developing in a flower head (Després and Cherif 2004). At low fly densities, there is virtually no competition among larvae, and insect reproductive success is high, leading to an increase in pollinator population size the following generation. At high fly densities, larvae compete intensively but never to the point of destroying all the developing seeds: flower heads release part of their seeds before full development of larvae (Jaeger et al. 2001), therefore controlling pollinator population size by starving the larvae. Variation in carpel number leading to non-uniform egg distribution among globeflowers could be another plant trait involved in the manipulation of a pollinator's behaviour, enhancing the efficiency of pollination and intensifying larval competition. Furthermore, preliminary experiments suggest another way for globeflowers to control over-exploitation by larvae: infected globeflowers over-produce a C-glycosyl flavone, and the concentration of this induced chemical increases with increasing number of larvae co-developing in a flower head (unpublished

results). C-glycosyl flavones were shown to be involved in larval growth inhibition in the corn earworm (Wiseman et al. 1993).

In conclusion, this study shows that the globeflower–globeflower fly association is strongly mutualistic over a wide geographic area, with on average 74% of seeds initiated (range 49–94%), from which 64% (range 44–92%) are devoted to plant reproduction. The benefit-to-cost ratio varies little across populations, as previously found in other obligate mutualisms involving a plant and a seed-eating pollinator. Moreover, most analysed plants (92%) were parasitized by at least one larva, i.e. most plants contribute to sustain the pollinator population. However, costs are not equally distributed across globeflowers: large plants host more larvae and lose a higher absolute number of seeds to predation than smaller plants, but the proportion of seed eaten is independent from flower size. Flower size reflects the level of resource a plant can allocate to its reproduction in a given year, which is likely to vary throughout an individual's life. Individual plants pay proportionally for their current resource status to sustain the pollinator population.

Acknowledgements We thank N. Jaeger and J.-F. Desmet for field assistance and A. Herre for helpful comments on the manuscript. L. D. was supported by the French Centre National de la Recherche Scientifique (CNRS) and Å. M. H. by the Royal Swedish Academy of Sciences, the National Research Foundation in South Africa, and the Swedish Foundation for International Cooperation in Research and Higher Education (STINT).

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