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Evolutionary conflict between *Trollius europaeus* and its seed-parasite pollinators *Chiastocheta* flies

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ABSTRACT

Mutualisms are characterized by balanced reciprocal exploitation. This creates an evolutionary conflict in that selection will favour individuals that increase their fitness at the cost of the mutualist partner. To counter this evolutionary instability, each partner must be able to prevent over-exploitation by the other. In plant/seed–parasite pollinator mutualisms like that involving the globeflower *Trollius europaeus* and the globeflower fly (*Chiastocheta* spp.), ovipositing females can have a more or less mutualistic/antagonistic effect on plant seed output, depending on the amount of pollination achieved during oviposition, the number of eggs laid and seed predation per larva. We found that flowers with no *Chiastocheta* egg had a high seed set and there was no significant increase in seed set before predation with increasing egg load, suggesting that most pollination is achieved by non-ovipositing visitors (males and/or non-ovipositing females). Hence, additional eggs do not lead to higher pollination, oviposition is a non-mutualistic behaviour and, therefore, there is a conflict between *T. europaeus* and *Chiastocheta* flies for the number of eggs laid. Egg load increases throughout flower lifespan. No mechanism seems to have evolved to regulate the number of eggs laid on *T. europaeus*. For example, controlled pollination experiments showed that *T. europaeus* cannot limit *Chiastocheta* oviposition by triggering flower senescence as soon as full pollination has been achieved. In this context, the high average number of eggs per flower observed in alpine populations is not surprising. Finally, the decrease in net seed production with increasing egg load was weak. We discuss the other factors involved in the regulation of the conflict between *T. europaeus* and *Chiastocheta* flies.

Keywords: *Chiastocheta*, conflict, flower senescence, mutualism, oviposition, seed predation, *Trollius europaeus*

INTRODUCTION

In obligate plant/seed–parasite pollinator systems, such as fig–fig wasp, yucca–yucca moth and globeflower–globeflower fly, the visit of ovipositing females benefits the plant through pollination but is costly because the resulting larvae consume a fraction of the seeds produced; therefore, there is a potential conflict of interest between the two partners of the

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interaction (Thompson, 1982, 1994; Janzen, 1983; Addicott, 1986; Pellmyr, 1989; Bronstein, 1992; Anstett *et al.*, 1996a). Individuals of each interacting species are selected for higher exploitation of individuals of the other species. This can lead to the demise of cooperation or mutual extinction in the case of an obligate interaction, which could be one cause of the scarcity of obligate plant–animal mutualisms (Pellmyr, 1997).

The interaction between the globeflower *Trollius europaeus* and its seed parasite pollinators *Chiastocheta* spp. has been less extensively studied than fig–fig wasp and yucca–yucca moth, but it was shown to be an obligate mutualism in Finland (Pellmyr, 1989) and in the Alps (Bratteler and Widmer, 1998; Jaeger and Després, 1998): *T. europaeus* is only pollinated by *Chiastocheta* flies, whose larvae feed only on *T. europaeus* seeds. In the fig–fig wasp and yucca–yucca moth systems, only ovipositing females pollinate, so that only infested fruits will produce seeds. In those systems, early visits are more beneficial than late visits because the number of ovules remaining to be fertilized decreases with visit rank: there is an optimal number of eggs laid corresponding to the equilibrium between the number of ovules fertilized by the ovipositing female and the number of seeds destroyed by its progeny. As soon as this optimum is reached, each additional egg will lead to increased costs for the plant and the conflict arises. Theoretical stability of obligate plant/seed–parasite pollinator interactions requires a mechanism limiting over-exploitation of one partner by the other. Several plant traits have been invoked to regulate the number of eggs laid, such as differential abortion/development of highly infested fruits (Bronstein, 1992; Pellmyr and Huth, 1994; Richter and Weis, 1995; Wilson and Addicott, 1998) and/or flower morphology or position (West and Herre, 1994; Anstett *et al.*, 1996b; Nefdt and Compton, 1996). Unlike in these two well-studied systems, the globeflower–globeflower fly interaction is unique in that both males and females visit flowers and the role of ovipositing versus non-ovipositing flies in passive pollination is unclear. If non-ovipositing flies do indeed pollinate, then maximum ovule fertilization is achieved more quickly and the conflict is likely to be more intense than if only ovipositing females were pollinators. Moreover, since emerging larvae will not carry pollen of their natal plant (in contrast to the fig–fig wasp system), there is no gain in oviposition for *T. europaeus* male reproductive success: seed loss through predation is not compensated by an increase in pollen export of the parasitized plant, reinforcing the intensity of the conflict.

Based on a positive correlation he observed in Finland between the number of *Chiastocheta* eggs laid and the percentage of seed initiation per flower, Pellmyr (1989) suggested that oviposition plays a major role in pollination. Moreover, assuming a constant amount of seeds eaten per larva, Pellmyr predicted that predation cost imposed by each additional egg was compensated by additional fertilization of ovules during oviposition, and suggested that there was no conflict between the plant and its seed–parasite pollinators, at least under the *Chiastocheta* densities observed in Finland. However, in populations experiencing high pollination rates, like in the Alps (Jaeger and Després, 1998; Hemborg and Després, 1999), late-ovipositing females are likely to have a more detrimental effect on seed set after predation than early-ovipositing females, as maximum ovule fertilization is already achieved at the time of their visit, so that they will only increase the predation load (Després and Jaeger, 1999). In such a context, one way *T. europaeus* could retaliate against late-ovipositing females is by accelerating flower senescence as soon as full pollination is achieved, not only to decrease the costs of flower maintenance, but also to prevent subsequent ovipositions leading to an increase in seed destruction. Indeed, several studies of other plant species suggest that flower senescence can be triggered – or, at least, accelerated – in the case of high pollen deposition (Primack, 1985; Ashman and Schoen, 1994; Proctor and Harder, 1995).

In the present study, we first analyse the relationship between the number of *Chiastocheta* eggs laid per flower and seed set before and after predation by larvae to assess the intensity of the conflict between the plant and the insect. Second, we analyse the increase in the number of *Chiastocheta* eggs laid throughout flower longevity and, finally, test for the possibility of a control by the plant itself on this increase through an acceleration of flower senescence when a large (non-limiting) amount of pollen is deposited on stigmas.

MATERIALS AND METHODS

Trollius europaeus L. (Ranunculaceae) is a hermaphroditic self-incompatible arctic–alpine species found in moist meadows above 700 m in the European Alps. More details concerning the biology of this species in the Alps are given in Jaeger and Després (1998). A plant (genet, *sensu* Harper, 1977) usually produces only a single flower a year so that experiments were performed on different plants. A flower contains about 30 carpels dehiscing 3 weeks after the end of flowering and about 160 stamens dehiscing sequentially throughout flower longevity (typically 5–8 days). In the Alps, the plant is passively pollinated by six species of *Chiastocheta* flies (Anthomyiidae): *C. rotundiventris*, *C. dentifera*, *C. inermella*, *C. macropyga*, *C. setifera* and *C. trollii*. *Chiastocheta* flies are the only pollinators of *T. europaeus* and *Chiastocheta* larvae feed only on *T. europaeus* seeds. Flies penetrate inside the globe-shaped flower where they eat nectar and pollen (Hagerup and Peterson, 1956) and they mate. Visitation patterns differ between the sexes: males visit many flowers searching for females and spend a few seconds in each flower, whereas females spend much more time in the globe whether they oviposit or not. Females deposit one to several eggs on or between the carpels at various flower stages, depending on the species (Pellmyr, 1989; Després and Jaeger, 1999). Larvae can move freely from one carpel to another and were shown to exhibit a specific mining pattern (Pellmyr, 1989). Each larva eats several seeds and falls into the soil to pupate.

Oviposition and seed set frequency before predation

Six sites (Cherlieu, Cottaves, Crolles, Som, Galibier and Lac) from different parts of the French Alps were previously analysed for egg number per flower and seed set frequency before predation (number of seeds/number of ovules) in 1995, 1996 and 1997 (Jaeger and Després, 1998). We used these data to determine the effects of population, year and egg number (covariate) on seed set frequency. The relationship between egg number and seed set frequency was tested within each population for each year (linear regression) because the different populations experience different ecological conditions (Jaeger and Després, 1998).

Oviposition and seed set frequency after predation

Flowers with a number of *Chiastocheta* eggs (range 1–19) were selected 1–2 weeks after the end of flowering in three populations ($n = 110$ at Lac in 1996; $n = 69$ at Galibier in 1997; $n = 125$ at Som in 1997). We counted the number of carpels of these flowers and placed them in nylon bags to avoid subsequent oviposition and to collect dispersing seeds. Intact seeds were counted after all larvae had left the fruit (4–5 weeks after the end of flowering). We estimated the seed set frequency after predation as the number of intact seeds remaining after predation divided by an estimate of the number of ovules. To estimate the number of ovules, we counted the number of ovules per carpel from five carpels and the number of carpels on 30–50 other flowers from each of the three populations. The number of ovules per carpel was positively correlated with the number of carpels (Lac 1996: $r = 0.36$, $n = 50$, $P < 0.05$; Galibier 1997: $r = 0.35$, $n = 30$, $P < 0.05$; Som 1997: $r = 0.34$, $n = 49$, $P < 0.05$). Hence, for each flower, the number of ovules was estimated from the number of carpels based on an empirically fitted curve in each population.

Number of Chiastocheta eggs laid throughout flower longevity

Mean flower longevity is 7 days (Jaeger and Després, 1998). Hence, we estimated the number of *Chiastocheta* eggs laid on flowers each day for 7 days at Cherlieu, Som and Lac in 1997. For this purpose, in each population we tagged 350 flowers on their first day of flowering (i.e. the first day of anther dehiscence). For days 1–7 of flowering, 50 flowers were bagged each day to prevent subsequent visits. The total number of *Chiastocheta* eggs laid was counted on each flower. These flowers were used in Després and Jaeger (1999).

Influence of pollination rate on flower longevity

The possibility of accelerated flower senescence due to high levels of pollination was tested by tagging 120 flowers on their first day of flowering at Galibier in 1997. From the first to the fifth day of flowering, 20 flowers per day were saturated with a mixture of pollen from 5 to 10 donors of the population. Twenty other flowers were left unmanipulated as a control. Each flower was checked for sepal retention every day. In a previous study on naturally pollinated flowers, we found a strong correlation between the end of flower longevity and the end of anther dehiscence (Jaeger and Després, 1998). This suggests that accelerated senescence of the flower should be associated with accelerated anther dehiscence. Hence, each flower was also checked for anther dehiscence every day.

Data analysis

To normalize the data, frequencies were arcsine square root-transformed for all analyses and egg counts were square root-transformed only for analyses of variance. Flower age, day of pollen saturation, population, year and interactions were all considered fixed effects and type III mean squares were used in the analyses of variance. The number of eggs was used as a covariate in the analyses of seed set before and after predation. All analyses were performed using SAS statistical programs (REG and GLM procedures, SAS Institute Inc., Version 6, 1994).

RESULTS

Oviposition and seed set frequency before predation

There were significant effects of year and number of eggs on seed set frequency before predation (Table 1). However, when each population was considered separately each year, the relationship between seed set frequency before predation and the number of *Chiastocheta* eggs laid per flower was not significant ($P > 0.05$) in 16 of 18 records (Table 2),

Table 1. Results of analysis of covariance on seed set frequency, using number of eggs as a covariate before predation in six populations analysed for three consecutive years (population and year considered as fixed effects) and after predation in three populations (population considered a fixed effect)

	Seed set frequency					
	Before predation			After predation		
Source of variation	d.f.	MS	F	d.f.	MS	F
Egg number	1	0.58	9.76**	1	0.65	23.8***
Population	5	0.08	1.42	2	0.02	0.77
Year[a]	2	0.40	6.67**			
Population × year	10	0.04	0.73			
Population × egg number	5	0.09	1.54	2	0.11	3.89*
Year × egg number	2	0.03	0.45			
Population × year × egg number	10	0.03	0.5			
Error	817			300	0.027	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[a] In the analysis of seed set frequency after predation performed at Lac in 1996, at Galibier in 1997 and at Som in 1997, no effect of year can be tested. d.f. = degrees of freedom; MS = mean square (type III).

and number of eggs only accounted for 0–20% of the variation in seed set frequency. Moreover, although they were rare, flowers without eggs had a high seed set frequency, ranging from 0.27 to 0.98 (Table 3).

Oviposition and seed set frequency after predation

There were significant effects of number of eggs and interaction between population and egg number on seed set frequency after predation (Table 1), so that each population was analysed separately. In the three populations, seed set frequency after predation decreased with the number of *Chiastocheta* eggs laid on the flower (Lac 1996: $y = -0.0164x + 0.46$; Galibier 1997: $y = -0.0109x + 0.51$; Som 1997: $y = -0.0054x + 0.44$; Table 2), but this decrease was weak. As the number of eggs was found to be positively correlated with the number of carpels in the three populations (Lac 1996: $r = 0.18$, $n = 110$, $P < 0.05$; Galibier 1997: $r = 0.26$, $n = 69$, $P < 0.05$; Som 1997: $r = 0.31$, $n = 125$, $P < 0.05$), a multiple linear regression of seed set after predation was performed against the number of eggs and the number of carpels: seed set decreased with the number of eggs laid on the flower for any given number of carpels (Table 4).

Number of Chiastocheta eggs laid throughout flower longevity

There were significant effects of population ($F_{2,648} = 13.72$, $P = 0.0001$), flower age ($F_{6,648} = 51.04$, $P = 0.0001$) and population × flower age ($F_{12,648} = 4.42$, $P = 0.0001$) on the number of *Chiastocheta* eggs laid. There was a progressive increase in the number of *Chiastocheta* eggs laid per flower throughout flower longevity in the three populations, and this increase was significant until the sixth day of flowering (Duncan's multiple-range tests; Fig. 1).

Table 2. Linear regressions between the number of *Chiastocheta* eggs laid per flower and seed set frequency before predation at six sites in 1995, 1996 and 1997, and after predation at Lac in 1996, Galibier in 1997 and Som in 1997

		Seed set before predation			Seed set after predation		
Population	Year	*n*	R^2	*F*	*n*	R^2	*F*
Cherlieu	1995	93	0.05	4.90*			
	1996	49	0.06	3.47			
	1997	49	0.0006	0.03			
Cottaves	1995	37	0.0009	0.03			
	1996	49	0.006	0.28			
	1997	48	0.0008	0.04			
Crolles	1995	34	0.20	7.97**			
	1996	49	0.02	0.91			
	1997	42	0.03	1.22			
Som	1995	44	0.08	3.53			
	1996	49	0.04	2.01			
	1997	49	0.02	1.14	125	0.03	3.94*
Galibier	1995	48	0.007	0.35			
	1996	49	0.008	0.37			
	1997	23	0.01	0.28	69	0.06	4.16*
Lac	1995	45	0.001	0.04			
	1996	49	0.006	0.29	110	0.20	28.3***
	1997	29	0.02	0.55			
Total		835					

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
n is the number of flowers sampled and R^2 is the proportion of seed set variance that is predictable from egg number.

Table 3. Seed set frequency of flowers without *Chiastocheta* eggs

Population	Year	*n*	Seed set frequency
Cherlieu	1995	6	0.72 (0.57–0.84)
Cherlieu	1996	3	0.69 (0.60–0.78)
Cottaves	1995	1	0.62
Cottaves	1996	4	0.70 (0.51–0.98)
Cottaves	1997	3	0.84 (0.77–0.91)
Crolles	1996	2	0.73 (0.64–0.82)
Crolles	1997	7	0.59 (0.27–0.86)
Galibier	1996	6	0.66 (0.29–0.92)
Galibier	1997	1	0.87
Lac	1995	3	0.75 (0.54–0.85)
Lac	1996	3	0.63 (0.47–0.85)

[a] In some populations × years, all flowers harboured at least one *Chiastocheta* egg. Means are given with minimum and maximum values in brackets. *n* = sample size.

Table 4. Multiple linear regression of seed set after predation against the number of *Chiastocheta* eggs and the number of carpels

Population	n	Parameter	Mean ± s	t for H_0: parameter = 0
Lac 1996	110	Intercept	−10.9 ± 18.1	−0.6
		No. carpels	5.77 ± 0.55	10.5***
		No. eggs	−7.3 ± 1.1	−6.6***
Galibier 1997	69	Intercept	−1.1 ± 18.8	−0.06
		No. carpels	5.8 ± 0.79	7.2***
		No. eggs	−3.3 ± 1.6	−2.1*
Som 1997	125	Intercept	22.9 ± 19.2	1.19
		No. carpels	4.38 ± 0.84	5.2***
		No. eggs	−1.19 ± 0.87	−2.2*

* $P < 0.05$, *** $P < 0.001$. n = the sample size.

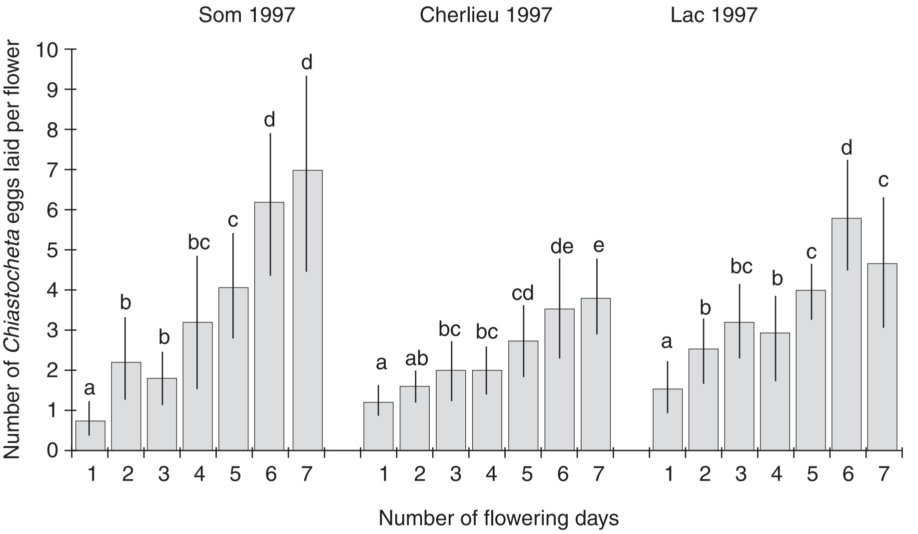

Fig. 1. Variation in the total number of *Chiastocheta* eggs laid throughout seven consecutive flowering days at Som, Cherlieu and Lac in 1997. 1 = first day of anthesis, 2 = second day of anthesis, and so on. Significantly different values of egg load are denoted by different letters (Duncan's tests; $P < 0.05$).

Influence of pollination rates on flower longevity

The day on which pollen saturation was performed had no effect on flower longevity ($F_{5,120} = 1.10$, $P = 0.37$) or the duration of anther dehiscence ($F_{5,120} = 1.18$, $P = 0.32$). Whatever the day of pollen saturation, the end of anther dehiscence occurred about 1 day before the end of flower longevity (Fig. 2). There was a strong positive correlation ($r = 0.42$, $n = 117$, $P < 0.05$) between the end of flower longevity and the end of anther dehiscence.

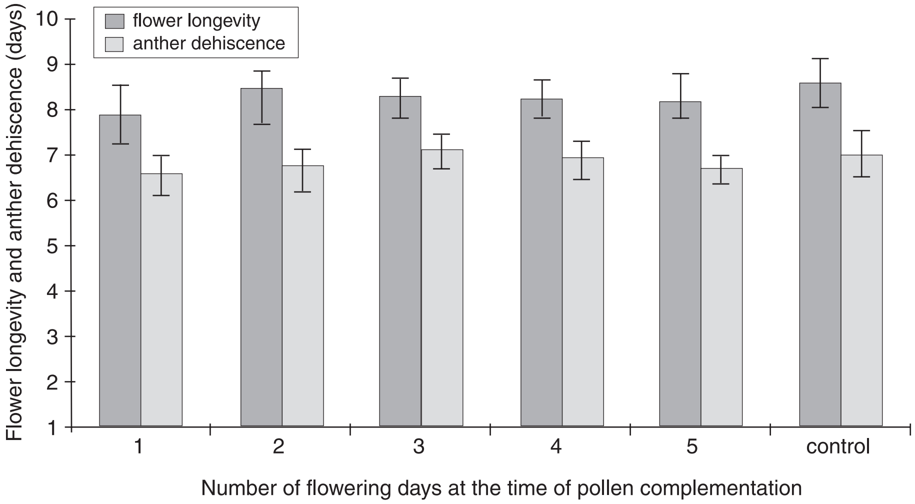

Fig. 2. Longevity and anther dehiscence of flowers saturated with pollen on their first to fifth days of flowering at Galibier in 1997. Flower longevity and anther dehiscence are not significantly different among treatments ($P > 0.05$).

DISCUSSION

Our results show that there is no direct and strong relationship between oviposition and seed set before predation: oviposition and pollination are independent. Hence, additional eggs do not lead to higher pollination: oviposition is a non-mutualistic behaviour and there is a conflict between *T. europaeus* and *Chiastocheta* flies for the number of eggs laid. The conflict was previously assumed to be stronger between *T. europaeus* and the late ovipositing species: females of *C. inermella*, *C. trollii* and *C. dentifera* lay their eggs from the fifth to the seventh day of flowering, after most flowers have been saturated with pollen (Després and Jaeger, 1999). The results of the present study show that early oviposition events should also be avoided by *T. europaeus* because enough pollen transfer is achieved by visits without oviposition. Indeed, seed set frequency of flowers without eggs was high, ranging from 0.27 to 0.98. Due to egg loss and possible counting errors, we cannot exclude having missed a few eggs actually laid on flowers. However, our results suggest that visits of males and/or non-ovipositing females significantly contribute to pollination. Unlike in the fig–fig wasp and yucca–yucca moth systems, in which active pollination has evolved, the globeflower–globeflower fly interaction is based on passive pollination only. In such a system, not only oviposition, but also nectaring, mate searching or mating can potentially lead to pollination (Pellmyr *et al.*, 1996). *Chiastocheta* visitation rates were observed to be high in these six populations (up to two visits per flower every 10 min; Jaeger and Després, 1998) and most of the visits were achieved by males moving rapidly among flowers. The high number of male visits could lead to significant pollen transfer in spite of their short stay in the flower. Moreover, pollen was observed on all 100 captured males (L.D. and N.J., personal observations).

In a previous study on *T. europaeus* in Finland where reproduction was pollen-limited, Pellmyr (1989) found a significant positive relationship between the number of *Chiastocheta* eggs laid and seed set frequency before predation. However, number of eggs accounted for only 12.9% of the variation in seed initiation frequency in Finnish populations and for 0–20% in Alpine populations. Differences observed between populations may reflect different fly densities and thus different overall pollination efficiencies. In contrast to Finnish populations, hand-pollination experiments showed that seed set was not limited by pollen transfer in the Alpine populations (Jaeger and Després, 1998). Moreover, even in populations where the relationship was significant, this does not prove that females are responsible for most pollination. Alternatively, females may prefer to lay eggs in well-pollinated flowers and/or the most parasitized flowers just happen to be the most visited (Zimmerman, 1980; Pettersson, 1992; Brody and Waser, 1995). Ovipositing females of all *Chiastocheta* species except *C. rotundiventris* show a preference for flowers with a high number of carpels (Pellmyr, 1989; Johannesen and Loeschcke, 1996; present study). This preference could be mediated through large flower size, which was found to favour egg laying in another anthomyiid fly species (Brody, 1992).

Although there is no direct causal relationship between oviposition and pollination, the increases in both the number of eggs laid per flower and pollination rates (Després and Jaeger, 1999) are progressive throughout flower longevity: an increase in the number of seeds produced cannot be achieved without a parallel increase in the number of eggs laid and, hence, predation pressure. Only 7% of 1803 flowers from 20 populations in the French Alps did not harbour any *Chiastocheta* egg (Jaeger, 1998). One way *T. europaeus* could limit the increasing predation pressure due to late antagonistic oviposition is by triggering senescence of its flower when full pollination has been achieved. In a previous study in the same populations, we found that full natural pollination was achieved on days 4–6 of flowering (Després and Jaeger, 1999). *Chiastocheta* densities can be very high in natural populations – up to 16 eggs per flower in the Alps (Jaeger and Després, 1998) and up to 20 eggs per flower in Denmark (Johannesen and Loeschke, 1996) – suggesting that maximum pollination can sometimes be achieved even earlier. However, we did not observe any acceleration of flower senescence when maximal pollination was achieved: flower longevity remained the same regardless of the day the flower became fully pollinated. Furthermore, flower longevity is not significantly longer in the case of no or little pollen deposition (Jaeger, 1998), suggesting that it does not depend on the amount of pollen deposited on the stigmas. The coincidence observed between anther dehiscence and flower longevity suggests that flower senescence occurs as soon as all pollen has been released. Further studies should consider the effect of pollen removal on flower longevity. Indeed, flower longevity is adapted to maximize both seed set after predation (female fitness) and pollen removal by *Chiastocheta* through gradual presentation (male fitness), and there might be a strong selection pressure for flowers to remain attractive until all pollen has been removed. In arctic–alpine species, for which environmental variance is high and pollination efficiency is unpredictable, long-term selection usually favours long flower longevity (Bingham, 1998). Accordingly, the minimum individual flower longevity observed during our 3 year study was 4 days. Most of the variability observed for this trait between populations and years (from 4 to 16 days) is likely to be due to weather conditions – more precisely, to rain and cold temperatures increasing flower lifespan – rather than to genetic factors (Jaeger and Després, 1998). Finally, constraints leading to long flower longevity independently of pollination conditions may favour the co-existence of several *Chiastocheta* species on *T. europaeus*, allowing time partitioning for oviposition. The age of the flower selected by females to lay their eggs differs among *Chiastocheta* species

(Pellmyr, 1989; Després and Jaeger, 1999). A shortening of flower longevity under good pollination conditions would lead to an increase in interspecific competition for oviposition sites, and possibly the exclusion of one or several *Chiastocheta* species. There seems to be a minimal flower longevity of 4–5 days (Jaeger and Després, 1998). Hence, a long fixed flower longevity could be a prerequisite for the co-existence of the six species in the Alps.

Other factors potentially limiting predation pressure can be considered. An increase in the number of carpels leads to an increase in the total number of seeds produced before predation. Hence, for a given number of eggs laid (i.e. a given number of seeds destroyed), flowers with a higher number of carpels are expected to have a higher seed set frequency after predation (Pellmyr, 1992). However, *Chiastocheta* oviposition also increases with increasing number of carpels, leading to higher predation pressure. As a result, we did not observe any significant increase in seed set frequency after predation with increasing number of carpels. Moreover, we found no obvious limitation to the number of eggs laid per flower by *Chiastocheta* flies: females of all species except *C. rotundiventris* usually lay several eggs per flower and females do not space their eggs to all vacant flowers (Johannesen and Loeschcke, 1996). Hence, regulation of the conflict between *T. europaeus* and *Chiastocheta* was not found to occur during oviposition.

Finally, the conflict observed between *T. europaeus* and *Chiastocheta* flies for the number of eggs laid is not as intense as might be expected based on Pellmyr's model: an increase in the number of *Chiastocheta* eggs laid on the flower led to a decrease in seed set frequency after predation in the three populations studied, but this decrease was weak. As opposed to Pellmyr's assumption of a fixed amount of seed consumption per larva, we counted the number of seeds remaining after predation, so that competition between larvae was taken into account in our estimation of predation costs. In another study, we showed that both interference between larvae (insect trait) and early carpel dehiscence preventing a fraction of seeds from being consumed (plant trait) appear to be the main factors regulating the conflict between the globeflower and globeflower flies (Jaeger *et al.*, in press). As a result, despite a mean number of eggs per flower much higher in the Alps (up to 16 eggs per flower; Jaeger and Després, 1998) than in Finland or Sweden (about two eggs per flower; Pellmyr, 1989; Hemborg and Després, 1999), the predation costs imposed by larvae on seeds are always limited, and seed set after predation is relatively stable whatever the number of eggs laid in the flower. In the yucca–yucca moth interaction, some regulation also occurs after oviposition: selective abortion of fruits with heavy egg loads (Pellmyr and Huth, 1994; Richter and Weis, 1995; Wilson and Addicott, 1998), non-random location of infertile seeds and thick membranes (Ziv and Bronstein, 1996) and larval mortality (Keeley *et al.*, 1984; Addicott, 1986) seem to reduce the predation costs imposed by larvae on seeds. In the globeflower–globeflower fly system, the plant cannot regulate seed predation by selectively aborting over-parasitized fruits or carpels, as there is usually only a single fruit produced in a given year, and larvae can move freely from one carpel to another.

In conclusion, there are two causes of evolutionary instability in the interaction between *Trollius europaeus* and *Chiastocheta* flies. First, *T. europaeus* has no advantage in insect oviposition in terms of female fitness (unlike in the *Ficus* and *Yucca* cases) or in insect breeding in terms of male fitness (unlike in the *Ficus* case). Second, fly densities were observed to be very high in several populations, potentially leading to over-exploitation of the host plant. However, the interaction is evolutionarily stable because *T. europaeus* cannot prevent *Chiastocheta* flies from ovipositing and because *Chiastocheta* larvae can consume a fraction of but not all seeds, so that although highly conflictual, the system cannot go to extinction.

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