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Ecological conditions promoting plant specialization on a seed-eating pollinator differ from those stabilizing the interaction

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ABSTRACT

Question: What are the ecological conditions that promote plant specialization on a seed-eating pollinator when less costly alternative pollinators are present?

Mathematical method: An adaptive dynamics model including the ecological dynamics of a plant–seed-eating pollinator mutualistic system.

Key assumptions: Plants are initially pollinated by specialist seed-eating pollinators and by generalist co-pollinators. Plant specialization (floral morphology continuously ranging from closed to open) and seed-eating pollinator morphological preference co-evolve, while co-pollinators always prefer open flowers. When seed-eating pollinators and co-pollinators have similar preferences, seed-eating pollinators are less effective. The functional relationship linking plants and seed-eating pollinators involves pollination efficiency, oviposition rate, the range of floral morphologies an insect is able to deal with (its degree of specialization), and the pollination and oviposition handling times.

Conclusions: Specialization evolves only if pollinators interfere, and it is favoured when co-pollinators' efficiency is low, when seed-eating pollinators' oviposition rate is low, and when the range of floral morphology they deal with is greater for oviposition than for nectar- or mate-searching visits. Moreover, although high pollination efficiency of seed-eating pollinators is a key factor in the persistence of the specific mutualism nowadays, the first steps of the evolution of specialization require an intermediate pollination efficiency of seed-eating pollinators.

Keywords: adaptive dynamics, co-pollination efficiency, evolution, floral morphology, *Trollius europaeus–Chiastocheta* mutualism.

INTRODUCTION

Over the last decade, our view of plant–pollinator interactions has shifted from the long-standing concept of 'pollination syndromes', reflecting extreme specialization as the common outcome of natural selection in those systems, to the widely accepted idea that

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generalization is widespread in pollination ecology, and that specialized plant–pollinator interactions might be the exception rather than the rule (Waser *et al.*, 1996; Johnson and Steiner, 2000; Waser and Ollerton, 2006). Pollination always implies a cost for the plant, such as nectar or pollen lost to feed pollinators, but the cost is especially severe in plant–seed-eating pollinator mutualisms where pollinator offspring feed on potential plant offspring (Pellmyr, 1989; Jaeger *et al.*, 2000; Bronstein, 2001). It is therefore especially intriguing that these mutualisms are among the few examples of extreme pollination specialization. If specialization on specific seed-predators is ubiquitous in all the species of *Yucca* and *Ficus*, this is not the case for the *Trollius* genus, where only the European species, *Trollius europaeus*, is exclusively pollinated by specialized seed-predators (*Chiastocheta* Pokorny, Anthomyiids). All *Trollius* species are perennial herbs, 5–12 cm tall, growing in moist, boreal, montane or subalpine habitats throughout the temperate and arctic regions of Asia, Europe, and North America. Some *Trollius* species are not visited at all by *Chiastocheta* flies, such as *T. pumilus*, *T. ranunculinus*, and *T. laxus*, all of which present an open flat corolla: these species are likely to be pollinated by a wide range of insects (Pellmyr, 1989). Other *Trollius* species, including *T. altaicus*, *T. europaeus*, *T. ledebouri*, *T. asiaticus*, *T. chinensis*, and *T. riederianus*, are visited both by *Chiastocheta* and other pollinators. Molecular phylogenetic analysis showed that an open flower is the ancestral state in the *Trollius* genus and that all species visited by *Chiastocheta* flies form a derived clade (Despres *et al.*, 2003). The only morphological character clearly linked to the presence of *Chiastocheta* is the bowl shape of the corolla, which reaches its extreme in *T. europaeus* by forming a totally closed globe. The globular shape of *T. europaeus* is thus likely to be responsible for the exclusion of pollinators other than *Chiastocheta* flies. These small flies enter the globe by spirally crawling between sepals, whereas the sepals prevent larger insects from entering. Furthermore, a field experiment involving floral morphology manipulation has demonstrated that present-day *Chiastocheta* prefer to visit closed phenotypes (Ibanez *et al.*, 2009). We hypothesize that *T. europaeus* and *Chiastocheta* flies co-evolved reciprocal specialization through various traits, one of them being the closed flower shape co-evolving with the insect flower shape preference. According to this hypothesis, ancestral *Chiastocheta* flies were adapted to the open floral morphology they dealt with. Indeed, all of the *Chiastocheta* species described to date [11–17, depending on authors (Pellmyr, 1992; Despres *et al.*, 2002)] lay eggs specifically on *Trollius* species regardless of the floral morphology of the host-plant, their larvae feeding only on *Trollius* seeds, on globular flowers for European *Chiastocheta* species (six species described) or on open flowers for Asiatic species. We therefore assume that ancestral *Chiastocheta* flies were dependent on an ancestral open-flower, generalist, *Trollius* species.

Plant specialization on seed predators is especially intriguing when co-pollinators feeding only on nectar and not on developing seeds are also present in the community, which is often the case (Holland and Fleming, 2002; Thompson and Cunningham, 2002). If seed-eating pollinators consume more seeds than they fertilize ovules, they act as parasites rather than mutualists (Holland and Fleming, 2002; Thompson and Cunningham, 2002; Thompson and Fernandez, 2006). Plants are then expected to develop traits that allow pollination by generalist co-pollinators and to minimize the costs imposed by specialized seed-eating pollinators. Therefore, the presence of co-pollinators is predicted to prevent the evolution of plant specialization on seed-predators, even when those are highly efficient pollinators, as observed for example in the *Lithophragma parviflorum–Greya politella* interaction (Pellmyr and Thompson, 1992). However, other potential pollinators are present in *Trollius europaeus* populations and were presumably also present in past populations, raising the question of why specialization on seed-predators evolved in the European globeflower and not in other *Trollius* species. We built an adaptive dynamics model to understand how specialization might have co-evolved in the initial context of an open flower pollinated by both *Chiastocheta* and generalist pollinators. Our aim was to determine under which ecological conditions closure of the flower and *Chiastocheta*'s preference for closed flowers could co-evolve.

MODEL

Background

We consider a single community composed of a *Trollius* species labelled T, the *Chiastocheta* genus labelled C, and other potential pollinators, such as bumblebees and syrphids, labelled P. The density of P is assumed to be constant, as these pollinators are generalists and mostly rely on flowers other than T, which they visit only occasionally, whereas the density of C varies along the ecological time scale and depends entirely on T for reproduction. T is pollinated by both C and P, and only suffers from seed predation from C. Both C and P are assumed to be nectar consumers of equivalent intensity, thus nectar-producing costs are not incorporated in the model.

Interactions between species are assumed to be mediated by the degree of phenotypic matching between quantitative traits (Dieckmann *et al.*, 1995; Nuismer and Doebeli, 2004). T, C, and P traits are x, y, and z, respectively. We interpret x as the degree of flower opening. The values $x = 0$ and $x = 1$ correspond to completely closed and wide open flowers respectively, with any morphologically possible value between 0 and 1. y and z are the insects' preferences when looking for a flower to visit. When traits x and y match (respectively x and z), C (respectively P) pollinate T at maximum efficiency, and when y and z match, the negative effect of P on pollination by C reaches its maximum. Starting with all traits close to 1, the model assesses the co-evolution of x and y, while z remains constant and equal to 1 because pollinators other than *Chiastocheta* are assumed to be generalists.

Ecological dynamics

The ecological dynamics are given by:

$$\frac{dT(x,t)}{dt} = T(poll(T,C,P,x,y,z)(1 - pred(T,C,x,y))(1-T) - d), \tag{1a}$$

$$\frac{dC(y,t)}{dt} = C\left(b_c T poll(T,C,P,x,y,z) pred(T,C,x,y)\left(1 - c_c\frac{C}{T}\right) - 1\right), \tag{1b}$$

where $poll(T, C, P, x, y, z)$ is the pollination probability function, depending on the densities of T, C, and P and their respective traits; and $pred(T, C, x, y)$ is the predation probability function, independent of P and z because generalist pollinators do not feed on seeds. The parameters d, b_c, and c_c represent respectively globeflower mortality, *Chiastocheta* fecundity, and *Chiastocheta* intra-specific competition depending on the ratio C/T. Fixed parameters, ecological variables, evolutionary variables, and their signification are summarized in Table 1. Equations (1) in this paper have some similarities with equations (5) and (6) of Holland *et al.* (2002) and equation (3) of Morris *et al.* (2003). In both equations, the right-hand sides correspond to population density multiplied by the per-capita growth rate, which is decomposed into birth and death rates. The T per capita birth rate equals the probability of being pollinated multiplied by the probability of escaping predation by larvae and by an intra-specific competition factor. The C per capita birth rate equals the amount of available seeds multiplied by the predation probability and by an intra-specific competition factor that depends on the number of flies per flower. Equations (1), as well as equations (2) below, have some degree of generality and could also describe the ecological dynamics of other plant–seed-eating pollinator systems.

The pollination and predation functions are:

$$poll(T, C, x, y) =$$

$$1 - Exp\left(-\frac{\alpha(x-y, \sigma_{c_1})a_{c_1}\, C(1 - c_p\, \alpha(y-z, \sigma_p)P)}{1 + a_{c_1}\, h_1 T} - \alpha(x-z, \sigma_p)\, a_p P\right), \qquad (2a)$$

$$pred(T, C, x, y) = 1 - Exp\left(-\frac{\alpha(x-y, \sigma_{c_2})a_{c_2} C}{1 + a_{c_2}\, h_2 T}\right). \qquad (2b)$$

The functional responses of equations (2) are derived from DeAngelis and Holland's (2006) equation (5) and have some similarity with the model of Morris *et al.* (2003). The term in the exponential function is the rate at which unpollinated ovules (respectively undamaged seeds) are fertilized (respectively eaten) during a short time scale; it corresponds to a standard Holling type 2 (prey-dependent) functional response (Begon *et al.*, 1996). The pollination rate includes a ratio-dependent term corresponding to the *Chiastocheta* flies' contribution and a pollinator-dependent term corresponding to the other pollinators' effect. The predation rate only includes a ratio-dependent term corresponding to larval feeding. Equations (2) are obtained by integrating pollination and predation rates over the whole period of blooming. In the following, we detail the meaning of all the parameters used.

a_{c_1} is the intensity of pollen transfer per visit ['quality' component *sensu* Herrera (1987)] and a_{c_2} is the oviposition rate. h_1 and h_2 are the intensities of C/T ratio-dependence for pollination and predation; h_1 is the time spent by a fly handling each flower (DeAngelis and Holland, 2006) and h_2 is the time a female requires to lay eggs. If h_1 is low, a single fly is able to pollinate a large number of flowers and pollination efficiency in a globeflower population will depend mainly on the density of *Chiastocheta* with globeflower population size having a limited impact, whereas if h_1 is high, pollination efficiency will depend on the pollinator-to-flower ratio. If h_2 is low, the intensity of predation will essentially depend on the number of ovipositing females, whereas if h_2 is high, predation intensity will depend on the C/T ratio. Extreme cases where h_1 (respectively h_2) is equal to zero correspond to pollinator- (respectively predator-) density dependence. For the analyses, we chose $h_2 > h_1 > 0$ because field data suggest that the intensity of ratio-dependence is stronger for oviposition than for pollination (Despres *et al.*, 2007). c_p is the effect P exerts on C pollination through interference when larger pollinators on the flower chase *Chiastocheta* flies away (S. Ibanez, personal observation) or through pollen waste by generalist pollinators ['ugly' pollinators (Thomson and Thomson, 1992)], and/or stigma clogging with incompatible pollen grains. a_p is the pollination efficiency of P insects, which is expected to be low because generalist pollinators visit a wide range of flowers and therefore transfer many incompatible pollen grains to globeflowers.

Strength of the ecological interactions

The Gaussian function $\alpha(w, \sigma)$ is used to measure the interaction strength between two species (Doebeli and Dieckmann, 2000; Egas *et al.*, 2004, 2005):

$$\alpha(w, \sigma) = \frac{1}{\sqrt{2\pi}} Exp\left(-\frac{w^2}{2\sigma^2}\right), \tag{3}$$

where w is the difference between two traits (the smaller the difference, the stronger the interaction) and σ is a variance-like parameter (when σ is large, the interaction is more tolerant to trait mismatch). Interaction strength concerns pollination and predation. When the pollinator trait (y or z) and the plant trait x match, the visitation rate of pollinating insects reaches its maximum ['quantity' component *sensu* Herrera (1989)]. In the same way, when the *Chiastocheta* trait y and the plant trait x match, the visitation rate of ovipositing flies reaches its maximum. When traits y and z match, the other pollinators have the same morphological preference as *Chiastocheta* flies, so they exert maximum negative effect on their pollination ability.

σ_{c_1} reflects the tolerance of *Chiastocheta* in their pollination behaviour with regard to floral morphology: for high σ_{c_1} values, visitation rate will be close to its maximum even if phenotypes do not match. Similarly, σ_{c_2} is the tolerance of ovipositing flies in their visitation rate with regard to floral morphology: for high σ_{c_2}values, females will still visit flowers, which morphology does not match with their preference. σ_p is the other pollinators' visitation behaviour tolerance with regard to floral morphology. We assume it is equal to the interaction strength between *Chiastocheta* and other pollinators with different floral morphology preferences: if a P pollinator matches the plant trait and occupies the inside of the globe, it will prevent *Chiastocheta* flies from doing so. Finally, we assume that trait match does not influence handling times, and that larval predation efficiency is independent of floral morphology and adult morphological preference.

Evolutionary dynamics

Traits x and y are susceptible to evolutionary change. Our aim is to analyse their co-evolution using the adaptive dynamics mathematical framework, a deterministic approximation of individual-based models, describing evolution in asexual populations under frequency-dependent selection (Metz *et al.*, 1992; Dieckmann and Law, 1996; Geritz *et al.*, 1998). Assuming that evolution is mutation-limited and that mutational steps are small, P and C fitness landscapes are determined by the invasion fitness of mutant phenotypes, defined as the long-term per capita growth rate of an initially rare mutant facing the resident population at its ecological equilibrium [denoted $s(x' \mid x, y)$ and $s(y' \mid x, y)$ (Metz *et al.*, 1992)]. We used the canonical equation of adaptive dynamics (Dieckmann and Law, 1996, equations 4.12 and 6.1), which here is a set of two differential equations describing the co-evolution of x and y traits (equations 4). The canonical equation takes into account the local selection gradients, the mutation rates and the variance–covariance matrix of both traits, and the density of each population at ecological equilibrium [denoted $T^*(x, y)$ and $C^*(x, y)$]. For simplicity, mutation rates and variances were assumed equal to 2 and 1 respectively, and because traits x and y concern different species, they were considered to be independent. As a result, the canonical equations (4) below are simple products of population densities by local fitness gradients:

$$\frac{dx}{dt} = T^*(x, y)\,\frac{\partial s(x' \mid x, y)}{\partial x'}\bigg|_{x' = x}, \tag{4a}$$

$$\frac{dy}{dt} = C^*(x, y)\,\frac{\partial s(y' \mid x, y)}{\partial y'}\bigg|_{y' = y}. \tag{4b}$$

The canonical equations (4) were discretized using the Runge-Kutta (RK2) method: at each time step, population sizes and local fitness gradients around the resident strategies were determined.

We ran co-evolutionary calculations until no further evolutionary change occurred: such steady states are co-evolutionary singular strategies (CoESS), and their property is to attract co-evolutionary trajectories. CoESS can either be stable (co-evolutionary continuously stable strategies, CoCSS) or unstable with disruptive selection acting on one or both traits (evolutionary or co-evolutionary branching points, CoEBP). We checked the stability of the CoESSs by determining the sign of the second partial derivative of $s(x' \mid x, y)$ and $s(y' \mid x, y)$ with respect to x' and y', evaluated at x and y respectively (Leimar, 2009).

We calculated deterministic co-evolutionary runs with x and y initially set to 0.99, a value close enough to $z = 1$ so that the effects of other pollinators are close to their maxima, but slightly different from 1 to determine whether traits converge towards 1 or diverge from it. Parameter values were chosen to obtain realistic values at the ecological equilibrium for pollination and predation rates, and for the C/T ratio – that is, within the range of those observed in natural populations (Jaeger and Despres, 1998; Jaeger *et al.*, 2001; Despres *et al.*, 2007). They were also chosen to maintain the system in the absence of *Chiastocheta*, so that at the beginning of the co-evolutionary process, the mutualism between *Trollius* and *Chiastocheta* is optional; indeed, ancestral open flower species in the *Trollius* genus persist without *Chiastocheta* (Pellmyr, 1992). Sensitivity analyses were conducted using all relevant parameters (the ranges used are as described in Table 1). All calculations were performed using the software Mathematica 5.1.1.0 (Wolfram Research)

RESULTS

Short-term co-evolutionary dynamics

Short-term co-evolutionary dynamics were used to understand the first steps of the specialization versus generalization evolution. At the beginning, trait x always evolved towards 1 while y evolved away from 1, and after a while, two types of short-term co-evolutionary dynamics were observed (Fig. 1): (a) y stopped evolving away from 1 and reversed its evolutionary course, and (b) x was driven away from 1 and evolved in the same direction as y. In both scenarios, the difference between x and y was always very small (<0.0005) compared with the tolerance parameters (range 0.1 to 1).

Long enough after the short-term dynamics presented in Fig. 1, the co-evolutionary dynamics remained monotonous, so that long-term evolution towards specialization or generalization can be predicted from the gradient at the end of the short-term dynamics.

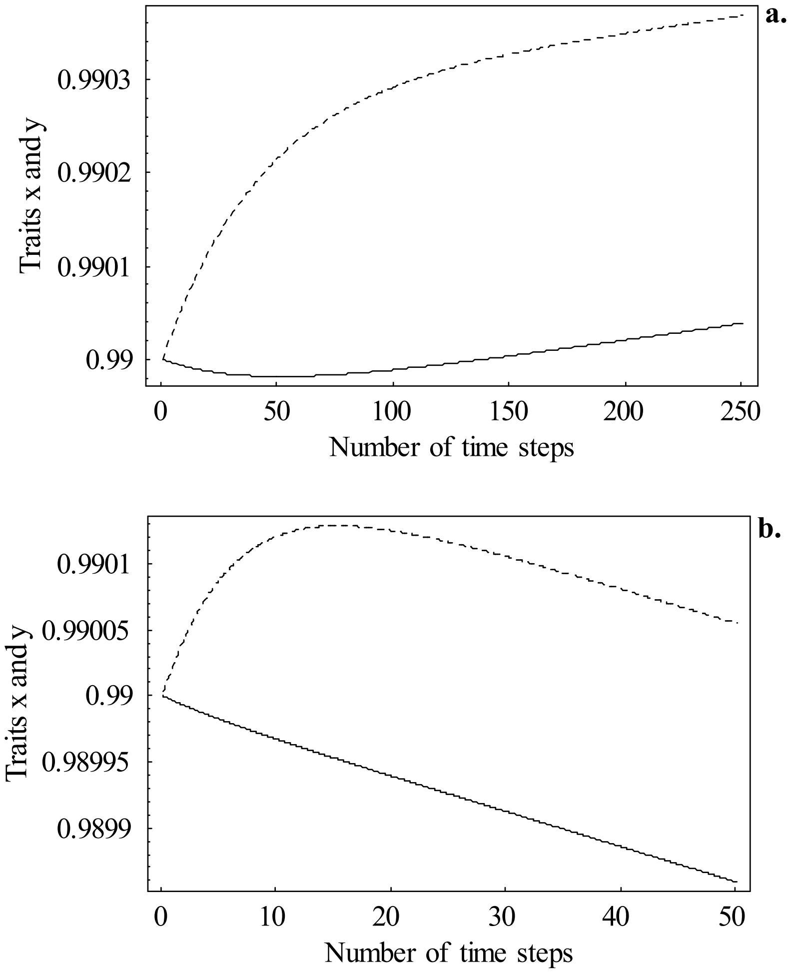

Fig. 1. Examples of short-term co-evolutionary dynamics when traits first evolve in opposite directions until one trait changes its course. Solid line: *Chiastocheta* preference y; dashed line: *Trollius* morphology x. *a* corresponds to the short-term dynamics of Fig. 2c with $h_1 = 0.8$ and *b* to Fig. 2a with $h_1 = 1$.

Long-term co-evolutionary dynamics

Traits x and y always co-evolved close to each other, so that in the long term co-evolutionary trajectories appeared confounded (Fig. 2). Two main co-evolutionary trends were observed: either other pollinators had an attractive evolutionary effect and both x and y traits co-evolved generalization towards 1; or they had a repelling evolutionary effect and both traits co-evolved away from 1 (Fig. 2). In both cases, second partial derivatives were negative (not shown), so the co-evolutionary attractors were stable (co-evolutionary continuously stable strategies, CoCSS). The speed of the co-evolutionary process depended upon parameter values and could change by an order of magnitude (see, for example, Figs. 1a and 1c with $h_1 = 1$), reflecting the variable strengths of local selection gradients. When

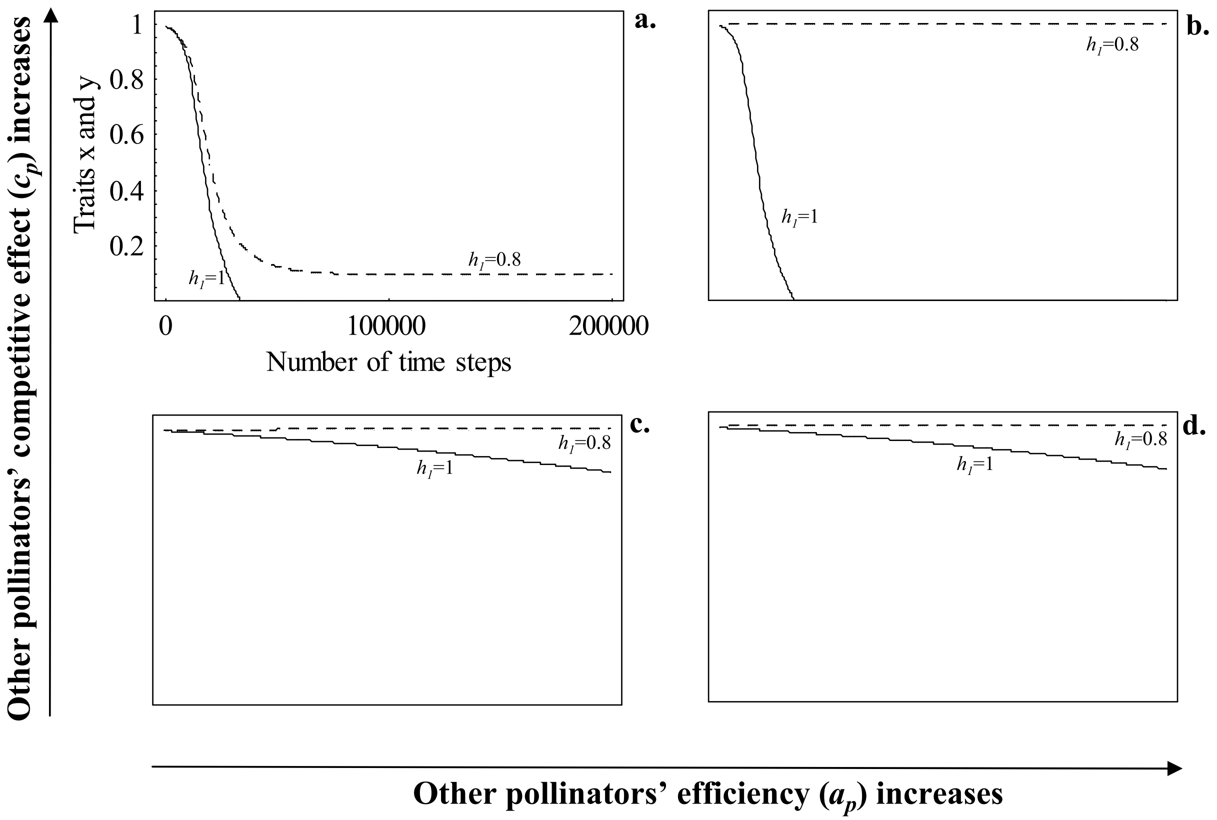

Fig. 2. Representative gallery of the co-evolutionary dynamics encountered in the model for different parameter combinations of other pollinators' effect on *Chiastocheta* pollination (vertical, c_p ranging from 0.3 to 0.7) and pollination efficiency (horizontal, a_p ranging from 0.3 to 1), and of the intensity of *C*/*T* ratio-dependence for pollination (solid lines, $h_1 = 1$; dashed lines, $h_1 = 0.8$). Each line represents the dynamics of both traits x and y, because the difference between traits never exceeded 0.001. Dynamics can lead to generalization (Fig. 2b, c, and d with $h_1 = 0.8$) or specialization. In the case of specialization, co-evolution can be fast (a) or slow (Fig. 2c and d with $h_1 = 1$) and reach the phenotypic boundary 0 (Fig. 2a with $h_1 = 1$) or stabilize between 0 and 1 (Fig. 2a with $h_1 = 0.8$). Other parameter values are as in Table 1, with $a_{c_1} = 8$, $a_{c_2} = 5$, $\sigma_{c_1} = 0.2$, $\sigma_{c_2} = 0.8$, $\sigma_p = 0.5$, $h_2 = 2$.

both traits co-evolved away from 1, they could reach the phenotypic boundary (see Figs. 1a and 1b with $h_1 = 1$) or stabilize at a CoCSS between 0 and 1 (see Fig. 1a with $h_1 = 0.8$); in both cases, *Trollius* and *Chiastocheta* traits matched and escaped from the other potential pollinators, and the result was reciprocal specialization.

Sensitivity analysis

We allowed most parameters of the model to vary (see Table 1) and conducted a sensitivity analysis, a sample of which is presented in Fig. 3. Co-evolution towards specialization or generalization was inferred from the short-term dynamics to reduce computation time. Specialization never occurred when the other pollinators did not have any negative effect on *Chiastocheta* pollination: such an effect was a necessary condition for the co-evolution of specialization. Specialization occurred only when the strong effect of other pollinators on *Chiastocheta* pollination was combined with an intermediate pollinating visits rate (Fig. 3a).

Table 1. Parameters used in the model

Parameter	Description	Range or value
Ecological densities		
T	Density of *Trollius europaeus*	R^{+*}
C	Density of *Chiastocheta* spp.	R^{+*}
P	Density of other pollinators	1
Traits		
x	T degree of morphological closure (subject to evolution)	[0; 1]
y	C flower morphology preference (subject to evolution)	[0; 1]
z	P flower morphology preference (fixed)	1
Parameters linked with *Chiastocheta* flies (C)		
b_c	C birth rate	5000
c_c	C intraspecific competition coefficient	0.4
a_{c_1}	C intensity of pollen transfer per visit	[0; 15]
σ_{c_1}	Tolerance of C in their pollination behaviour as regards floral morphology	[0.1; 0.5]
a_{c_2}	C oviposition rate	[0; 15]
σ_{c_2}	Tolerance of ovipositing flies in their visitation rate as regards floral morphology	[0.3; 1]
Parameters linked with other pollinators (P)		
c_p	P competitive effect on C pollination effectiveness	[0; 2]
a_p	P intensity of pollen transfer per visit	[0; 2]
σ_p	Tolerance of P in their pollination behaviour as regards floral morphology; equal to the interaction strength between C and P	0.4
Other parameters		
d	T death rate	0.2
h_1	Intensity of C/T ratio-dependence for pollination	[0; 2]
h_2	Intensity of C/T ratio-dependence for predation	[0; 2]

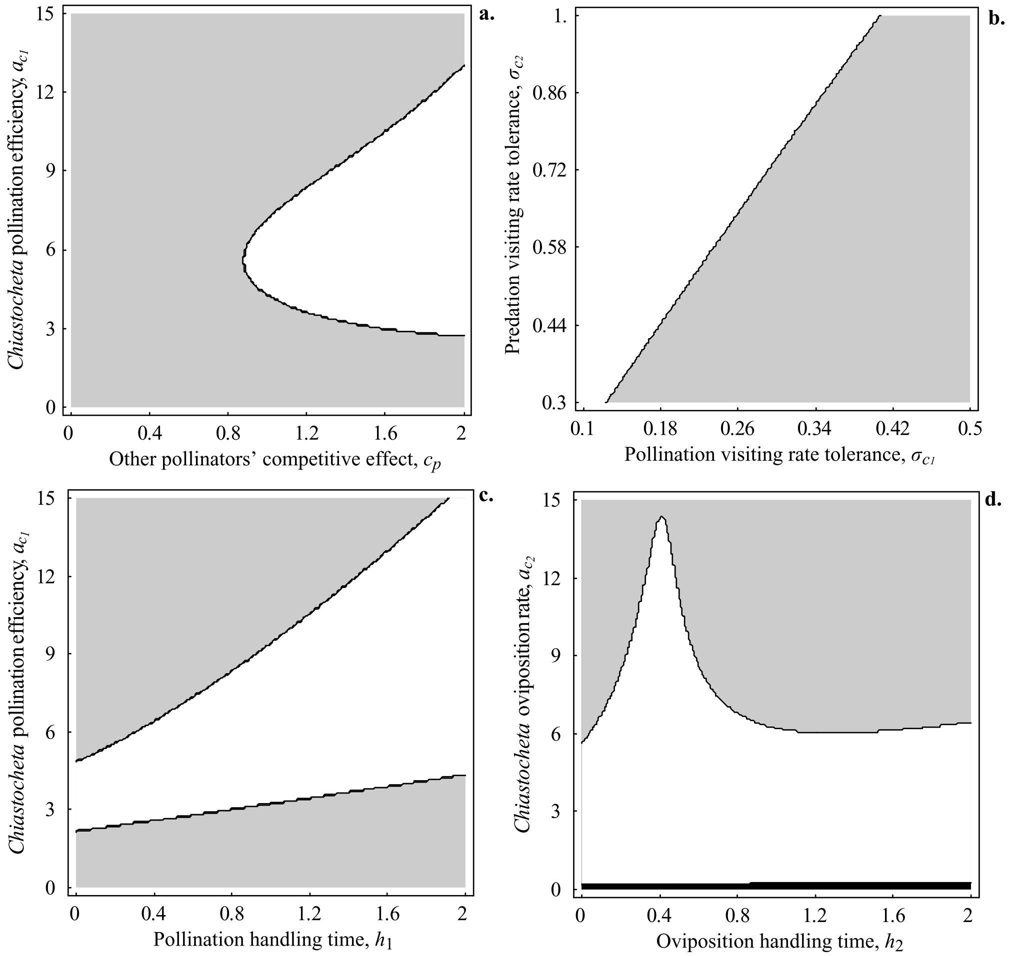

Fig. 3. Conditions for ecological viability of the *Trollius–Chiastocheta* spp. system (black, unviable parameter values) and for the co-evolution of specialization (white) versus generalization (grey) derived from short-term co-evolutionary dynamics. The effects of parameters c_p and a_{c_1} (Fig. 2a), σ_{c_1} and σ_{c_2} (Fig. 2b), h_1 and a_{c_1} (Fig. 2c), and h_2 and a_{c_2} (Fig. 2d) become visible. Other parameter values are as in Table 1, with $a_{c_1} = 8$, $a_{c_2} = 5$, $\sigma_{c_1} = 0.2$, $\sigma_{c_2} = 0.8$, $\sigma_p = 0.5$, $h_1 = 1$, $h_2 = 2$, $a_p = 0.3$, $c_p = 0.7$, except when those parameters are concerned with the sensitivity analysis.

Figure 3b shows that specialization occurred only above a given $\sigma_{c_2}/\sigma_{c_1}$ ratio. Figure 3c shows the effect of pollination parameters. As in Fig. 3a, specialization occurred at intermediate *Chiastocheta* pollination efficiency. a_{c_1} had to reach a given level for specialization to evolve, and this level increased with higher handling times. At the same time, a_{c_1} had to remain below a given level for specialization to evolve, and this level increased with higher h_2 values. Figure 3d shows the effect of predation parameters. The ecological system collapsed due to very rare oviposition rates. Specialization occurred for low oviposition rates, except within a narrow handling time range (around 0.4 with our parameter choice) in which the occurrence of specialization increased.

DISCUSSION

Mechanisms of the co-evolution of specialization

Trollius and *Chiastocheta* traits always co-evolved close to each other, which is not surprising: *Chiastocheta* flies rely entirely on *Trollius* for the development of their larvae, so they have no choice but to be specialized. The short-term co-evolutionary dynamics show that *Chiastocheta* flies try to escape the negative impact of other pollinators. Once *Trollius* morphology lies between the other pollinators' and *Chiastocheta* flies' preferences, *Trollius* has to choose between possible partners. If *Trollius* chooses to specialize on *Chiastocheta*, the system will keep co-evolving away from the other pollinators because of their negative impact on *Chiastocheta*. If *Trollius* chooses to match the other pollinators' morphological preference, *Chiastocheta* flies will have no choice but to follow this 'unfortunate' decision, despite the negative impact of other pollinators. This is why we never observed disruptive selection and evolutionary branching. In short, either *Trollius* or *Chiastocheta* drives the co-evolutionary process. Although we cannot speak about *reciprocal* specialization because only *Trollius* can choose between different partners, we have to take into account the evolution of both partners' traits (de Mazancourt *et al.*, 2005) and build a co-evolutionary model.

Emergence of a trade-off

Specialization often implies trade-offs (Schemske and Bradshaw, 1999; Aigner, 2001; Egas *et al.*, 2004), although this is not always the case (Aigner, 2004). In our model, *Trollius* does not face any trade-off at the beginning of co-evolution, since the differences between the traits are very small compared with the pollination tolerance parameters (σ_{c_1} and σ_p). When the system evolves generalization, the differences between traits become even smaller, but when specialization evolves, a trade-off emerges from the co-evolutionary process. When the system reaches the CoCSS state, the difference between the other pollinators' and *Chiastocheta* flies' preferences is large; *Trollius* now faces a strong trade-off. At this point, the ecological conditions necessary for the initiation of the specialization process can change without affecting the stability of the CoCSS. Field experiments conducted on *T. europaeus*, the only *Trollius* species that evolved specialization, showed that the closed phenotype is highly stable and that the specialization trade-off between *Chiastocheta* and other pollinators is very strong (Ibanez *et al.*, 2009). Artificially opened globeflowers produced less seeds and exported far less pollen than controls, because of a dramatic decrease in visitation rate by *Chiastocheta* flies, which was not compensated by visits by other pollinators.

Ecological conditions for specialization to evolve

In the following, we detail four ecological conditions leading to specialization. First, specialization only occurs when pollination services are not additive (Aigner, 2001; Lau and Galloway, 2004) – that is, when other pollinators prevent *Chiastocheta* flies from achieving their maximum pollination contribution. Additionally, specialization is favoured when the other pollinators' efficiency is low. In present-day globeflower populations, interference between *Chiastocheta* and other insects was occasionally observed, usually when large Diptera, Coleoptera or bumblebees landed on globeflowers and chased *Chiastocheta* away (personal observation), and/or prevented *Chiastocheta* from entering the globe by occupying the space. In past open-flower populations, such interference was likely to have been more frequent and perhaps even stronger because flies could not hide inside the globe. Furthermore, pollen waste by generalist pollinators ['ugly' pollinators (Thomson and Thomson, 1992)], and/or stigma clogging with incompatible pollen grains transferred by generalists, is a likely outcome of the co-occurrence of specialists and generalists on a plant. Transfer of pollen between different plant species reduces the pollination efficiency of generalist pollinators.

Second, specialization is favoured when the *Chiastocheta* flies' oviposition rate is low. A high oviposition rate increases seed predation and therefore reduces the mutualistic benefit of the interaction. Seed consumption per larva is another factor increasing total seed predation, thus we expect that low seed consumption per larva would favour specialization. The production of adonivernith, a flavonoid close to luteolin, is enhanced when larval density is high in a single *T. europaeus* flower (Gallet *et al.*, 2007), and an increase in this plant chemical compound results in a decrease in seed consumption per larva (Ibanez *et al.*, 2009). Paradoxically, this plant defence mechanism acting on an antagonistic trait (fly predation) might have favoured mutualistic specialization. Interestingly, adonivernith is absent from other *Trollius* species, which have not evolved specialization on *Chiastocheta* (Gallet *et al.*, 2007).

Third, flies must be less selective on floral morphology for oviposition than for nectar- or mate-searching visits. When oviposition is poorly selective, the plant pays the costs of the mutualistic interaction whatever its strategy is, and it pays to specialize on *Chiastocheta* through corolla closure, because the benefits gained from increased pollination will exceed the cost incurred due to increased predation.

Fourth, specialization does not occur when *Chiastocheta* effectiveness is too low, or when it is too high. The latter result is counterintuitive, since specialization is expected to occur with the most effective pollinator [according to the 'most effective pollinator principle' (Stebbins, 1970)]. In our model, when *Chiastocheta* are highly effective, pollination is fully achieved despite the negative effect of other pollinators on *Chiastocheta* flies and the plant does not specialize. The condition of intermediate pollination effectiveness is linked with the condition of a negative effect of the other pollinators on *Chiastocheta* pollination. If other pollinators do not prevent *Chiastocheta* from achieving pollination, there is no reason for the plant to select for their exclusion. This surprising finding might explain why *T. ledebouri* and *T. riederianus*, which are visited by *Chiastocheta* flies in Japan, did not evolve pollination specialization on *Chiastocheta*. In these species, *Chiastocheta* pollination effectiveness is much higher than on *T. europaeus* (Pellmyr, 1992), which might paradoxically prevent the evolution of specialization.

Distinguishing between past and present mechanisms promoting specialization

Field experiments show that *Chiastocheta* flies are highly effective pollinators in present-day globeflower populations (Ibanez *et al.*, 2009), which is essential for the current stability of this highly specialized mutualism. However, the present model shows that both intermediate *Chiastocheta* pollination effectiveness and negative interactions among co-pollinators are required at the beginning of the interaction for specialization to evolve. Present conditions for the stability of the interaction are the result of the specialization evolutionary history, and contrast with the past mechanisms acting at the beginning of specialization. Following Levins and Lewontin (1985), 'the conditions necessary to the initiation of some process may be destroyed by the process itself'.

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