

Reproductive biology of the Iberian species of *Potentilla* L. (Rosaceae)

by

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Abstract

Several processes related to the reproductive biology of the Iberian species of the genus *Potentilla* L. subg. *Trichothalamus* (Spreng.) Rchb., *Comarum* (L.) Syme and *Fragariastrum* (Heist. ex Fabr.) Rchb. are studied. We provide data concerning compatibility, agamospermy and hybridization along with pollination and fruit dispersal. No agamospermy mechanisms were found in any of the taxa, nor were there signs of any hybridization, although potential signs of self-incompatibility were detected. Regarding to pollination, a marked tendency toward autogamy was observed in certain groups. Finally, some mechanisms for the release and dispersal of diaspores are described in relation to diaspore and flower receptacle morphology.

Keywords: agamospermy, dispersal, elaiosome, hybridization, Iberian Peninsula, pollination, *Potentilla*, self-incompatibility, subg. *Trichothalamus*, subg. *Comarum*, subg. *Fragariastrum*.

Introduction

The genus *Potentilla* L. is a complex group including over 300 species mainly distributed on the temperate regions of the northern hemisphere. The extreme variability of some of its characters makes it a highly polymorphic genus; some of its species have a rather uncertain taxonomic position which is partly attributable to the existence of intra- and interspecific hybridization processes and apomictic phenomena. Hybridization has been mainly described on taxa of subg. *Potentilla* (Müntzing, 1928, 1931, 1958; Müntzing & Müntzing, 1941; Smith, 1963), *P. tabernaemontani* Asch. (Rousi, 1965), *P. anserina* L. (Soják, 1985,

Resumen

Se exponen los resultados del estudio de algunos mecanismos de la biología reproductiva en las especies ibéricas de *Potentilla* L. subg. *Trichothalamus* (Spreng.) Rchb., *Comarum* (L.) Syme y *Fragariastrum* (Heist. ex Fabr.) Rchb. Se aportan datos que afectan a la compatibilidad, agamosperma e hibridación a lo largo de las fases de polinización y dispersión del fruto. No se observaron mecanismos de agamosperma en ninguno de los táxones estudiados ni tampoco se comprobó hibridación alguna, aunque se detectaron signos potenciales de autoincompatibilidad. Relacionado con la polinización, se observó en ciertos grupos una clara tendencia a la autogamia. Se describen, por último, algunos mecanismos de liberación y dispersión de diásporas, que están relacionados con la morfología de la propia diáspora y del receptáculo floral.

Palabras clave: agamosperma, autoincompatibilidad, dispersión, elaiosoma, hibridación, Península Ibérica, polinización, *Potentilla*, subg. *Trichothalamus*, subg. *Comarum*, subg. *Fragariastrum*.

1986), or the more recent papers on *P. hookeriana* Lehm. and *P. uniflora* Ledeb. (Eriksen, 1996) or *P. nivea* L. and *P. crantzii* (Crantz) G. Beck (Eriksen & Popp, 2000).

In the most comprehensive monograph written on the genus, Wolf (1908) accepted 305 species and divided the genus in two sections –*Trichocarpae* Wolf (1908) and *Gymnocarpae* Wolf (1908)– and many subsections, series and tribes or groups of species. The division into subgenera was consolidated following the criteria of Ball & al. (1968). According to Guillén & Rico (1998) there are 30 species of *Potentilla* in the Iberian Peninsula, pertaining to 4 subgenera: *Trichothalamus* (Spreng.) Rchb., *Comarum* (L.) Syme,

Fragariastrum (Heist. ex Fabr.) Rchb., and *Potentilla*. Of them, the latter includes most of the species (22 in the Iberian Peninsula) and is the most complex.

Our research was focused on the species belonging to the subgenera *Trichobalamus*, *Comarum* y *Fragariastrum*, that have a floral morphology much more heterogeneous and diversified than any species in subgenus *Potentilla*. In order to interpret the significance of this diversification and to understand better the reproductive biology of the genus as a whole, some experimental tests and field observations were made.

These tests were intended to detect apomixis, hybridization or self-incompatibility processes (Tables 1 and 2). Observations on pollination and fruit dispersal were periodically done on different Iberian populations of each species in the study. The present paper focuses on the following species (Fig. 1a-h) of the subgenus *Fragariastrum*: *P. caulescens* L., *P. nivalis* Lapeyr., *P. alchimilloides* Lapeyr., *P. montana* Brot., *P. sterilis* (L.) Garcke, and *P. micrantha* Ramond ex DC.; although it also includes *P. fruticosa* and *P. palustris*, the only Iberian representatives of the subg. *Trichobalamus* and subg. *Comarum*, respectively.

Material and methods

The experimental work began in 1994 and lasted for four years. Several specimens of each taxon were grown in the laboratory to perform a number of controlled crossing tests to study the aforesaid reproductive processes. In situ experiments were performed in natural populations, and despite major obstacles due to the difficulty of access to the sites under study, it was possible to monitor the manipulated specimens to determine the existence or not of self-incompatibility, agamospermy or hybridization mechanisms.

In order to detect a potential dependence on pollination vectors, a test of spontaneous self-pollination was made. The flowers were isolated before anthesis with cylinders of translucent sulfite paper sealed at the top with adhesive tape and closed around the pedicel at the bottom. To detect apomictic processes, the flowers were emasculated before encapsulation. For hybridization, the same system was used, but the styles of the selected flowers were sprinkled with fresh pollen of other species of the same subgenus or studied group with a fine brush.

Occasionally the paper cylinders broke because of weathering, or anthers were torn as flowers were emasculated. Only in these cases some achenes could be formed, which is marked with an asterisk (*) in Table 2.

The number of flowers used in each test is shown in Table 1. For each population several flowers were set

as control and not manipulated. When possible, test and control flowers were selected from different specimens. The number of achenes formed in the manipulated flowers was compared with the average number of achenes formed in the control flowers, according to the results in Tables 1 and 2. It should be noted that the number of achenes shown refers always to ripe and apparently well-formed achenes, but whose viability was not studied.

The three experimental tests were not always performed on all the studied species (see Tables 1 and 2). Although it is not possible to do a statistical analysis to get definitive conclusions due to the low number of flowers used, the tests provide interesting signs about the reproductive behavior of this group of plants.

Our pollination observations focused essentially on two aspects: development of the arrangement of flower parts during the flowering period, and presence or visits of pollinating agents. To know if this information could be relevant, we recorded the effective role of the flower visitor and the environmental conditions during the visit. Finally, in a study of fruit release and dispersal, we recorded the variations undergone by the receptacle core and the achenes in their development as well as the presence of external agents that may be involved in the process. Photographs were also taken to document all the processes.

Results and discussion

Self-incompatibility, agamospermy and hybridization

In *P. fruticosa*, both field observations and experimental results (Tables 1 and 2) seems to indicate the existence of self-incompatibility mechanisms and that self-fertilization is very uncommon. The small number of achenes formed in each encapsulated flower may be explained by the existence of partial self-incompatibility. The self-incompatibility of *P. fruticosa* has indeed already been demonstrated by Davidson & Lenz (1989), and according to these authors it may be of genetic origin. In our experimental work we detected no agamospermy phenomena in this species.

In *P. caulescens*, the results for two of the studied populations, where some specimens were transplanted, provided data of interest. It was observed that the carpels of the emasculated flowers underwent no changes, whereas fruits developed as a result of self-pollination in the flowers prepared for the spontaneous self-pollination test. According to field observations and experimental results, there appear to be no apomixis processes in this species, and any self-incompatibility that may exist is not total.

Table 1. Synthesis of tests and observations performed with the eight Iberian species of *Potentilla* under study.

SPECIES	Main population studied ¹	Type of test performed ²	No. of flowers used / Test	Results ³	Control test	Pollination / Dispersion observations
<i>P. fruticosa</i>	León: Busdongo, 29TTN7661*	SI	6	+	+	Yes
	León: Picos de Europa, 30TUN5079	AG	6	-		"
	Vitoria: Arlucea, 30TWN3733					"
<i>P. palustris</i>	León: Pto. de S. Isidro, 30TUN0570	observations without manipulation of SI and AG		see text		Yes
	León: Picos de Europa, 30TUN5079					"
	Asturias: Pto. Leitariegos, 29TQH1162					"
<i>P. caulescens</i>	Jaén: Pico Gillillo, 30TWG0091*	SI	12	(+)	+	Yes
	Cuenca: Hoz de Priego, 30TWK5976*	AG	12	(-)		"
		HB	12	(-)		"
<i>P. nivalis</i>	León: Pto. de las Señales, 30TUN1969	observations without manipulation of SI, AG and HB	-	apparently negative in the three cases (see text)	+	Yes
	Santander: Picos de Europa, 30TUN57		-			"
	Huesca: Coll. de Sahún, 31TBH8618		-			"
<i>P. alchimilloides</i>	Navarra: Pico Orhi, 30TXN6360*	SI	6	-	+	Yes
		AG	6	-		
		HB	6	-		
<i>P. montana</i>	Asturias: Candás, 30TTP7829* (in laboratory)	SI	6	+	-	Yes
		AG	6	-		
		HB	6	-		
<i>P. sterilis</i>	Asturias: Candás, 30TTP7829* (in laboratory)	SI	6	-	+	Yes
		AG	(2)	(-)		
		HB	2	-		
<i>P. micrantha</i>	Asturias: Pajares, 30TTN7464* (in laboratory)	SI	4	-	+	Yes
		AG	(2)	(-)		
		HB	2	¿+?		

¹ See full list in Appendix 1.² Detection of: SI, self-incompatibility; AG, agamospermy; HB, hybridization.³ Overall non-quantified results indicated in brackets.

* Populations in which flowers were manipulated.

Table 2. Results of self-incompatibility, agamospermy and hybridization tests on the species of *Potentilla* under study.

SPECIES	CONTROL FLOWERS		SI: SELF-INCOMPATIBILITY		AG: AGAMOSPERMY		HB: HYBRIDIZATION	
	average no. of achenes per flower / average no. of carpels per flower	% of successes	average no. of achenes per flower / average no. of carpels per flower	% of successes	average no. of achenes per flower / average no. of carpels per flower	% of successes	average no. of achenes per flower / average no. of carpels per flower	% of successes
<i>P. fruticosa</i>	30.1/34	88.5%	(1.5/32)*	4.7%	(0.5/34)*	1.4%	x <i>P. caulescens</i> and x <i>P. alchimilloides</i> apparently nonexistent, results not quantified	
<i>P. caulescens</i>	35.2/38	92.6%	partial self-incompatibility, results not quantified		apparently nonexistent, results not quantified		x <i>P. fruticosa</i> and x <i>P. alchimilloides</i> apparently nonexistent, results not quantified	
<i>P. nivalis</i>	29.3/33.2	88.25%	self-compatible flowers, results not quantified		apparently nonexistent, results not quantified		apparently nonexistent, results not quantified	
<i>P. alchimilloides</i>	34.8/35.1	99.1%	19.6/28.1	69.7%	(0.6/33.1)*	1.8%	x <i>P. caulescens</i> (0.6/26.6)*	2.2%
<i>P. montana</i>	0/22.6	0%	0/22.6	0%	0/21.8	0%	x <i>P. sterilis</i> 0/20.3	0%
<i>P. sterilis</i>	33.8/38	88.9%	22.3/34.5	64.6%	apparently nonexistent, results not quantified		x <i>P. montana</i> (5.5/28.5)*	19.3%
<i>P. micrantha</i>	31/32.2	96.2%	self-compatible flowers, results not quantified		apparently nonexistent, results not quantified		x <i>P. sterilis</i> (5.5/24)*	22.9%

In brackets or marked with an *: Accidental results owing either to tearing of cells exposed to the elements or to breaking of anthers as stamens were manipulated.

Within the group of species studied, *P. nivalis* is the only species in which we have observed, without manipulating flowers, that self-incompatibility mechanisms can be ruled out. This could be in part due to the particular arrangement of the floral structures (Fig. 1e): The perianth assembly adopts a conical shape, wide at the bottom and progressively narrower farther up, where there is an opening in the form of a small orifice. With this arrangement the reproductive structures are practically closed (flowers are almost cleistogamic) and it is very difficult for external pollinating agents to come into play, and if they do (e.g. bees or wind), they are likely to favour autogamy, as explained below.

For *P. alchimilloides* the data shown in Table 2 indicate an absence of agamospermy processes (no achenes were formed in any of the emasculated and isolated flowers, except for one in which four appeared owing to a tear in the protective cell). Although the results obtained suggest the possibility of partial self-incompatibility, as described above for *P. fruticosa* or reported for *P. palustris* (Olsen & Warncke, 1992), and indeed our self-incompatibility tests showed almost 70% success in pollination, self-pollination also occurs. Yet there seems to be no hybridization with other species of the same group with very similar floral morphology, and in particular with *P. caulescens*.

Regarding *P. montana* it has been shown in field-work that, in the natural populations from which the cultivated specimens were taken, the percentage of achenes formed in each flower ranges from 70 to 100%. But no achenes were formed in any of the laboratory tests performed with these plants – not even in the control tests, or with manual self-pollination. These results seem to indicate the presence of total self-incompatibility mechanisms such as not detected in any of the other species studied. No agamospermy processes were found and our attempts at hybridization with pollen from *P. sterilis* had no result. In the populations studied of *P. montana*, which grow in close proximity to those of *P. sterilis*, no intermediate forms were found to indicate the existence of hybrids between these two similar taxa.

The results of our experiments with *P. sterilis* show an absence of self-incompatibility processes. It was possible to carry out hybridization tests, using pollen from *P. montana*, only on very few flowers, and the results were negative. A few achenes, always peripheral, appeared in some flowers as a result of an anther being torn during emasculation. In the flowers emasculated for hybridization tests the results obtained also served to indicate an absence of agamospermy processes.

In *P. micrantha*, an absence of self-incompatibility was also established. The only two flowers that could be emasculated for hybridization tests with pollen from *P. sterilis* formed a small number of peripheral achenes (accidentally, as in the case of *P. montana*). These also served to establish the absence of agamospermy processes. Although *P. micrantha* × *P. sterilis* hybrid forms are described in literature, these two species coexist in close proximity, often at the same sites (as shown in Table 1 and Appendix 1), but not intermediate forms were detected.

Pollination

Arrangement of flower parts

Flower structure in the taxa under study is closely related to the pollination mechanisms of each species. Around the receptacle core, which bears the carpels, are the stamens (generally 20), with nectaries or a nectar disc at their base, and a pentamerous perianth, consisting of a calyx with an epicalyx and the corolla. The flowers of all studied species remained habitually open for 4-8 days. But there are significant variations within the three subgenera studied as to the arrangement of the parts of the androecium, perianth and nectaries (Fig. 1), which will be analyzed together with our remarks on pollination in each case.

The position of the stamens and perianth parts during the flowering period varies among species and may determine their pollination mechanisms. In *P. fruticosa*, *P. alchimilloides* and *P. montana* (Fig. 2a) the anthers usually mature immediately after anthesis; as the thecae dehisce in these species the stamen filaments lean progressively outward – the anthers do not remain in contact with the styles – and finally adopt an almost radial arrangement.

In *P. palustris* and *P. caulescens* (Fig. 2b1, b2), during anthesis the stamen filaments are arranged parallel to the styles, so the anthers may even brush against the stigmas, but after a while they move away from the stigmas to the periphery, and are finally arranged radially. In *P. sterilis* (Fig. 2c1, c2) the stamens are initially parallel to the styles or converging with them, even though the thecae have not yet opened; later, as the anthers dehisce, they move toward the styles and come into close contact with them; finally the stamens separate and adopt a radial arrangement. When the flower opens in *P. micrantha* (Fig. 2e), the stamens move toward the centre of the flower, forming a conical structure with the anthers at the tip, surrounding and touching the stigmas. The thecae dehisce while so arranged, and later on the stamen filaments slowly radially diverge to become vertical. In *P. nivalis*,

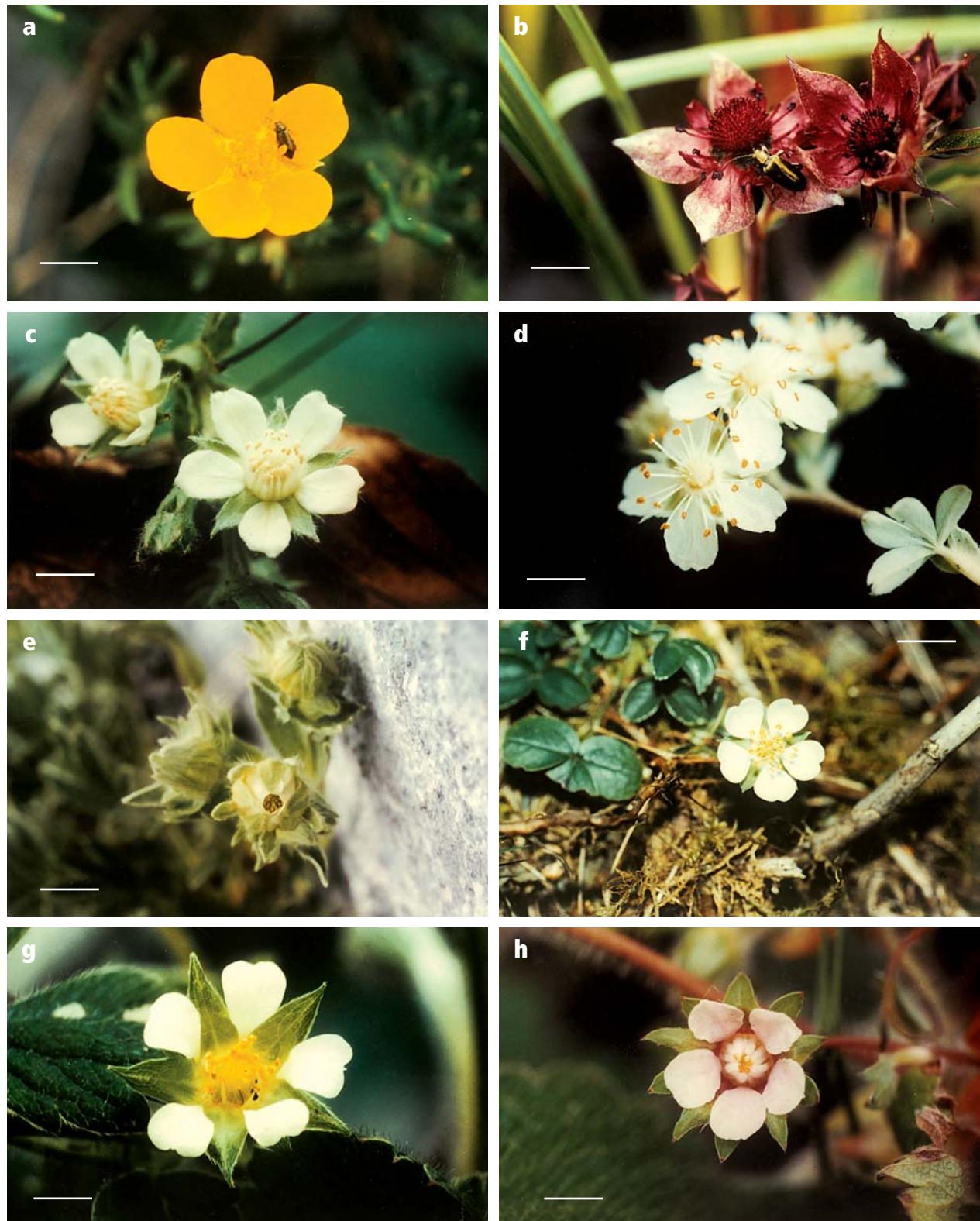


Fig. 1. Arrangement of flower parts and some pollinators of the species studied: **a**, flower of *Potentilla fruticosa* with a pollinating coleopteran; **b**, flower of *P. palustris* with one of its usual pollinators, the chrysomelid beetle *Donacia aquatica*; **c**, flowers of *P. caulescens* immediately after anthesis; **d**, allogamous flowers of *P. alchimilloides* with divergent stamens from the receptacle core; **e**, flowers of *P. nivalis* with stamens and stigmas enclosed in the perianth; **f**, flower of *P. montana* with divergent stamens from the centre of the flower; **g**, flower of *P. sterilis* with stamens initially applied on the styles; **h**, flower of *P. micrantha* with convergent stamens on the stigmas. Scales: a, b, g, h = 0,75 cm; c, d = 1,25 cm; e = 1 cm; f = 1,5 cm.

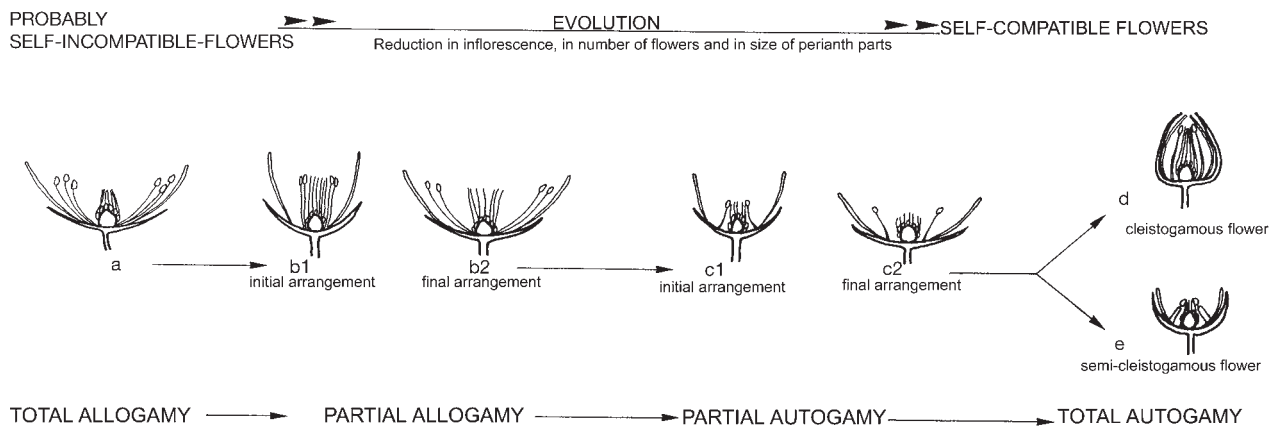


Fig. 2. Evolution and arrangement of flower structures in the subgenera *Trichothalamus*, *Comarum* and *Fragariastrum* of the genus *Potentilla* in relation to reproduction: **a**, *P. alchimilloides*, *P. fruticosa*, and *P. montana*; **b**, *P. caulescens* and *P. palustris*; **c**, *P. sterilis*; **d**, *P. nivalis*; **e**, *P. micrantha*.

(Fig. 2d) the perianth adopts an almost conical shape, wide at the base and narrow at the apex and virtually closed at the tip of the petals. Inside this structure the anthers and stigmas remain in contact throughout the flowering period. We have not observed any influence of the light or atmospheric conditions in the disposition (opening or closing of the flower) of sepals or petals.

External agents in pollination

Five of the studied species are markedly entomophilous. *Potentilla fruticosa*, *P. alchimilloides*, *P. caulescens* and *P. montana* are commonly pollinated by Hymenoptera, mainly wild bees, but also by Diptera (Syrphid flies), Lepidoptera and, occasionally, Hemiptera. All of these insects, except the Hemiptera, seek mainly nectar secretions either at the receptacle core (*P. fruticosa*), base of the petals (*P. alchimilloides* and *P. caulescens*), or nectar disc (*P. montana*). Although *P. sterilis* and *P. micrantha* also have a nectar disc of apparently similar characteristics to that of *P. montana*, they are not visited by the pollinators that frequent the latter.

In *P. alchimilloides* it is relatively common to find ants inside the flower buds seeking nectar and in this activity they become totally covered in pollen because it sticks on their hairy body. When the ants are trying to leave the flower, they leave the pollen on the style. In *P. caulescens* the still closed perianth is opened by small wild forager bees trying to reach the pollen and nectar enclosed within the flower.

In *P. palustris* the nectar held between the base of the receptacle and the stamens shines brightly, stand-

ing out against the dark purple background of the perianth, and especially of the sepals. These nectar secretions are a conspicuous lure for flying insects that are attracted in large numbers, especially Hymenoptera of the *Apidae* family (genera *Apis* and *Bombus*, etc.), Diptera, mainly Syrphid and Muscid flies, and also some Lepidoptera. The anthers of *P. palustris* and *P. fruticosa* are characterized, unlike those of the other species studied, by a wide connecting structure situated between the two marginal thecae. In *P. palustris* the thecae shrink as they dehisce and the pollen grains are squeezed out as furry granules that stick to the sides of the anthers. This pollen is gathered directly by hymenoptera, but on many occasions it falls from the stamens to the sepals (or the stigmas, although this only occasionally since the filaments lean outward from the flower core). On the sepals the pollen may be easily consumed by small coleoptera, especially *Donacia aquatica* (Chrysomelid, Fig. 1b), which become covered on pollen.

Contrary to the previous cases, insects do not play an important pollination role in *P. nivalis*, as in this species the particular arrangement of flower parts makes it impossible for insects to get inside the small orifice in the upper part of the perianth. Bees collect the nectar introducing their mandibles between the chinks at the base of the petals and sepals, which moves the stamen bases. The transmitted movement of the anthers rubbing against the style favors self-pollination.

Pollination models

From the above field observations and analysis of morphological and functional characters of flower

structure in *Potentilla*, it is possible to establish various pollination models and their possible evolution (Fig. 2). These would range from total allogamy (Fig. 2a, b), mainly on the basis of entomophilous pollination mechanisms, to total autogamy (Fig. 2d, e), with intermediate models in-between (Fig. 2c). The first group would include *P. fruticosa*, *P. alchimilloides*, *P. montana*, *P. caulescens* and *P. palustris*; the second would contain *P. nivalis* and, to a lesser extent, *P. micrantha*; the intermediate model would be represented by *P. sterilis*.

Allogamy.—In *P. fruticosa* the reproductive success of self-pollination (essentially entomophilous) is conditioned by self-incompatibility (cf. Davidson & Lenz, 1989). Its complex inflorescence, long flowering period and multiple stamens indicate that sexual reproduction in this taxon requires much more energy than in any other of the studied species. We therefore consider it to be one of the least evolved taxa of all those studied.

Potentilla alchimilloides and *P. caulescens* are species in which a certain degree of self-incompatibility could exist. Both are mainly allogamous, have many flowers per inflorescence, and big anthers, which make them similar to *P. fruticosa*.

Within the Iberian members of Wolf's (1908) *Fragariastra genuina* section, *P. montana* is the least evolved form in terms of energy expenditure. It conserves flower characteristics (long threadlike stamen filaments, large petals, radial arrangement of stamens during anther dehiscence) which, besides a probable importance for self-incompatibility, make this species similar to other in the subgenus *Fragariastrum*, and in particular to *P. alchimilloides*. However, at the same time it exhibits, like *P. sterilis* and *P. micrantha*, evolved characters such as a clear tendency toward inflorescence and total number of flowers per plant reduction.

Among the studied species, *P. palustris* seems to follow an independent evolutionary course, as it conserves characters considered ancestral in the genus: a likely degree of self-incompatibility, predominance of allogamy and a long flowering period. Nevertheless, it has developed effective mechanisms to assist entomophily: inflorescences with fewer flowers than in most of the related species, sepals brightly coloured, petals relatively very small, and a lower number of stamens (only 10 in some flowers).

Tendency toward autogamy.—Pollination in *P. sterilis* is an outstanding example of transition from allogamy to autogamy within the genus, and it seems to represent the connection between both modes. Flowers in *P. sterilis* and *P. montana* are very similar. In both they

have relatively large petals, large anthers, and filaments threadlike almost throughout. However, in *P. sterilis*, as in *P. micrantha*, the stamen filaments move toward the centre of the flower and appear reinforced at the base, which is wider, facilitating contact between anthers and stigmas (Fig. 2c). This floral arrangement links *P. sterilis* to *P. micrantha* (Fig. 2e), which has developed advanced mechanisms to achieve effective autogamy.

Autogamy.—The arrangement of perianth parts in *P. nivalis* determines its autogamy and seems to mark an evolutionary tendency within the subgenus *Fragariastrum*, which also appears in other non-Iberian species such as *P. grammopetala* Moretti, *P. valderia* L., *P. haynaldiana* Janka, *P. doerfleri* Wettst., *P. apeninna* Ten., *P. kionaea* Halácsy, and *P. deorum* Boiss. & Heldr. These species have usually smaller anthers, and sometimes less flowers per inflorescence than other members of the subgenus. This tendency allows us to point to a degree of evolutionary advancement in comparison with the allogamous species (e.g. *P. caulescens* and *P. alchimilloides*). Self-pollination seems to be in this case the most efficient way of guaranteeing reproduction in areas where life is relatively difficult for some pollinating insects owing to weather conditions at high altitudes. Moreover, reproductive structures could be damaged by extended exposure to the elements. Self-pollination mechanisms may be reinforced—and this is exceptional within the genus—by wind or bees, which, as we have seen, stay at the base of the flowers where there is nectar between the stamen filaments while the anthers are protected in the upper part of the perianth, in close contact with the styles and stigmas.

In *P. micrantha* the efficacy of autogamy mechanisms guarantees the reproductive success of the species. Such mechanisms entail a significant energy saving: its anthers are smaller than those of almost all representatives of the *Fragariastrum* subgenus, and it produces considerably fewer pollen grains (the size of the anthers is much smaller than in any other species of the genus, while the pollen grains have similar dimensions). The success of its autogamy mechanisms could be possibly related with the diminution of the number of flowers and the drastic reduction on inflorescence and flower size. These tendencies have been observed within the group of similar species, but in *P. micrantha* they are more clearly expressed.

Fruit release and dispersal

The release of fruits from the flower receptacle core and the dispersal thereof are closely related to flower architecture (Figs. 3, 4), and on this basis we have es-

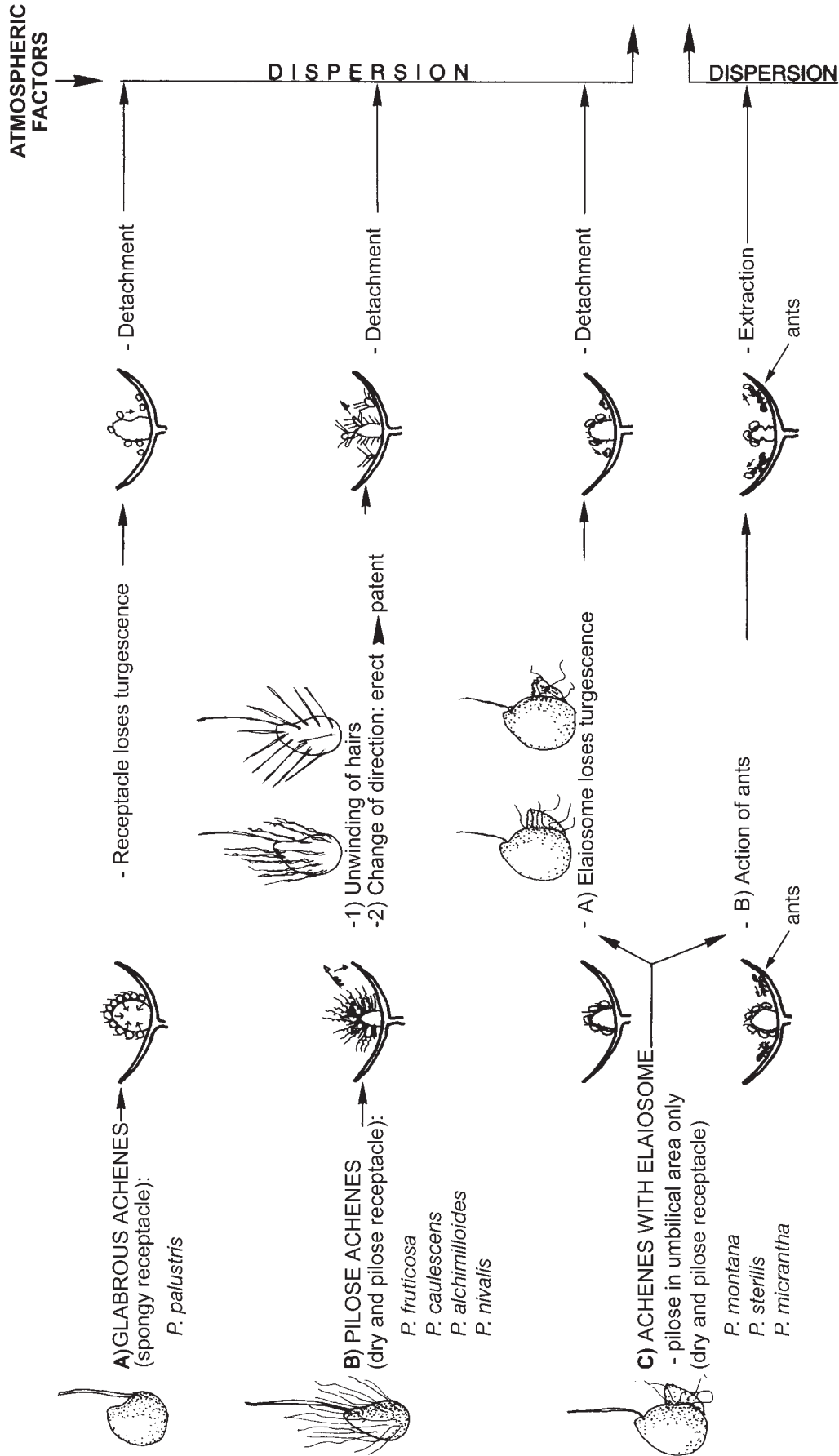


Fig. 3. Achene detachment and dispersal in Iberian species of subgenera *Trichothalamus*, *Comarum* and *Fragariastrum* of the genus *Potentilla*.

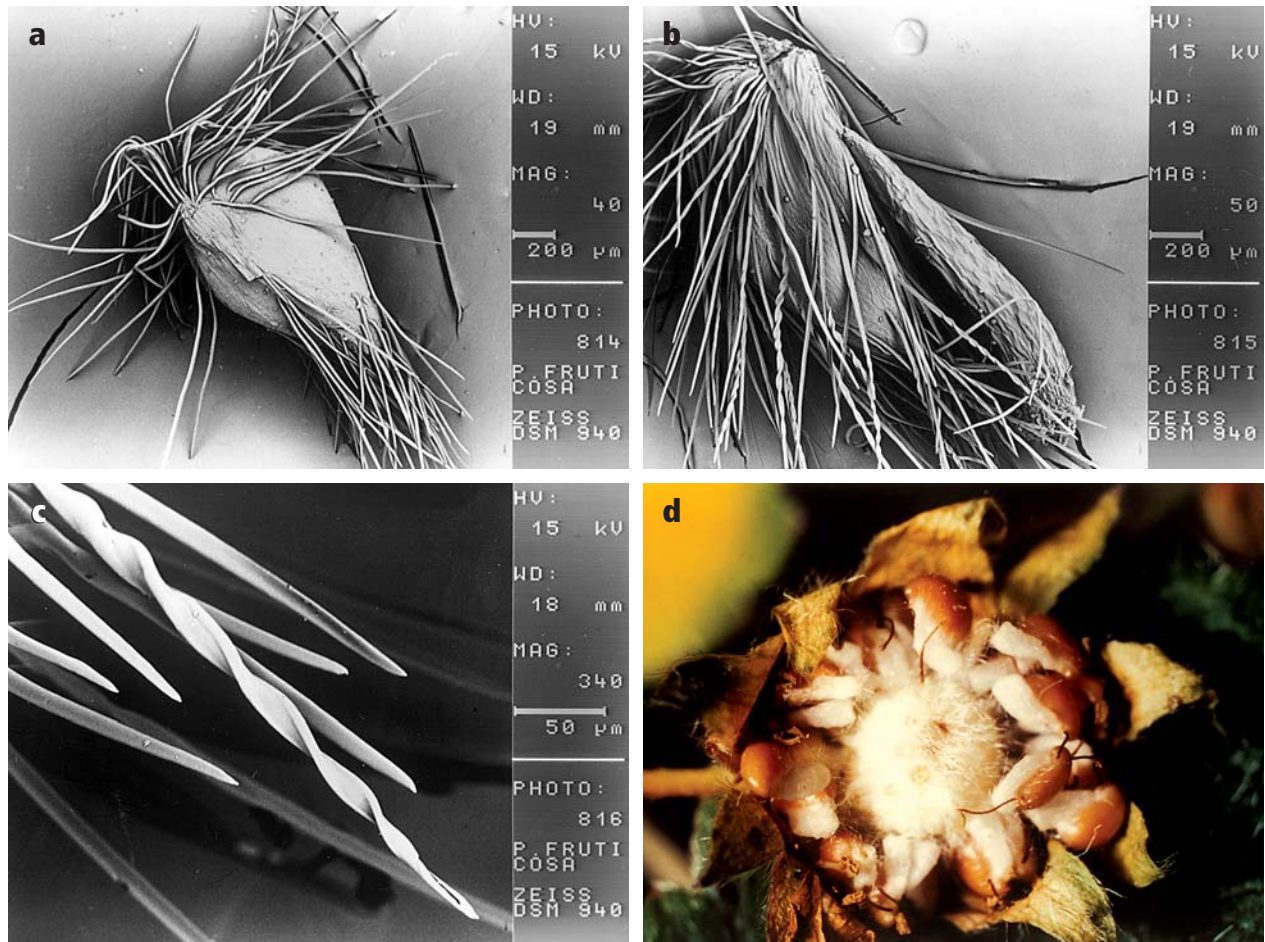


Fig. 4. *Potentilla fruticosa*: **a**, ripe achene; **b**, unripe achene; **c**, detail of wound hair in an unripe achene. *Potentilla sterilis*: **d**, receptacle with achenes dragged out by ants.

established three clearly differentiated groups for the studied species:

- Species with totally glabrous achenes with no elaiosome (Fig. 3A).
- Species with pilose achenes with no elaiosome (Fig. 3B).
- Species with achenes having elaiosome and pilose only in the umbilical area (Fig. 3C).

Species with totally glabrous achenes with no elaiosome (Fig. 3A).—This group only includes *P. palustris*, which also has a glabrous, rather spongy, receptacle core. In *P. palustris* the fruits become fully ripe protected by the calyx parts, which curve toward the centre of the flower. The receptacle core dries gradually, losing its turgescence and shrinking a little so that by mid or late summer the achenes are slightly detached from it. Finally, the break-up of the calyx caused by atmospheric factors allows the achenes—now almost unprotected—to fall easily to the ground and to germinate.

Species with pilose achenes with no elaiosome (Fig. 3B).—*Potentilla fruticosa*, *P. caulescens*, *P. nivalis*, and *P. alchimilloides* share these characteristics and also, broadly speaking, their mode of diaspore dispersal. The dispersal mechanism of *P. fruticosa* may illustrate that of this group (Figs. 3B, 4a-c): The mature achenes are covered with an indumentum of long, upright, flattened and wound hairs, parallel to the style or slightly divergent from it from the base. As the fruits progressively ripe, the hairs become increasingly turgescence, straight, and circular in cross-section, and form a wider angle in relation to the style. This increasingly wider angle produces a perpendicular and outward force in relation to the receptacle surface, which separates the fruits from it assisting subsequent dispersal. Coincident with fruit ripening, weathering of calyx produces its break, ensuring dispersal, usually toward the end of summer.

Species with achenes having elaiosome and pilose only in the umbilical area (Fig. 3C).—Three Iberian

species pertain in this group: *P. sterilis*, *P. micrantha* and *P. montana*, with *P. alba* L. and *P. carniolica* A. Kerner as the only non-Iberian members. The presence of elaiosome is exceptional in the genus; elaiosome appears exclusively in the previous five taxa and is found in trace form in only two species of subg. *Potentilla*: *P. crantzii* (Crantz) G. Beck and *P. neumaniana* Rchb. (pers. obs.). All share a herbaceous and creeping habit also seem in species of other genera with the same unusual dispersal mode (e.g., *Viola*, *Polygala*, *Corydalis*, *Primula*, etc.) growing in the herbaceous layer of woodland.

In these plants the fertilized and fully formed fruits remain attached to the receptacle by a conical, fleshy, whitish and rather hairy structure situated in their umbilical area, a structure that morphologically resembles an elaiosome. The somewhat accrescent calyx parts finally move toward the centre of the flower and protect the fully formed achenes, which may be disseminated when the flower structures have dried up, or before that happens.

If the achenes remain in the receptacle until the flower structures completely dry up, the elaiosome progressively dehydrates, shrinks and takes the same light brown colour as the rest of the fruit; thus the drying up and loss of turgescence in the elaiosome facilitates the liberation of the achenes from the receptacle. Moreover, the flower pedicels, which are very slender in these three species, usually break down at an early stage, so the detached fruit heads are exposed to the elements, which complete their dispersal.

We have also occasionally seen in *P. sterilis* and *P. micrantha* –and we suppose it may also occur in *P. montana*– how, at the end of the fruit formation process, ants seek out the still-fleshy elaiosomes and drag the fruits out of the receptacle, not yet withered, to their nests (Figs. 3C, 4d). The ants' appetite for the fleshy tissue of *P. sterilis* and *P. micrantha* is not limited to the fruit; it extends also to the receptacle core and nectar disc, structures that are sometimes cut up by the ants' mandibles.

Conclusions

In the studied groups, we have seen a progressive tendency to reduction in the reproductive process through various strategies: reduction in the number of flowers per inflorescence as a way of reducing the number of carpels and stamens (Fig. 3); reduction in the number and size of stamens with no decrease in the size of pollen grains; shortening of the flowering period; acquisition of mechanisms to ensure efficient pollination; diversification of the ways in which ach-

enes are dispersed; and, occasionally, elimination of self-incompatibility mechanisms.

No agamospermy mechanisms were observed in any of the species on which we carried out experiments, in contrast with what is indicated by other authors for some species included in subg. *Potentilla* (Müntzing, 1928, 1931, 1958; Müntzing and Müntzing, 1941; Smith, 1963; Rousi, 1965; Holm & al., 1997).

– Experimental hybridization was unsuccessful, either between species of the same subgenus (e. g., *P. alchemilloides* and *P. caulescens*), same section (*P. sterilis* and *P. micrantha*), or different subgenera (*Trichothalamus* and *Fragariastrum*).

– Our experiments revealed possible self-incompatibility mechanisms in *P. fruticosa*, *P. caulescens*, *P. alchimilloides* and *P. montana*. These species are essentially allogamous and entomophilous, although their populations are often endogamous because of geographical isolation.

Pollination types and pollination strategies in the species under study are independent of their phylogenetic position within the genus. Thus, within the subgenus *Fragariastrum* we found contrasting tendencies between the most closely related species, *P. montana* and *P. micrantha*; and in each of the three subgenera studied there are taxa with similar pollination mechanisms, e.g. *P. fruticosa* (subg. *Trichothalamus*), *P. alchimilloides* (subg. *Fragariastrum*) and *P. montana* (subg. *Fragariastrum*).

– We observed two clear and contrasting pollination tendencies: allogamy and autogamy. The allogamous species exhibit total or partial self-incompatibility mechanisms.

– There is an apparent tendency toward the acquisition of autogamy: reduction in the number of flowers per inflorescence, lack of self-incompatibility, reduction in stamens number and anther size, and development of mechanical devices that hinder allogamy and assist self-pollination.

– Some taxa are or may be autogamous. This is strictly the case in the cleistogamous *P. nivalis*; almost always the case in *P. micrantha*, which has developed an advanced mechanical system that guarantees self-fertilization; and often the case in *P. sterilis*.

– *Potentilla montana*, *P. alchimilloides*, *P. caulescens*, *P. fruticosa* and, especially, *P. palustris*, are allogamous and, essentially, entomophilous. *Potentilla palustris* shows mechanisms favoring entomophily that are completely different from the mechanisms adopted by the remaining species.

Contrary to what happens in pollination, fruit release and dispersal mechanisms are similar in species within groups. *Potentilla montana*, *P. sterilis* and *P. micrantha* follow the same model: their achenes have elaiosome and may consequently be dispersed by myrmecochory. *Potentilla caulescens*, *P. nivalis* and *P. alchimilloides* conform to a different model similar to that of *P. fruticosa*, in which the pilosity of the achenes plays an important role in the mechanics of their detachment. Finally, *P. palustris* follows a third model, somewhat different from the previous two.

In all the studied taxa the diaspores are polychorous, although barochory predominates over myrmecochory, anemochory, or hydrochory.

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Appendix 1. Populations studied

Potentilla alchimilloides

Gerona: La Molina, Pic. Niu D'Aliga, 31TDG1086, 2235 m (MACB 56782). **Huesca:** Collado de Tella, 31TBH6921, 2400 m (SALA 60192); Panticosa, 30TYN0932, 2100 m (SALA 60189); Collado de Sahún, 31TBH8618, 2400 m (SALA 60193). **Navarra:** Pico Orhi, 30TXN6360, 1900 m (SALA 60194); Isaba, Belagua, 30TXN7656, 1900 m.

Potentilla caulescens

Albacete: Calar del Río Mundo, 30SWH4956, 1150 m (SALA 60166). **Cuenca:** Hoz de Priego, 30TWK5976, 1150 m (SALA 60178); Hoz de Beteta, 30TWK736892, 1200 m. **Gerona:** Bassegoda, 31TDG6984, 1250 m (MA 528884). **Granada:** Monachil, Dornajo, 30SVG6008, 2000 m. **Huesca:** Anisclo, La Selva, 31TBH5915, 1000 m (SALA 60168). **Jaén:** Sierra de Cazorla, Pico Gilillo, 30SWG0091, 1750 m (SALA 60167); Sierra de Cazorla, Puente de las Herrerías, 30SWG0593, 1200 m (SALA 60170); Sierra de Cazorla, source of the Guadarquivir 30SWG0288, 1650 m (SALA 60174); Villanueva del Arzobispo, Pico Roblehermoso, 30SWH0619, 1750 m (SALA 60175).

Potentilla fruticosa

Álava: Arlucea, Barranco del Molino, 30TWN3733, 800 m. **Gerona:** between Nuria and Caralps, 31TDG3093, 1875 m (MA 528966). **León:** Picos de Europa, Vega de Liordes, 30TUN5079, 1890 m; Villanueva de la Tercia-Camplongo, 30TTN7661, 1170 m (SALA 60195).

Potentilla micrantha

Asturias: Pajares, 30TTN7464, 1200 m; Puerto de Cubilla, 30TTN6866, 1500 m (SALA 60206); Teverga, Puerto de San Lorenzo, 29TQH3674, 1490 m (SALA 60213); Puerto Ventana, 29TQH4672, 1600 m; Somiedo, Saliencia, Lago del Valle 29TQH3170, 1600 m (SALA 60212); **Huesca:** Panticosa, 30TYN2234, 1800 m (SALA 60211); El Portalet, 30TYN1440, 1760 m (SALA 60210). **León:** Mirador de Panderrueda, 30TUN4967, 1200 m (SALA 60214); Viadangos de Arbas, 30TTN7659, 1800 m. **Navarra:** Solana de Belagua 30TXN7050, 1400 m.

Potentilla montana

Asturias: Candás, La Braña, 30TTP7829, 40 m; Teverga, alto de San Lorenzo, 29TQH3674, 1490 m (SALA 60180); Somiedo, Saliencia, Lago del Valle, 29TQH3169, 1500 m (SALA 60181); Cangas de Onís, Valle de Orandi, 30TUN3290, 1200 m (SALA 60182). **Navarra:** Puerto de Ibañeta, 30TXN3662, 1058 m (SALA 60179); Sierra de Urbasa, Monte Limitaciones, 30TWN6738, 950 m. **Zamora:** Mombuey, 29TQG1756, 1250 m.

Potentilla nivalis

Gerona: Nuria, Nou Creu, 31TDG3094, 2000 m (MA 528924); Sierra del Cadí, 31TCG8383, 1890 m (MA 529243). **Huesca:** Bielsa, Barranco de Montinier, 31TBH6922, 1870 m (MA 544749); Bielsa, Circo de Pineta, 31TBH6030, 2300 m; Collado de Sahún 31TBH8618, 2400 m (SALA 60183). **León:** Puerto de las Señales 30TUN1969, 1650 m (SALA 60184); Picos de Europa, Collado de Remoña 30TUN57, 2100 m; Picos de Europa, Vegarredonda, 30TUN3888, 1700 m; Palencia, Velilla del Río Carrión, 30TUN5443, 1850 m (MA 493793).

Potentilla palustris

Asturias: Puerto de Leitriegos, Laguna del Cueto de Arbás, 29TQH1162, 1700 m (SALA 94809). **León:** Picos de Europa, Vega de Liordes, 30TUN5079, 1900 m (SALA 94811); Puerto de San Isidro, near Lago Ausente, 30TUN0570, 1750 m (SALA

94812). **Zamora:** Ribadelago, Laguna de Peces, 29TPG8867, 1600 m.

Potentilla sterilis

Asturias: Candás, La Braña, 30TTP7829, 40 m (SALA 60203); Luanco, Moniello, 30TTP7334, 15 m (SALA 60202); Gamoniteiro, 30TTN6484, 800 m (SALA 60205); Cangas de Onís, Valle de Orandí, 30TUN3290, 1200 m (SALA 60197); **Gerona:** Beget-Oix, 31TDG5784, 560 m (MA 528911). **León:** Puerto de San Isidro, Iso-ba, Lago Ausente, 30TUN0871, 1400 m (SALA 94815). **Navarra:** base of Pico Orhi, 30TXN6360, 1000 m (SALA 60198); Roncesvalles, 30TXN3869, 1300 m (SALA 60204); Solana de Belagua, 30TXN7040, 1200 m (SALA 60199); Iranzu, 30TWN8535, 800 m. **Zamora:** Sotillo de Sanabria, 29TPG8763, 1200 m.

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