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Effects of fragmentation on pollination ecology and genetic diversity in endemic Mediterranean species

Abstract

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One of the expected consequences of global change (due to climatic change or to human activities, such as changes in land use) is the fragmentation of plant populations in small and isolated units more suitable to disappear because the possibilities of gene flow are interrupted and the reduced number of individuals is not enough to face environmental, genetic or demographic stochastic events. These effects are expected to be higher in rare or endemic species with a limited distribution, since these organisms are more sensitive to this kind of changes. In the present work, we studied the effects of fragmentation of populations in three endemic plants from the Mediterranean region: *Delphinium bolosii* (*Ranunculaceae*), *Petrocoptis montsicciana* (*Caryophyllaceae*) and *Seseli farrenyi* (*Apiaceae*). They are threatened plants and protected by law. They also have different degree of floral complexity and different degree of pollinator specialization. We analyzed the effects of fragmentation using comparisons of pairs of populations (large-small) at two levels: the reproductive success (measured through pollinator visitation rates, quantity and quality of pollination services and seed set) and the parameters of genetic diversity (using allozyme electrophoresis). Results of this study will allow postulate bases for the management and conservation of these species.

Introduction

Human activities on Earth have been recognized as responsible of global change (Vitousek 1994), which includes the climatic change and the change in land use that often lead to the decline and deterioration of many natural ecosystems (Cincotta & Engelman 2000 a,b). One outcome of this global change is the loss or fragmentation of habitats. In general, loss of habitat produces a decline in total population size of plant species and fragmentation of habitat can isolate small populations from each other. Currently, fragmentation of populations in small units induced by man is one of the main threats to biodiversity on the planet (Wilcox & Murphy 1985; Saunders & al. 1991).

Fragmentation processes reduce the size and increase spatial isolation of plant populations. As a consequence of that, the possibilities of genetic flow are interrupted and the

number of individuals to face stochastic changes decreases. The endemic and threatened plants are expected to be more sensitive to fragmentation effects. Three different types of stochasticity can be distinguished: genetic, demographic (both due to intrinsic factors) and ecological or environmental (due to extrinsic factors). Theory predicts the inexorable extinction of isolated and small remnants because of these factors (Barret & Kohn 1991; Young & al. 1996; Bijlsma & al. 1997; Dunham & al. 1999). However, that will depend on the minimum viable population size for each species (Gilpin & Soulé 1986; Menges 1991; Iriondo 1996). What seems clear, though, is high genetic variation is desirable for maximizing population viability, its fitness and its evolutionary potential (Hamrick & al. 1979; Young & al. 1996). In this study we have focused in effects of fragmentation on pollination (ecological factor) and on genetic diversity (genetic factor).

In insect-pollinated plants, several consequences of fragmentation on pollination are expected: the decrease of population size produces changes in population attractiveness to pollinators, which can imply a reduction of visitation rates and lower constancy (Kwak & al. 1998). In turn, this involves changes in quantity and quality of pollination services leading to lower conspecific pollen loads on the stigmas and higher heterospecific pollen loads by competing pollen deposition (Galen & Gregory 1989; Murcia & Feisinger 1996), and subsequent reduction of reproductive success in terms of lower seed set, lower seed viability due to genetic impoverishment, and lower recruitment, increasing the extinction risk (Byers 1995; Kunin 1997).

Several consequences of fragmentation processes on genetic diversity are also expected. By one hand, the reduction of population size promotes an increase of random genetic drift, which can imply loss of alleles translated in a decrease of mean number of alleles per locus (A), percentage of polymorphic loci (P) and expected heterozygosity (H_e) (Barrett & Kohn 1991; Young & al. 1996). In apomictic plants a reduction of expected heterozygosity no necessarily has to occur (Ellstrand & Rose 1987; Wolf & al. 2000). On the other hand, the decrease of population size promotes mating between relatives ("biparental inbreeding"), increasing the genetic erosion (inbreeding) and decreasing the observed heterozygosity (H_o). Finally, isolation between remnants promotes a reduction of gene flow (Nm), increasing proportion of interpopulation differentiation (G_{ST}) and genetic distances (D) between remnants (Barrett & Kohn 1991; Young & al. 1996). These effects may be more buffered in polyploid plants (Soltis & Rieseberg 1986; Soltis & Soltis 2000).

The aim of this work is attempt to characterize the fragmentation effects in endemic Mediterranean species, which are poorly reported in the literature, based on ecological and genetic parameters: the influence of pollinators in the quantity and quality of pollination and allozyme variation, respectively. We need to point out that we are studying the consequences of fragmentation on existing populations but we do not know exactly how they were before, in other words, we have not a baseline to know the starting point. On the other hand, we try to know long-term effects of fragmentation, by addition of historical factors, so our approach is far distant from those involving experimental manipulations of populations, which obviously cannot be addressed to endangered species.

The Mediterranean area is considered a hotspot of world biodiversity because of significant concentrations of endemic species: 4.3 % of world endemisms (Myers & al. 2000). From a total of 25,000 species, 13,000 are endemic from that area. In addition, this area has experienced an important loss of habitats in the recent times. Land transformation by

agricultural, industrial or mainly tourism activities notably reduced the extension of wild communities, leading to a progressive loss of available habitats, for strictly adapted species. Lots of examples are available in conservation literature.

Material and Methods

The studied plants

For this study we selected threatened narrow endemic species, which are the most presumably sensitive plant species to these changes. To explore the fragmentation consequences, they must be structured in large and small populations. We choose 3 different entomophilous species with a gradient of complexity in the floral morphology and different expected degree of pollinator specialization. These species were *Delphinium bolosii* C. Blanché & Molero (*Ranunculaceae*), *Petrocoptis montsicciana* O. Bolòs & Rivas-Mart. (*Caryophyllaceae*) and *Seseli farrenyi* Molero & J. Pujadas (*Apiaceae*).

All three species are endemic to Catalonia. They occur in areas more or less extent but they occupy little space, with few populations and a low number of total individuals (Table 1). All these species are threatened, as is specified in the recent Red List of Spanish Vascular Flora (Aizpuru & al. 2000). *D. bolosii* is endangered EN (B1 + 2cde, C1), *P. montsicciana* is vulnerable VU (D2) and *S. farrenyi* is endangered EN (B1 +2c), according to UICN categories. They are protected by law, by the governments of Spain (Catálogo Nacional de Especies Amenazadas, Real Decreto 439/90), Europe (Habitats Directive, 92/43/CEE) and Catalonia (PEIN, Decret 328/1992), respectively. Habitat protection is provided by the Natural Park of Cape Creus in *S. farrenyi*, some populations of *P. montsicciana* are PEIN areas and there is a proposed reserve for *D. bolosii*.

Delphinium bolosii is a dysploid diploid belonging to a group of plants with oriental affinities, which arrived to the Iberian Peninsula in the Messinian period (Blanché 1991). It is closely related with *D. fissum* Waldst. & Kit. (euploid diploid), which extends until the western of the Iberian Peninsula, but always in small and isolated populations. Currently *D. bolosii* has 3 populations, one extinct (last record was in 1912, Blanché 1991). Our team has data from the other two populations in the last twenty years, and although some fluctuations occurred, they have maintained more or less constant. It grows in fresh and protected spots. One population is in a *Rubus canescens* hedge in a narrow ravine over schists.

Table 1. Distribution and size of studied species.

	D. bolosii	*P. montsicciana*	*S. farrenyi*
Extent of occurrence (km^2)	64	910	1
Occupation area (km^2)	3	32	0.5
Number of populations	3 (1 extinct)	13	3
Total species size (N)	3,000	11,000	2,000

The other consists of a few lines of plants occurring on ledges of calcareous cliff facing the North over the Segre river. We sampled the only two existing populations with a total of 360 (LNO) and 100 (PRI) reproductive individuals, respectively (Table 2).

Petrocoptis montsicciana is a diploid species that belongs to an emblematic genus endemic to the Northern of the Iberian Peninsula. It has a mosaic of species with a complicated taxonomy: some authors consider the genus belonging to *Silene* (Mayol & Roselló 1999). *P. montsicciana* is a relict chasmophyte strictly restricted to vertical calcareous rock walls and caves of limestone. It grows over crevices, ledges and overhangs. This species is less threatened than the other two (with the exception of construction of new roads, mountain climbers' activity, or other potential dangers). However it has a very low renewal rates (Mayol 1998). Currently, it has 13 populations isolated among them and often fragmented. We sampled two of the most accessible populations with similar orientation and different population size: 600 (CAM) and 100 (TER) reproductive individuals, respectively (Table 2).

Seseli farrenyi is also diploid. It is a very isolated species, with a narrow distribution restricted only to Cape Creus area, in the Northern coast of Catalonia (Northeastern of the Iberian Peninsula). It grows in fissures of schistous rocks on cliffs very close to the sea, on weakly acidic and sandy soils. It has uncertain affinities with *S. montanum* L. or alternatively with *S. praecox* (Gamisans) Gamisans and *S. bocconi* Guss. from Corsica, Sardinia and Sicily. One of the only 3 known populations is progressively declining in the last twenty years. We sampled 2 of them with 150 (SCM) and 10 (SES) reproductive individuals (Table 2). Each year only about 27 % of total rosettes bloom effectively (unpubl. data).

All 3 species are markedly protandrous perennial herbs. *D. bolosii* has the most complex floral morphology. The flower is zygomorphic with 5 petaloid sepals; one prolonged in a long spur. The two upper petals are partially included in the spur, and produce large amounts of nectar. The two lateral petals provide a horizontal land-platform for pollinators. Flowers are grouped in racemose inflorescences. *P. montsicciana* has purple-pink pentameric flowers, arranged in dichasia inflorescences. Each flower has 10 stamens and 5 styles emerging inside a central crown produced by appendixes soldered to petals. Disposition of petals is slightly zygomorphic. The flowers of *S. farrenyi* are white, small and symmetric and grouped in compound umbels.

Table 2. Location and codes of studied populations.

Species	Large	Code	Small	Code
D. bolosii	Hs: Lleida, La Noguera, Rubió de Baix	LNO	Hs: Tarragona, Priorat, Ulldemolins	PRI
	UTM: 31TCG3441		UTM: 31TCF2377	
P. montsicciana	Hs: Lleida, La Noguera, Camarasa gorges	CAM	Hs: Lleida, La Noguera, Terradets gorges	TER
	UTM: 31TCG2541		UTM: 31TCG2556	
S. farrenyi	Hs: Girona, Alt Empordà, Cap de Creus, Es Camallerús	SCM	Hs: Girona, Alt Empordà, Cap de Creus, Ses Estenedors	SES
	UTM: 31TEG2685		UTM: 31TEG2685	

Methods

Fragmentation effects on pollination were surveyed by comparison of pairs of large-small populations previously established, where we analyzed: 1) pollinator visitation rates through successive censuses of standard periods of 15 minutes to identify and quantify pollinators and observing their behavior on the flowers; 2) pollination services through the analysis of stigmatic pollen loads from senescent flowers stained with basic fuchsin. Heterospecific pollen was identified by comparison with a pollen collection reference; 3) reproductive success measuring all good expanded seeds and undeveloped ovules, always in amounts not dangerous for the population.

To analyze allozyme variation, we collected leaf fragments in the field along a longitudinal transect from pairs of referred large and small populations and they were conserved in cold. Extracts were obtained with Tris-citrate buffer. Starch gel electrophoresis was performed using the procedures described by Soltis & Soltis (1989) with slight modifications (Bosch 1999; López-Pujol 2000). A total of 21 enzymes were assayed. After optimizing the conditions for each species, 12 loci were solved for *D. bolosii (aat, aco-1, aco-2, adh-1, mdh-1, mdh-2, me-1, 6 pgd-1, 6 pgd-2, pgi-1, pgi-2,pgm-2)* 16 for *P. montsicciana (aat, aco-1, aco-2, adh, dia-2, dia-3, mdh-1, mdh-4, me, 6 pgd-1, 6pgd-2, pgi-2, pgm-1, pgm-2, prx-1, prx-2)* and 14 for *S. farrenyi* (*aat, adh, dia-1, dia-2, idh, mdh-1, mdh-3, mdh-4, 6 pgd-1, 6 pgd-2, pgi-1, pgi-2, pgm-2, rbc*).

Pollination effects

Dependence on pollinators

To survey fragmentation effects on pollination mechanisms we previously need to know the dependency on entomophilous pollination. Thus we will be able to understand in which degree fragmentation effects may be derived from pollination. We conducted an insect exclusion test, bagging flowers in the field. All three species are insect-dependent. In absence of insects *D. bolosii* set only 20% of seeds (n = 82 flowers), *P. montsicciana* 30 % (n = 75) and *S. farrenyi* only 3 % (n = 2934). These results suggested that dependence of insects to set seeds in the studied species is not correlated with the degree of flower morphological complexity. Insect pollination disruptions under fragmentation, then, should be more pronounced in *S. farrenyi*.

General pattern of pollinators

Despite its complex floral morphology and expected specialized pollination, *Delphinium bolosii* is visited by a broad spectrum of pollinators, possibly due to a large diversity of local fauna (Table 3). Flower morphology requires robust visitors, which are able to separate the floral pieces, with a long proboscis to reach the nectar hide in the spur. The most ideal seem to be bumblebees (such as *Bombus terrestris* and *B. pasquorum*), however some of them act as nectar robbers. Other bees with similar body size (such as

Table 3. Visitors of *D. bolosii.*

Insects	PRI	LNO	P.E.	Reward
HYMENOPTERA	**66.1**	**42.6**		
Apidae				
Bombus terrestris terrestris Krüger	4.2	15.4	R	Pollen+Nectar
Bombus pasquorum rufocitrinus L.	1.5	1.0	++	Nectar
Xylocopidae				
Xylocopa violacea L.		12.1	++	Nectar
Anthophoridae				
Anthophora dispar Lep.		1.8	++	Nectar
Ceratina dentiventris Gerst.		0.2	+	Pollen
Megachilidae				
Osmia submicans Morawitz		1.2	++	Nectar
Scoliidae				
Scolia flavifrons Fabricius		0.4	+	Pollen
Eumenidae				
Alastor atropos Lep.	31.2	8.2	r	Nectar
Halictidae				
Lassioglossum sp.	26.7	2.3	+	Pollen
Halictus sp.	2.0		+	Pollen
LEPIDOPTERA	**21.2**	**45.6**		
Papilionidae				
Papilio machaon L.	0.2	0.8	+	Nectar
Nymphalidae				
Cynthia cardui L.		0.2	+	Nectar
Hesperiidae				
Thymelicus sylvestris Poda	1.5	1.6	+	Nectar
Lycaenidae				
Polyommatus icarus Rottemburg		0.4	+	Nectar
Satyridae				
Pyronia bathseba Fabricius		0.4	+	Nectar
Brintesia circe Fabricius		0.8	+	Nectar
Melanargia lachesis Hübner	0.4	0.4	+	Nectar
Pieridae				
Gonopteryx rhamni L.	5	3.7	+	Nectar
Gonopteryx cleopatra L.	5.5	3.7	+	Nectar
Pieris brassicae L.		1.2	+	Nectar
Artogeia rapae L.		1.2	+	Nectar

Artogeia napi L.		0.4	+	Nectar
Euchloe ausonia Hübner		0.2	+	Nectar
Colias croceus Geofroy	0.4	0.6	+	Nectar
Sphingidae				
Macroglossum stellatarum L.	7.4	30.8	+	Nectar
DIPTERA	**10.4**	**11.0**		
Syrphidae				
Melliscavea auricollis Meigen		8.4	+	Pollen
Sphaerophoria scripta L.			+	Pollen
Eupodes corollae Fabricius			+	Pollen
Epysyrphus balteatus De Geer	2.5		+	Pollen
Eristalis tenax L.	0.4	1.6	+	Pollen
Bombylidae				
Bombylius sp.	5.7	1.0	+	Nectar
Antrax sp.		1.0	0	
Calliphoridae				
Brachycera sp.		0.4	0	
Therevidae		0.4	0	
HETEROPTERA	**0.4**	**0.6**		
Pentatomidae				
Graphosoma linetaum italicum Müller		0.4	0	
Rhopalidae				
Chorosoma schillingi Schummel		0.2	0	
Coreidae				
Coreus marginatus L.	0.4		0	
COLEOPTERA	**1.9**	**0.3**		
Scarabaeidae				
Oxythirea funesta L.		0.3	0	
Meloidea				
Mylabris sp.	1.9		0	

PRI, N records: 478, Total censuses: 29 h, LNO, N records: 487, Total censuses: 32 h,
Explanation to signs in "pollination efficiency" (P.E.): ++ = effective pollinator; + = occasional pollinator; 0 = hardly ever pollinating; R = primary robber; r = secondary robber.

Xylocopa violacea, *Anthophora dispar* or *Osmia submicans*) and a high number of Lepidoptera, in particular the hawkmoth *Macroglossum stellatarum*, also visit it. This latter is less efficient in relation to the amount of pollen deposited per visit but they compensated with a high visitation frequency. Halictid bees forage and move into the stamens

area, favoring self-pollination. The small bee *Alastor atropos* act as a secondary robber using the holes made by other robbers. Some Diptera (such as *Bombylius* and syrphid flies) also visit *D. bolosii*.

The most frequent pollinators of *Petrocoptis montsicciana* are Hymenoptera, mainly

Table 4. Visitors of *P. montsicciana.*

Insect	TER	CAM	P.E.	Reward
HYMENOPTERA	**66.7**	**81.5**		
Anthophoridae				
Anthophora sp. (*)	38.9	64.7	++	Nectar
Eucera nigrilabris	2.8	0	++	Nectar
Melecta sp.	2.8	0	++	Nectar
Halictidae				
Lassioglossum sp.	8.3	10	+	Pollen
Apidae				
Bombus terrestris Kruger	2.8	2.6	R	Nectar
Apis mellifera L.	2.8	1.1	++	Nectar
Xylocopidae				
Xylocopa violacea L.	0	2.6	++	Nectar
Vespidae	5.5	0.5	+	Pollen
Andrenidae				
Andrena sp.	2.8	0	++	Nectar
Megachilidae				
Anthidium stiticum Cockerell	0	0.5	++	Nectar
LEPIDOPTERA	**11.1**	**8.5**		
Sphingidae				
Macroglossum stellatarum L.	11.1	8.5	+	Nectar
DIPTERA	**22.2**	**9.5**		
Bombylidae				
Bombylius sp.	2.8	6.9	+	Nectar
Syrphidae	16.6	2.1	+	Pollen
Muscidae	2.8	0.5	+	Pollen

TER, N records: 36, Total censuses: 20 h, CAM, N records: 190, Total censuses: 25 h. Conventions for P.E. follow table 3. (*) *A. dispar* Lep., *A. plumipes* and *A. biciliata.*

long-tongued bees of genus *Anthophora*: *A. dispar* and *A. plumipes* and more sporadically *A. biciliata* (Table 4). Other bees, some Diptera, in particular species of *Bombylius* and the hawkmoth *Macroglossum stellatarum* also visited regularly *P. montsicciana* (Table 4). Most pollinators collected nectar placed at the corolla basis region except Syrphid flies that collected pollen. *Bombus terrestris* always behaved as nectar robber making a hole at the basal calyx zone to reach the nectar.

Seseli farrenyi has a very unspecific pollination system. It was pollinated by at least 28 different species of insects, including wasps, small bees, ants, flies, syrphid flies, sting bugs, and a great variety of beetles (Table 5). Except for wasps, bees and some flies (the winged ones), these pollinators are small insects and remained long periods on the same umbels.

Comparison between large and small populations

We did not detected significant differences in visitation rates between both *D. bolosii* populations, whereas in *P. montsicciana* and *S. farrenyi* the small population received less approaches per census (Fig. 1). In *P. montsicciana* we found some correlation between the number of visited flowers and the number of open flowers per census ($y = 0.3456x + 2.359$, $r^2 = 0.351$). In *S. farrenyi*, the small population received less approaches per census than the large one, but differences were not significant in the total of umbellules visited.

The amount of heterospecific pollen on the stigmas was in all cases relatively small (4 % in *D. bolosii*, 11 % in *P. montsicciana*), but larger in *S. farrenyi* (13 %), the plant with less complexity in floral morphology from the three studied ones and with a pollination system more unspecific. However, it was not so extremely high. The composition was different, depending on each species. It is remarkable the high amount of *Antirrhinum molle* pollen (61 %) found in *Petrocoptis* stigmas, another endemic chasmophyte that also live in the same walls.

In *D. bolosii*, as expected we detected less stigmatic pollen in the small population than the large one, and a higher proportion of heterospecific pollen (basically from *Rubus canescens* and *Psolarea bituminosa*). In the case of *S. farrenyi*, the smaller population has also less stigmatic pollen loads than the large one, but in this case with a small proportion of heterospecific pollen. In *P. montsicciana* the small population showed unexpectedly more pollen than the large one (Fig. 1).

In *D. bolosii* and *P. montsicciana* there is no statistically evident effect of population size on seed set, whereas in *S. farrenyi* data showed a notably decrease in seed production in the small population (Fig. 1).

Genetic effects

Allelic richness

Besides to present the results from comparisons between large-small populations, we also give information at species level to provide a more global vision of genetic diversity of each species. More extensive data on allozyme variation can be found in Bosch & al.

Table 5. Visitors of *S. farrenyi*.

Flower visitors	SES	SCM	P.E.	Reward
HYMENOPTERA	**55.2**	**42.3**		
Vespidae				
Polistes omissus Weranch	6.6	4.4	+	Nectar
Sphecidae				
Sceliphron destillatorium Illiger		1.9	+	Nectar
Eumenidae				
Ancistrocerus trifasciatus Müller		1.3	+	Nectar
Apidae				
Apis mellifera L.	1	0.3	++	Nectar
Halictidae	1.9	13.1		
Lasioglossum sp.			++	Nectar
Halictus sp.			++	Nectar
Chrysididae				
Chrysis sp.		4.7	+	Nectar
Formicidae	45.7	16.6	+	Nectar
Camponotus piceus Leach				
Camponotus lateralis Olivier				
Cataglyphis piliscapus Forel				
Messor bouvieri Bondr.				
Pheidole pallidula Nylander				
DIPTERA	**11.5**	**10.2**		
Syrphidae	1	0.9	+	Pollen
Sphaerophoria scripta L.				
Eristalis tenax L.				
Muscidae				
Musca domestica L.	3.8	0.9	+	Pollen
Callophoridae	1.9	7.5	+	Pollen
Tephritidae	4.8	0.9	0	
HETEROPTERA	**1**	**6.6**		
Pentatomidae				
Graphosoma lineatum italicum Müller		5.3	0	
Pentatoma rufipes L.		1.3	0	
Carpocoris sp.	1		0	
COLEOPTERA	**32.3**	**40.9**		
Coccinellidae				
Coccinella septempunctata L.	1.9	1.3	0	
Chrysomelidae	1	0.6	0	
Cantharidae		0.6	0	
Oedemeridae				
Oedemera flavipes F.	1.9	0.6	0	
Mordellidae	15.2	31.9	0	
Melyridae	12.3	5.3	0	
Danacea sp.				
Dasytes sp.				
Bruchidae		0.6	0	

SES, N records: 105, Total censuses: 12 h, SCM, N records: 320, Total censuses: 23 h. Conventions for P.E. follow table 3.

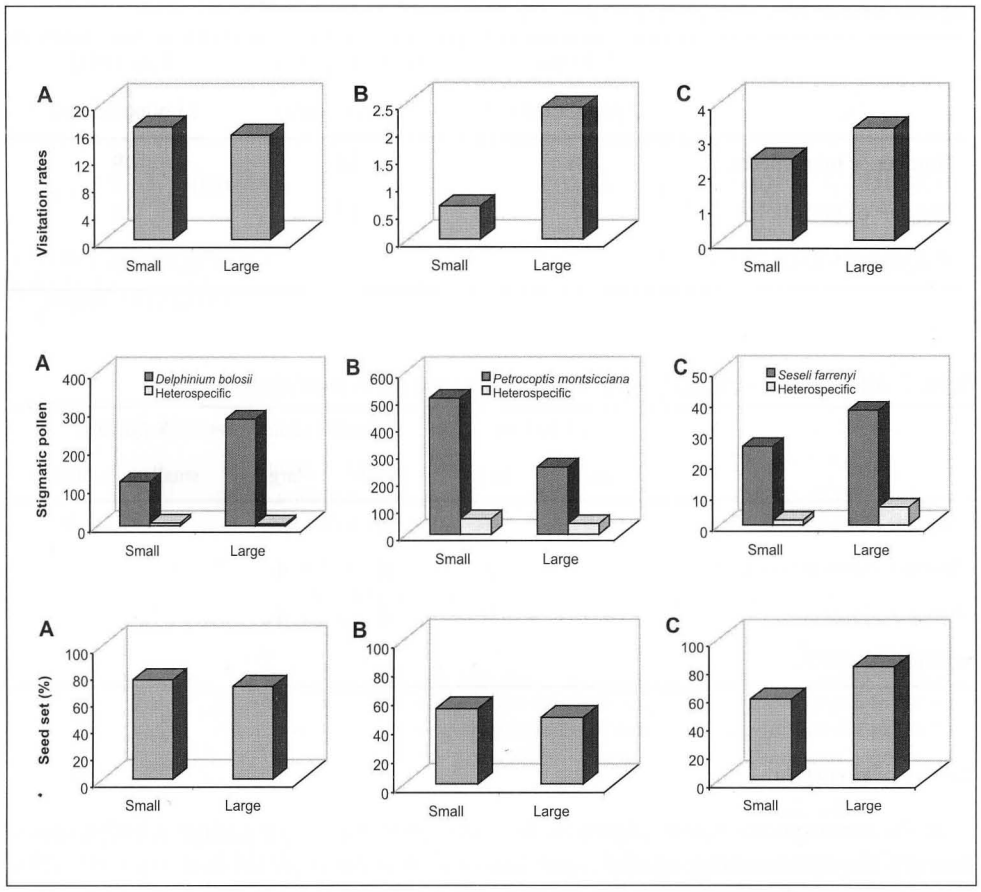

Fig. 1. Visitation rates, stigmatic pollen loads and seed set in the large and small populations of the three studied species: A) *Delphinium bolosii*, B) *Petrocoptis montsicciana* and C) *Seseli farrenyi*.

(1998) and in López-Pujol & al. (2001 & 2002). We have included additional data from other populations of *S. farrenyi* and *P. montsicciana*. At species level, data show that *S. farrenyi* and *P. montsicciana* have the same total number of alleles, much higher than *D. bolosii* (Table 6). Most of them are rare alleles, with frequencies lower than 0.05. These alleles can be lost relatively rapid as consequence of processes such as random genetic drift (Lawrence & Marshall 1997). At level of pair comparisons, in general, as expected by theory, the large populations have more alleles, and also more private alleles (Table 7). Except for *D. bolosii*, the large populations have more rare alleles than the smaller ones. The reduced number of alleles in small populations can be explained by their loss from the allelic pool induced by genetic drift (Barrett & Kohn 1991).

Table 6. Allelic richness at species level.

	D. bolosii (2 populations)	*P. montsicciana* (4 populations)	*S. farrenyi* (3 populations)
Number of total alleles	21	49	49
Number of rare alleles (frequency < 0.05)	2	17	22

Table 7. Allelic richness in comparison between large and small populations.

	D. bolosii		*P. montsicciana*		*S. farrenyi*	
	small	large	small	large	small	large
Number of total alleles	19	20	29	33	36	46
Number of private alleles	1	2	0	4	0	4
Number of rare alleles (frequency < 0.05)	2	0	2	4	8	17

Levels of diversity

In the three species, levels of genetic diversity, given by the percentage of polymorphic loci (*P*), the mean number of alleles per locus (*A*), and the expected heterozygosity (*He*), are higher than those expected for endemic species reported by Hamrick & Godt (1996) (Table 8). The low levels of genetic diversity in rare and endemic species have been related to different reasons: effects of small population size, isolation of populations (Barrett & Kohn 1991; Ellstrand & Elam 1993), and adaptation to uniform habitats (Babbel & Selander 1974; Gray 1996). However, values are really high in *S. farrenyi* but also in *P. montsicciana*. We must point out that there are plenty of rare alleles in these two species, and a decrease in population sizes will drive quickly to their loss and, consequently, the reduction in values of genetic diversity parameters. In the three species a general pattern of deficiency of heterozygotes take place, if we compare the observed values with the expected ones for heterozygosity. The deficiency of heterozygotes can be attributed to several causes, like preferential mating among similar genotypes, selection for homozygotes, and population structure in neighborhoods or subpopulations, among others (Oostermeijer & al. 1995; Elam 1998).

At level of pair comparisons, large populations have more genetic diversity than the smaller ones, as expected (Table 9). Values of *A* and *P* are higher for large populations in the three species, with the exception of expected heterozygosity, which is lower in the large population of *D. bolosii*. Observed heterozygosity is, in all cases, lower than expected het-

Table 8. Genetic diversity parameters at species level. *P*: percentage of polymorphic loci; *A*: mean number of alleles per locus; H_o: observed heterozygosity; H_e: expected panmictic heterozygosity.

	D. bolosii (2 populations)	*P. montsicciana* (4 populations)	*S. farrenyi* (3 populations)	Hamrick & Godt (1996)
P	41.7	70.3	83.3	26.3
A	1.65	2.2	3.0	1.39
He	0.117	0.239	0.297	0.063
Ho	0.056	0.121	0.120	---

Table 9. Genetic diversity parameters in comparisons between large and small populations. *P*: percentage of polymorphic loci; *A*: mean number of alleles per locus; H_o: observed heterozygosity; H_e: expected panmictic heterozygosity.

	D. bolosii		*P. montsicciana*		*S. farrenyi*	
	small	large	small	large	small	large
P	33.3	50	56.3	75.0	78.6	85.7
A	1.6	1.7	1.8	2.1	2.6	3.3
H_e	0.125	0.109	0.221	0.243	0.285	0.302
Ho	0.083	0.030	0.146	0.127	0.124	0.137

erozygosity, which draws a general pattern of deficiency of heterozygotes. This genetic erosion can be explained by increased levels of inbreeding in the three species, which can be attributed to several reasons depending on the species studied:

1. In *D. bolosii*, the increased selfing rates in the small population (Bosch & al. 1998) can explain the observed deficiency of heterozygotes.
2. In *P. montsicciana*, inbreeding is produced by some selfing and structuring of populations in genetic neighborhoods, where mating is among related individuals (López-Pujol & al. 2001).
3. In *S. farrenyi*, increased levels of inbreeding are probably caused by structuring of populations in spatial subpopulations (López-Pujol & al. 2002). Differences in phenology of individuals within subpopulations (only 27 % of individuals blooms and set seeds every year) also reduces the effective population size and increases inbreeding.

Distribution of genetic diversity

Distribution of genetic diversity is quite different in the three species (Table 10).

Table 10. Distribution of genetic diversity in the studied species. H_T: total genetic diversity; H_S: genetic diversity within populations; D_{ST}: genetic diversity between populations; G_{ST}: proportion of interpopulation differentiation; *Nm*: genetic flow.

Parameters	*D. bolosii* (2 populations)	*P. montsicciana* (4 populations)	*S. farrenyi* (3 populations)
H_T	0.142	0.384	0.310
H_S	0.118	0.239	0.297
D_{ST}	0.023	0.144	0.013
G_{ST}	0.166 (~17%)	0.376 (~40%)	0.041 (~4%)
Nm	1.26	0.415	5.85

In *D. bolosii*, values of genetic diversity between populations (D_{ST}) and proportion of interpopulation differentiation (G_{ST}) indicate that genetic diversity is mainly distributed within populations. G_{ST} value suggest that about 17 % of genetic diversity is attributable to differences between populations. Gene flow (*Nm*) is low, but enough (higher than one) to prevent divergence by genetic drift. Geographic isolation of populations – about 64 km between the only two existing populations – is probably the responsible for the low interchange of genes.

In *P. montsicciana*, the fraction of allozyme variation due to interpopulation differentiation is very high, near 40 %. Value of gene flow is extremely low, due to the isolation of populations – mean distance among studied populations is about 30 km – and limited seed and pollen dispersal. In this species gene flow appears not to be a strong force enough to deter the random loss of alleles.

In *S. farrenyi*, values of D_{ST} and G_{ST} indicate that most of the genetic diversity is distributed within populations and that there is very low divergence among populations (only about 4 %). The value of gene flow is very high, indicating a substantial interchange of genes among populations, and can be explained by relative large distances of seed dispersal and high proximity among populations – the range of distances are 1-3 km.

Conclusions

Depending on the particular evolutionary history, population biology and degree of disturbance of each species, a particular specific set of consequences can be drawn:

In *D. bolosii* no significant differences between both populations were detected in relation to pollination processes and those do not overpass the security threshold. The same results were obtained for allozymic diversity, although the small population is slightly more impoverished genetically. However, the large population has more inbreeding since a higher deficit of heterozygotes. One explanation might be pollinator behavior, which

have to move greater distances in a lineal distribution in the large population and individuals are maybe grouped in neighborhoods, whereas in the small population crossings could be more random.

P. montsicciana showed an unexpected behavior: the small population, despite to receive less visits, had more pollen on the stigmas and produced more seeds. One plausible explanation could be an increase of self-pollination rates in the small population. Insect exclusion tests carried out in both populations during different years always showed a higher seed set in flowers from the small population (unpublished data). The lower genetic diversity detected in relation to the large population gives support to this hypothesis.

In constrast, in *S. farrenyi* took place the expected consequences of fragmentation: in the small population we observed a reduction of pollination services due to a slightly reduction of visitation rates but also due to a lower pollinator effectiveness because a higher proportion of ants were detected in this population, whereas in the large one bees and wasps were more frequent and more effective pollinators (unpublished data). This was translated in a clear decrease of pollen on the stigmas and a reduction of seed set in the small population. Allozyme variation was also lower in this population. All these results support the idea that the small population is declining.

From the data obtained on these three endemic Mediterranean species surveyed in the present contribution, the following general concluding remarks can be stated:

* There is no general pattern of fragmentation effects on pollination and genetic diversity in Mediterranean endemics. A particular behavior taxon-specific is observed, including a wide set of consequences which incidence depends on pollination and reproductive systems, present day geographic distribution and evolution history of each species. Thus, the general diversity of Mediterranean flora is also reflected on levels of response to fragmentation.

* The effects on visitation rates are more pronounced in species particularly sensitive due to a high dependence on pollinator activity to be fertilized; the polyploid, vegetative reproducing and self-pollinated species are less affected by pollination consequences of fragmentation. These are identified by a decrease of visitation rates due to low attractiveness of small isolated populations (less flowers open per patch, a decrease of number of visits per census or a decrease of visited flowers rates). In spite of that, the time spent by each insect per visit is not correlated with the number of visits, and thus, the final effectiveness of the process is not necessarily reduced. In any case, the behavior of each visitor can act as a safety buffer.

* The final effectiveness of pollinator services lie in the quantity (total number of pollen grains deposited on stigmas) and quality (composition and percentage of conspecific pollen) in small populations. In our species, a combination of loss of quantity, quality or both have been detected, although critic levels are not reached and no obvious effect on final seed set can be assessed, this event take place and help to describe the consequences of fragmentation.

* A clear genetic consequence from this survey is the allelic impoverishment in small populations, both by loss of the total number of alleles per population and by low values of *A* and *P*. In addition, rare or private alleles were also lost in small populations. This is true in all the pairs of small-large populations investigated, except in the case of *D. bolosii*:

in this species, both populations have suffered a notably loss of alleles. On the other hand, in all cases, low values of observed heterozygosity (*Ho*) respect of expected heterozygosity (*He*), meaning a deficit of heterozygotes (or excess of homozygotes), where detected, a clear symptom of loss of genetic diversity. This can be explained by:

(1) an increase of selfing in small populations (monoparental inbreeding)

(2) an increase of mating between closely related individuals with similar genotypes (biparental inbreeding) due to a reduced number of available flowers to interchange genes and due to particular flight patterns of insects.

(3) a reduced effective population size: this is particularly evident in species with a rosette strategy, relatively frequent in Mediterranean flora: the total number of individuals is higher than those flowering at a given season, and thus, available gene sources are lower in number (although genotypes are extended in time). Polyploidy and vegetative propagation could buffer these effects (Simon & al. 2001), but fragmentation effects on reproduction are expected to be more severe in rosette plants than in species in which effective population size (*Ne*) is near to its total population size (*N*).

* Finally, fragmentation effects on genetic diversity clearly suggest that distance between populations is a factor determinant to block or to allow genetic interchanges between populations. The limit depends on dispersal capability of each species but, in the species surveyed, gaps of 30 km are enough to effectively isolate populations whereas gene flow is maintained in population pairs distanced by only 1-2 km.

We are convinced that further research is needed to increase the knowledge of fragmentation effects on Mediterranean endemics, provided the specific response of each taxon to restriction in population size and distance between remnant populations. Only when a substantially increased scientific background is achieved, management plans will be able to counteract efficiently fragmentation of endemic and endangered species and, thus, conservation biology investigation will meet the challenge of truly influence biodiversity conservation policies.

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