

Federico Selvi & Graziana Fiorini

***Carex grioletii* Roemer (Cyperaceae) in Tuscany and its conservation status**

Abstract

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On the base of the finding of new stations of *Carex grioletii* in Tuscany, our chorological, ecological and karyological knowledge of this rare plant of the mediterranean-pontic flora is brought up to date. In Tuscany there had been no data about this elusive species for almost a century, as a consequence of the difficult accessibility of the growing sites of the populations, their small area, and of the anthropic alterations occurred in two of the three localities in which it was found in the past. The populations are all restricted to shady and mesic forest habitats of the mediterranean and submediterranean belt with a conservative power for species of the tethyan-tertiary flora. *C. grioletii* has $2n = 48$ small-sized chromosomes, an uncommon number within the genus *Carex*. Although less rare than previously thought, this species could become vulnerable to extinction because of its low adaptive capacity to changed ecological conditions and expansion inability connected probably to its reproductive biology and to the absence of vegetative spreading systems.

Introduction

Carex grioletii belongs to sect. *Acrocystis* Dumort. of the genus *Carex* L., which includes the taxa with a terminal male spike and a variable number of lateral female inflorescences with tristigmatic flowers and ovoid to subglobose, often puberulent utricles (Stoeva & Popova 1993). It is a mediterranean-pontic species with a bipolar and disjunct distribution range stretching between the Black and the Caspian Seas (Pontic-Caucasian area) and in the Northern mediterranean basin, where it is known to occur only in a few, isolated localities of the Montenegro coast (Chater 1980), peninsular Italy and Sicily, South-Eastern France (Malinvaud & Heribaud 1901) and North-Eastern Spain (Velayos & al. 1991). This strongly fragmented type of distribution, the type of habitat in which the species grows and its systematic position within the genus have led various authors to consider it a preglacial remnant of the ancient mediterranean flora with a particular phytogeographical and historical significance (Sommier 1903, Montelucci 1952, Brilli Cattarini 1965, Pignatti 1982, Chater 1980). In Italy it was observed in the Penisola

Sorrentina (Guadagno 1918), where it has not been recently confirmed (Caputo & al. 1989), in a few localities of Latium (Béguinot 1901, Montelucci 1952, Lattanzi & Lucchese 1984, Lucchese 1986), Marche (Brilli Cattarini 1965, Brilli Cattarini & Ballelli 1979), Tuscany and Liguria (Burnat 1893), where the species was discovered by Griolet in the "Selva Lomellina" near the park of Villa Doria in Pegli in 1803 (Bertoloni 1854). Chiovena (1927) reported *C. grioletii* for Sicily on the base of a herbarium record referred to *C. distans* L. by Tornabene (1892); the plant was recently confirmed for the Sicilian flora by Rossitto & Ottonello (1986), who found a single population in a mesic habitat in the Caronie mountains (Messina). In Tuscany *C. grioletii* was first observed by P. Savi in 1843 (Caruel 1860), then collected by L. Doria on Isola del Giglio (Tuscan Archipelago) in April 1898 (Sommier 1902) and later by Sommier (1903) in two localities of Monte Argentario.

Despite the number of geobotanical studies carried out in Tuscany, no data about this elusive plant have been published for almost a century. Recent floristic researches on Monte Pisano (Del Prete & al. 1990) and Monte Argentario (Baldini 1995) gave negative results about its presence in the formerly known stations. Already Caruel (1871) supposed its disappearance from Mt Pisano, and the two localities of Mt Argentario have been reported to be currently too altered from an ecological viewpoint to shelter the populations discovered at the beginning of the century (Baldini 1995). On the contrary, the plant has been recently confirmed for Isola del Giglio (Brilli Cattarini, pers. comm., PESA !), where it still grows in the formerly known locality ("Acqua Selvaggia").

The recent discovery of *C. grioletii* in a remote and floristically unknown area of Northern Maremma (Selvi 1998) has suggested to investigate in greater detail the chorology, synecology, karyology and some demographic parameters of this rare species in Tuscany. The results of these researches are reported in this paper and discussed in relation to some conservation aspects which may help the management of the growing sites of the populations.

Results

New stations of Carex grioletii

Field researches were carried out in the years 1993-1996 in a large part of Central-Southern Tuscany, mainly in the forest hill areas of the mediterranean and submediterranean belt. *C. grioletii* was found in 8 new stations (Fig. 1), mainly in the Northern part of the Grosseto Province.

Herbarium records are currently kept in FI, SIENA and in Herb. Selvi. For each population, the approximate number of individuals and the surface occupied by the population were estimated, along with its distance from forest tracks, roads, buildings or other potential sources of external threat. A phytosociological relevè was also carried out to analyse synecological aspects.

In May 1994 *C. grioletii* was found in the Northern slopes of Mt Leoni (Grosseto), in a deep and narrow valley called "Fosso dei Roghiccioni" (IGM map n° 319 sect. 1) at an elevation of about 360 m a.s.l. The population was much localized and with a small number of individuals (Table 1), partly in flower and partly with already mature fruits.

Table 1. Demographic and vulnerability parameters for each verified growing site of *C. grioletii* in Tuscany.

Site	Approximate number of individuals	Approximate population area m ²	Distance from roads and forest tracks (m)	Type of anthropic activity in the site	Legal protection and property of the site
Mt Leoni, F.so Roghiccioni	6-10	150	600	None	None, private p.
Mt Leoni, F.so Carpineta	30-45	500	15	Coppicing	None, private p.
Mt Leoni, F.so Falsacqua	15-20	220	1	Coppicing	None, private p.
Mt Alma, F.so Santa Lucia	30-50	340	10	Turistic trekking and coppicing	Regional property
Farma valley	15-25	560	850	None	Regional nature reserve
Sassetta	2-4	110	50	None	Regional property
Valpiana, F.so Rigattaie	8-12	170	50	None	Regional property
Pecora valley	20-30	200	30	Coppicing	None, private p.

It was restricted to a very shaded slope with an elevated atmospheric and edaphic humidity and a brown forest soil derived from siliceous rocks of the "Verrucano" geological formation.

The vegetation was represented by a mesophytic pluristratified tall forest including both evergreen and deciduous species, with a sparse herbaceous layer (Table 2). The concentration of several evergreen mesohygrophytic shade species, such as *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, *Ficus carica* L., *Laurus nobilis* L., *Ruscus hypoglossum* L., *Polystichum setiferum* (Forssk.) Woynar, *Hypericum androsaemum* L. and *Daphne laureola* L., was particularly relevant

In the Mt Leoni area, *C. grioletii* was later found along Fosso della Falsacqua (IGM map n° 319 sect. 1) and Fosso Carpineta on the N. W. slopes of Poggio Val di Loria (IGM map n° 319 sect. 4), at an elevation between 300 and 400 m a.s.l. Both populations consisted of a good number of individuals (Table 1) and were restricted to mesic habitats with *Quercus ilex*, *Quercus cerris*, *Quercus suber* and in one case, *Ostrya carpinifolia* (Table 2).

Table 2. Vegetation sample plots of phytocoenoses with *Carex grioletii*.

Site	Leoni	Leoni	Alma	Farma	Farma	Valp.	Sass.	
Surface mq	400	350	250	600	400	250	200	
Altitude (m a.s.l.)	360	320	120	220	220	90	120	
Slope aspect	N	NW			N		E	
Inclination (°)	25	200			15		15	
Ground cover (%)	100	100	100	100	100	100	100	
Number of species	23	22	24	25	35	20	11	
Hm 22	<i>Quercus cerris</i> L.	4	2	5		4	5	2
	<i>Quercus ilex</i> L.	4	4	2		3	1	4
	<i>Quercus petraea</i> (Matt.) Liebl.	2			2			
	<i>Populus tremula</i> L.				3	4		
	<i>Fraxinus angustifolia</i> Vahl				2	1		
	<i>Alnus glutinosa</i> (L.) Gaertner				4			
Hm 14	<i>Hedera helix</i> L.	1		+	+	1	+	
	<i>Carpinus betulus</i> L.	2		2	3	4		
	<i>Fraxinus ornus</i> L.	2	1	1			1	2
	<i>Ostrya carpinifolia</i> Scop.	1	2			2		
	<i>Vitis vinifera</i> L. subsp. <i>sylvestris</i> (Gmel.) Hegi	+	+	+				
	<i>Castanea sativa</i> Mill.					1		
	<i>Acer campestre</i> L.					1		
Hm 7	<i>Ilex aquifolium</i> L.	+			1	2		
	<i>Taxus baccata</i> L.				2	3		
	<i>Corylus avellana</i> L.				1	+		
	<i>Ulmus minor</i> Mill.			1			1	
Hm 3	<i>Phyllirea latifolia</i> L.		+	1			+	1
	<i>Viburnum tinus</i> L.		2	1			+	
	<i>Cornus mas</i> L.				+	1	+	
	<i>Arbutus unedo</i> L.		+					2
	<i>Ficus carica</i> L.	1		+				
	<i>Laurus nobilis</i> L.	1						
Hm 1	<i>Ligustrum vulgare</i> L.			+		+	+	
	<i>Tamus communis</i> L.		+				+	+
	<i>Lonicera etrusca</i> Santi		+	r				
	<i>Polystichum setiferum</i> (Forssk.) Woynar	1			2			
	<i>Myrtus communis</i> L.							1
	<i>Carex pendula</i> Huds.				1			
	<i>Osmunda regalis</i> L.				2			
Hm 0,4	<i>Carex grioletii</i> Roemer	r	+	1	+	+	+	r
	<i>Rubia peregrina</i> L.	r	+	1		r	+	+
	<i>Hedera helix</i> L.	+	2	1	1	1	2	
	<i>Melica uniflora</i> L.	r		+	+	+	+	
	<i>Daphne laureola</i> L.	+	r		r	+		
	<i>Brachypodium sylvaticum</i> (Huds.) Beauv.		r	+		1	1	

Table 2. Vegetation sample plots of phytocoenoses with *Carex grioletii*.

Site	Leoni	Leoni	Alma	Farma	Farma	Valp.	Sass.
Surface mq	400	350	250	600	400	250	200
Altitude (m a.s.l.)	360	320	120	220	220	90	120
Slope aspect	N	NW			N		E
Inclination (°)	25	200			15		15
Ground cover (%)	100	100	100	100	100	100	100
Number of species	23	22	24	25	35	20	11
<i>Rosa sempervirens</i> L.		r	r			+	r
<i>Ruscus aculeatus</i> L.			+		1	1	
<i>Carex sylvatica</i> Huds.	+		r		+		
<i>Viola reichenbachiana</i> Jord.			+	+	+		
<i>Viburnum tinus</i> L.	1	+	+				
<i>Cyclamen hederifolium</i> Ait.		1	1		+		
<i>Anemone apennina</i> L.		+	1			+	
<i>Myosotis scorpioides</i> L.				+	+		
<i>Anemone nemorosa</i> L.	+				1		
<i>Mycelis muralis</i> (L.) Dumort	r				r		
<i>Asparagus acutifolius</i> L.						r	+
<i>Symphytum tuberosum</i> L.		+	+				
<i>Luzula forsteri</i> (Sm.) DC.	r	r					
<i>Hypericum androsaemum</i> L.	r			+			
<i>Ranunculus velutinus</i> Ten.		r				r	
<i>Carex distachya</i> Desf.		r					+
<i>Stachys sylvatica</i> L.					r		
<i>Galium palustre</i> L.				+			
<i>Carex digitata</i> L.					+		
<i>Luzula pilosa</i> (L.) Willd.					r		
<i>Vinca minor</i> L.					+		
<i>Physospermum cornubiense</i> DC.					r		
<i>Sanicula europaea</i> L.					+		
<i>Primula acaulis</i> L.					1		
<i>Rubus hirtus</i> W. et K.					+		
<i>Blechnum spicant</i> (L.) Roth				1			
<i>Athyrium filix-femina</i> (L.) Roth				1			
<i>Carex remota</i> L.				1			
<i>Carex pallescens</i> L.				r			
<i>Ajuga reptans</i> L.						r	
<i>Ruscus hypoglossum</i> L.	+						
<i>Epipactis microphylla</i> (Ehrh.) Swartz					r		

In June 1995, *C. grioletii* was discovered in the upper part of the Alma valley, that divides the two hill areas of Monte d'Alma at the North and Poggio Ballone at the South (IGM map n° 318 sect. 2). The population stretched over a few hundreds square meters and consisted of a good number of individuals (Table 1) localized along the banks of the stream called "Fosso di Santa Lucia" at an elevation of about 120 m a.s.l.

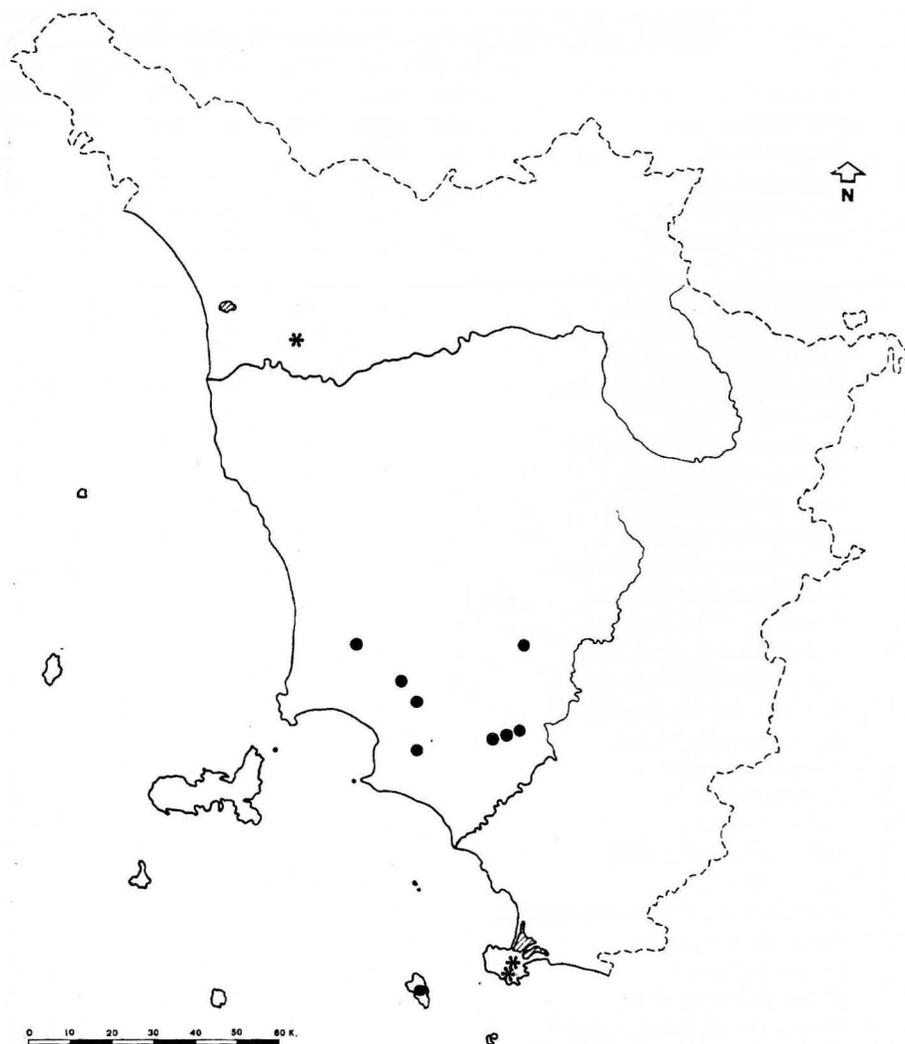


Fig. 1. Location of recently verified growing sites (circles) and of those not recently confirmed (stars) of *Carex grioletii* Roemer in Tuscany.

The vegetation was developed on a rich brown forest soil derived from a quartzitic-feldspatic sandstone and consisted of a stratified mixed community dominated by *Q. cerris* L., *Quercus ilex* L. and *Carpinus betulus* L. (Table 2).

A population of *C. grioletii* was found in June 1996 in the middle part of the Farma river (IGM map n° 307 sect. 2.), an East-West oriented valley separating the Grosseto and the Siena provinces. The plant was localized in the bottom of the valley along the southern bank of the river. The few individuals were scattered over a relatively small surface (Table 1) at the foot of a steep and humid slope. They were part of a rich herbaceous layer of a

very tall meso-hygrophytic vegetation that did not show any trace of past human disturbance (Table 2), with old trees of *Taxus baccata* L. and *Ilex aquifolium* L. and, in the proximity, *Osmunda regalis* L., *Blechnum spicant* (L.) Roth, *Athyrium filix-femina* (L.) Roth, and *Polystichum setiferum* (Forssk.) Woynar.

C. grioletii was then observed in the hill area of Sassetta (Livorno), in the locality "Pineta" (IGM map n° 306 sect. 4), a low sandstone relief (120 m a.s.l.) between the two fluvial depressions of the Lodano and Massera streams. The very small population (Table 1) was localized in the herbaceous layer of a mixed community dominated by *Quercus ilex* and small deciduous trees (Table 2).

In the same year, this species was found in the lower part of the valley called "Fosso delle Rigattaie" near the village of Valpiana (IGM map n° 318 sect. 1). Few individuals (Table 1), mostly in fruit, were restricted to the shady bottom of the narrow valley, in which the vegetation was represented by a mesophytic community dominated by *Quercus cerris* (Table 2).

A further station of *C. grioletii* has been discovered in the low part of the Pecora river, at the bottom of the valley of Fosso Borgognano near its confluence with "Botro alla Fonte" (IGM map n° 306 sect. 3). The population consisted of a good number of individuals (Table 1) completely in fruit and was part of the herbaceous layer of a mesotrophic community type dominated by *Quercus cerris*.

Karyology

Karyological analyses were carried out on a single plant collected in the Monte Leoni area (F.so Falsacqua), potted and currently cultivated in the Botanical Garden of Florence. Germination tests made on mature utricles taken from three provenances (Mt Leoni, Mt Alma and Farma valley) gave completely negative results, despite the different preparatory treatments to which the seeds were previously subjected (chilling). Therefore, observations were made on mitotic metaphase plates of meristematic cells taken from several apical portions of small adventive lateral roots. The chromosome number was determined from countings on 23 plates obtained from about 60 root apices. After a pretreatment of 2 h in a 8-hydroxyquinolin solution, the material was fixed in Carnoy 3:1, hydrolized in HCl 1N at 60° C for 6 min. and then stained with lactopropionorcein (18 h). Observations were made by means of a Zeiss Axiophot Light Microscopy at $\times 100$ magnification.

C. grioletii has $2n = 48$ chromosomes (Fig. 2) and our finding agrees with a single previous counting made on material from Val Seborino, Liguria (Dietrich 1964). The number is uncommon within the genus *Carex*, which is characterized by the presence of several basic numbers and presence of complex aneuploid series (Heilborn 1924, Davies 1956). Sect. *Acrocystis* in particular is characterized by relatively low chromosome numbers and includes the only other species for which $2n = 48$ has been ascertained, *C. tomentosa* L., which is also the phenotypically closest entity to *C. grioletii* (Stoeva & Popova 1993). The chromosomes are small-sized and dot-like, as in many other *Cyperaceae*. Despite the small size, however, the centromere area was in some cases recognizable in central position to the arms. As observed by Stoeva & Popova (1993) in *C. tomentosa*, chromosomes of *C. grioletii* could be grouped into three size classes: $1.5-1.7 \times 1.1-1.2 \mu\text{m}$, $1.2-1.4 \times 1.0-1.1 \mu\text{m}$, $1.0 \times 0.9 \mu\text{m}$, but the number of chromosomes per class

was very variable. In several plates chromosomes were observed to form groups of three or four.

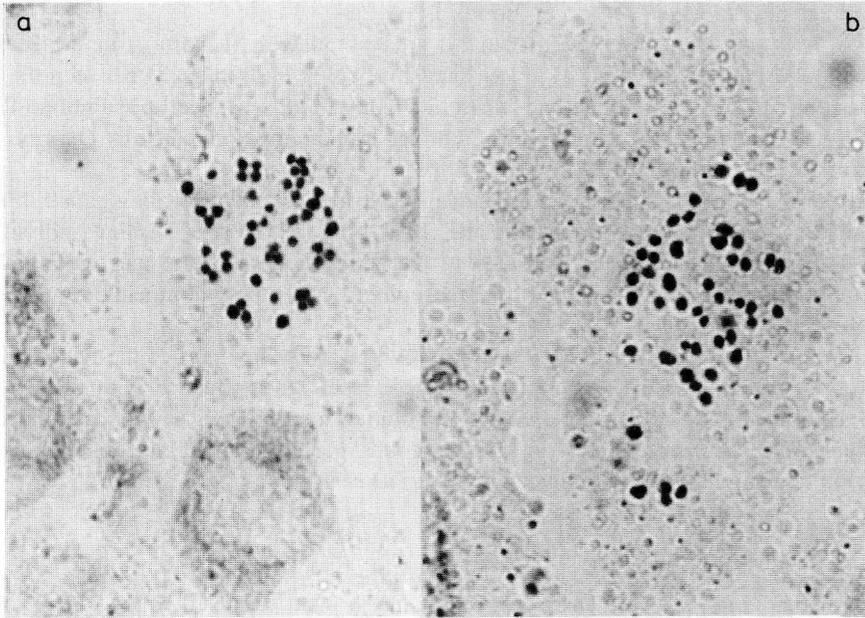


Fig. 2. Metaphasic chromosome plates (a, b) of *C. grioletii* from Mt Leoni (Grosseto). — Scale bar = 10 μ m.

Discussion

The results of this study show that *C. grioletii* is less rare than previously thought, at least in Tuscany. The reasons of its putative rarity are connected to the small size of the populations, their strong localization and their difficult accessibility. As observed by the authors who have previously treated the argument (Béguinot 1901, Sommier 1902, Montelucci 1952, Brillì Cattarini 1965, Rossitto & Ottonello 1986), *C. grioletii* is generally restricted to well-preserved, moist and shady habitats of the mediterranean or submediterranean belt. From a phytocoenological viewpoint, it seems linked to mesohygrophilous aspects of the *Quercus cerris* vegetation (*Melico uniflorae-Quercetum cerridis* subass. *carpinetosum betuli* Arrig.), in particular to those of low altitude that come into close contact with the mediterranean evergreen vegetation thanks to the peculiar conditions occurring at the bottom of narrow valleys. In these community types it can be associated to heterotopic populations of submontane forest species (as in the Farma Valley) or, more often, to nemoral plants of the hill deciduous belt. It can be found even in mesic aspects of mediterranean vegetation with a mixing of *Quercus ilex* and deciduous trees such as *Ostrya carpinifolia*, *Fraxinus ornus* or *Quercus cerris* (*Quercetum ilicis mediterraneo-montanum* Br. Bl.). A particular case is represented by the Farma valley, in

which *C. grioletii* grows in the understory of an hygrophytic community referable to the association *Osmundo regalis-Alnetum glutinosae* Vanden Berghen.

As all these habitat types still occur in several hill areas of Central-Southern Tuscany, it is likely that this species is more widely distributed than what currently known. On the other hand, it does not grow everywhere the habitat is apparently suitable for its ecological requirements. For example *C. grioletii* has not been observed during the recent geobotanical researches in the Farma valley (Chiarucci & al. 1993), suggesting that the plant is there extremely localized despite the presence of several sites with environmental conditions apparently similar to that of the single known population. Also in the Mt Leoni area it lacks from sites where several frequently associated species are instead present. This strong localization could be correlated to a particular sensitivity to ecological microvariations and/or to an expansion inability connected to its reproductive biology. Although the plant set a good amount of apparently good fruits, germination of the seeds was not successful under different experimental conditions. This behaviour might be a consequence of the putative relict status of the populations, which could have undergone increasing homozygosity and genetic depletion due to the small size and the reciprocal isolation. The conservativeness of forest habitats restricted at the bottom of narrow valleys in the Tuscan mediterranean belt is also indicated by the associated presence of shade plants of the tethyan-tertiary flora, such as *Laurus nobilis* L., *Ilex aquifolium*, L. *Osmunda regalis* L., *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, *Ficus carica* L., *Taxus baccata* L., *Ruscus hypoglossum* L. In other mediterranean stations *C. grioletii* is associated to *Pteris cretica* L. and *Woodwardia radicans* (L.) Sw. (Malinvaud & Heribaud 1901, Sommier 1902, Guadagno 1918) and, in the pontic area, to other species of the so called "colchian flora" (Béguinot 1901).

From a conservation viewpoint, *C. grioletii* is mostly dependent on the preservation of its nemoral habitat. Its disappearance from Mt Argentario and from sites recently subjected to forest coppicing in the Mt Leoni area provide circumstantial evidence of its low adaptive capacity to changed ecological conditions. The plant seems not to be capable of demographic expansions due to the absence of vegetative spreading systems and probably to a low germination capacity of the seeds. Although less rare than previously thought, *C. grioletii* could therefore reach a critical demographic threshold, if forest activities will continue in a such a delicate habitat as mesic and shady forests of the mediterranean belt. Future surveys on the populations here described will allow to assess their demographic tendencies and to evaluate their real survival chances in the long-term.

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Address of the authors:

Federico Selvi & Graziana Fiorini, Dipartimento di Biologia Vegetale dell'Università, Via La Pira 4, I-50121 Firenze, Italy.