Flowering dynamics and reproductive success of an *Erythronium dens-canis* population across years

PAOLO PUPILLO
Emeritus professor, Department of Pharmacy and Biotechnology, University of Bologna – Email: paolo.pupillo@unibo.it

Abstract

An *Erythronium dens-canis* population of site "Farneto-C" in the Parco dei Gessi Bolognesi was monitored for years to investigate the major factors impinging on flowering and species propagation. Here, the results for years 2016 and 2018 to 2021 are presented. While flowering/fruiting usually proceeds in parallel with the discoloration of leaf brown spots, both events being apparently steered by spring air temperature, plant emergence from bulb in late winter seems to depend on different environmental factors. About half of the plants are damaged by animals or give unfertile fruits, but the percentage of sound fruit set is increasing in recent years. Finally, the rare *Erythronium* variant with full-green leaves of Farneto-C (3% of total population) is confirmed to cohabit with the common silvery morph (78%) and a mixed ("tricolor") morph (19%).

Key words: dog's tooth violet, *Erythronium dens-canis*, population dynamics, reproductive success, spring ephemerals.

Riassunto

Dinamiche di fioritura e successo riproduttivo di una popolazione di* Erythronium dens-canis *nel corso degli anni
Una popolazione di *Erythronium dens-canis* (dente di cane) del sito "Farneto-C" nel Parco dei Gessi Bolognesi è stata monitorata per diversi anni per studiare i principali fattori che influenzano la fioritura e la propagazione della specie. Sono qui presentati i risultati per gli anni 2016 e dal 2018 al 2021. Mentre la fioritura/fruttificazione in genere procede parallelamente con lo scolorimento delle macchie fogliari brune, in quanto entrambi gli eventi sono apparentemente regolati dalla temperatura dell'atmosfera in primavera, l'emersione della pianta dal bulbo nel tardo inverno sembra dipendere da fattori ambientali differenti. Circa metà delle piante sono danneggiate da animali o danno frutti sterili, ma negli ultimi anni la percentuale di frutti sani è in aumento. Infine si conferma che la rara variante di *Erythronium* con foglie completamente verdi del Farneto-C (il 3% della popolazione complessiva) coabita stabilmente con la comune morfa "argentea" (78%) e una morfa mista ("tricolore") (19%).

Parole chiave: dente di cane, *Erythronium dens-canis*, dinamica di popolazione, successo riproduttivo, piante effimere primaverili.

Introduction

Erythronium dens-canis L., dogtooth's violet (family Liliaceae, tribe Tulipeae), is the only European species of a widespread genus of the northern hemisphere, with about 30 species of bulbous geophytes (Govaerts, 2017). This species is discontinuously present in Italy in hill and mountain districts in fresh deciduous woods or shady meadows at the southern edge of the Alps and in northern Apennines (Kleih, 2010). All *Erythronium* species are typical spring ephemerals, i.e. perennial plants emerging from soil in late winter that carry out their yearly photosynthetic and reproductive life in a couple of months by exploiting full sunlight in the understory of leafless woods, to disappear at the time of tree leaf flushing. *E. dens-canis* is a species within the family Liliaceae with a strong photosynthetic capacity, like other spring ephemerals (Recchia *et al.*, 2017). The flower, a small lily white to rosy in color, stands atop a red scape flanked by two elongated, nonidentical leaves with a lively camouflage variegation (Givnish, 1990). However, immature nonflowering plants, which represent the majority of the population in the study area, are simpler still and show a single, wide leaf. They are thought to reach sexual maturity at a mean age of 7 years when, still in the heart of winter, a rolled leaf erupts from soil, then a second leaf soon sprouts within the first one with a flower bud inside. Later, both leaves will expand to a lanceolate shape at the base of the flower.

The normal variegation of *E. dens-canis* consists of an array or network of brown spots upon a grey-glaucous (silvery) leaf ground, due to light reflection. The brown color of the spots is created by a single layer of mesophyll cells containing a red vacuolar anthocyanin, above the underlying green cell layers (Esteban *et al.*, 2008). The spots will vanish in a few weeks leaving place to bright green areas (La Rocca *et al.*, 2014). Contrary to American and Asiatic *Erythronium* species, the biology and ecology of European dog's tooth violet has been little explored (Guitiàn *et al.*, 2008). We therefore considered it of interest to report on the phenology of this plant over years, also in relation to current climate changes to which *Erythronium* is particularly exposed by reason of its ecology and lifecycle (Lambert *et al.*, 2010; Gandin *et al.*, 2011; Thomson, 2019). And *Erythronium* merits further consideration according to recent reports on relief effects of its extracts on human health, i.e. inflammation (Park & Kim, 2015) and breast cancer (You *et al.*, 2020).

Materials and Methods

Site and sampling. Data were collected beginning 2011 and systematically since 2016 in the site named Farneto-C, which hosts one of a few scattered populations occurring in the nature park Parco dei Gessi Bolognesi (44°25'N 11°24'E, 270-290 m altitude). Farneto-C is a moderately drooping area at the edge of a closed karst valley (Buca dell'Inferno) with a thin soil layer on chalk substrate (PUPILLO & ASTUTI, 2017) and a low, discontinuous covering of low trees, mainly powder oak (*Quercus pubescens*), hop-hornbeam (*Ostrya carpinifolia*) and flowering ash (*Fraxinus ornus*), with an understory of *Ruscus aculeatus*, *Asparagus acutifolius* and perennial herbs (*Cyclamen hederifolium*, *Helleborus viridis*, *Scilla bifolia, Anemone nemorosa*). Flowering individuals of *Erythronium dens-canis* flush from bulb in late winter. Those surviving animal predation carry on their short aboveground cycle until the end of April, then they disappear concomitant with tree canopy closure to emerge again next February.

Photographic surveys with weekly field monitoring were conducted every year from February to April (8-15 surveys according to years) on all flowering *Erythronium* plants on both sides of a small dirt road (via Gaibola) and along an adjoining track for a total length of 93 m (only 71 m in some years, as seen from the variable number of plants reported, 72 to 153) within a depth of 2 m from the edges. The group of new plants found during a weekly visit is defined a "cohort". In this way, a series of up to 15 weekly pictures ("occurrences") for individual plants was obtained each year. Plants documented by a single picture were not considered for statistics. Photographs were usually taken with a Nikon D90 camera equipped with DX 18-105 mm objective. About 1300 to 2100 photos were shooted each year from plant emergence to senescence, selected, trimmed and inspected as a database for statistics. About 6880 archive images are still available for five vintages reported in this paper: years 2016, 2018, 2019, 2020 and 2021. Nonflowering *Erythronium* plants, which represent the majority of the population, are not considered in this study.

Leaf decoration. *Erythronium* leaves are categorized according to three basic variegation types: the dominant silvery (*SLV*) type with grey-green leaf background; a less frequent silvery-and-green type (*S&G*); and a rare full-green variant (*GRN*) found only in this site as far as known. All three types exhibit brown spots or complex brown patterns (later turning to a vivid green) on the basal ground color. Spot discoloration was followed using three descriptors: brown spots (*brs*), partially depigmented spots (*pid*), wholly green-discolored spots (*whg*).

Reproductive success. A number of flowering *Erythronium* individuals are predated by mammals, which surgically remove bud or fruit ("damaged/detopped" plants, *dam*), or effect whole plant extirpation ("missing" plants, *mis*). Moreover, some plants produce unfertile capsules which later yellow and fall (aborted plants, *abo*). For calculation of reproductive (in)success, *dam*, *mis* and *abo* percentages are sometimes pooled together.

Results

The population of dog's tooth violet *Erythronium dens-canis* at the Farneto-C site has been the object of our studies since 2011, and results of relatively complete surveys in definite areas of the site performed in years 2016, 2018, 2019, 2020 and 2021 are reported below. Growth and development of all flowering erythroniums of the study area (72 to 153 plants according to years) were followed from the day of discovery to senescence (or disappearance) through a dozen of weekly field surveys with photographic recording. The data for five years of investigation are organized under three headings: Fig. 1 population dynamics, Fig. 2 plant growth and lifecycle, Fig. 3 leaf spot discoloration. The statistics of Figs. 1-3 feature a cumulative plot of plant responses over 11 weeks, i.e. week 5 (early February) to week 15 (mid April) for a each year.

As previously reported (LA ROCCA *et al.* 2014) flowering individuals represent a minority in Farneto-C population, that largely consist of young or semimature-nonflowering plants with a single leaf. However, a significant increase of flowers has occurred since 2015 to about 8-17% of the total *Erythronium* population according to locations (average 11%). Flowering specimens are usually the first erythroniums flushing from soil, even under snow covering and well before most younger plants, and are among the earliest plants to show up at the end of winter in these woods. Fig. 1 A-E represents the trend of population (POP) dynamics, with a population maximum always at weeks 8-9 (late February). This effect is clearly due to early plant emergence (*new* plants) mainly at weeks 5-7 (yrs. 2016, 2018) or 7-8 (yrs. 2019-2021), although effects of ungulate predation will soon become manifest. The plant disappearance parameter (*mis*) can be taken as a reliable albeit indirect measure of predation pressure, other possible causes (landslides, wildboar trampling) being unlikely in this season. Parameter *mis* suggests that grazing was heavier in yrs. 2016 and 2018 when more than one-third of all plants had vanished at fruiting stage, and again in 2021, less in intermediate years, but ascertained partial dam-

Year	*% dam*	*% mis*	*% abo*	*% dam + mis*	*% tot. failed*
2016	6.8	34.2	19.2	41.0	60.2
2018	11.1	33.3	10.9	44.4	55.3
2019	8.1	24.3	18.9	32.4	51.3
2020	17.8	17.0	7.4	34.8	42.2
2021	7.2	27.4	6.5	34.6	41.1

Table 1. Reproductive failure in *Erythronium dens-canis*. Percentages of damaged-detopped plants (*dam*), of missing plants (*mis*) and of aborted fruits (*abo*) in the Farneto-C population.

ages by ungulates (*dam*) tend to level up these figures (Table 1). The plant developmental progress during late winter and early spring (buds first, then flowers and fruits) is outlined in Fig. 2 A-E containing a phenology (PHEN) survey. Here, the complex event of early plant growth is represented under the term *bud* from the time of sprouting to the stage of reclined blossom at the tip of a long, upward stretched scape. Not surprisingly, the precocious *bud* stage is normally more or less overlapped with newly emerged plants (*new*, Fig. 1 A-E) although it can be retarded by cold or snowy weather. Flowering, however, is the central event in the aboveground life of *Erythronium*, and manifests itself as a single flower wave or outbreak peaking in 1-2 weeks' time. Later, isolated flowering specimens tend to be reduced in size and rarely get to fruit ripening (fruit abortion). Flowering process itself appears to be markedly dependent on climate conditions, for example it was strongly delayed in year 2018 as a consequence of snow and cold rain (Fig. 2B). In general, bad weather spells resulted in significant shifts of flowering and fruiting times, and by preventing pollination they were a major cause of fruit loss in some years, up to some 19% of all plants (Table 1). Adverse climate was not the only cause of abortion, however; another one was clearly spring drought. In Fig. 2 A-E fruit failure by abortion is added up to losses by ungulates; roe deer can nibble flower buds or fruits with surgical aim. Reproductive failure by about half of all plants is the cumulative consequence of abortion and grazing, the latter being by and large the prevalent factor. While many data in Table 1 are apparently random as they afford little insight (if any) into the possible causes, it is interesting that the sum of damaged/detopped and missing (i.e., wholly grazed) plants is almost constant over years. Also notable is the gradual reduction of total fruit losses since 2016, mainly due to decreasing abortion events.

Another event in *Eythronium* life history is spot discoloration (La Rocca *et al.* 2014). Starting from the known circumstance that all mature *Erythronium* leaves present brown spots (*brs*) from birth to flowering at least, it was noted that within one month from flushing the pigment was usually vanished. The pigment resorption process was completed in a few days, such that the intermediate stage ("pigment disappearing" *pid*), when the spots were fading away, was often overlooked (Fig. 3 A-E). A brilliant green area of the original shape remains in the place of the spot after completion of the process (leaf "wholly converted to green", w*hg*). Discoloration in flowering *Erythronium* never occurs at *bud* stage but may sometimes occur at late flower (*flo*) stages, typically at fading, or still beyond at an early capsule stage (compare Figs. 2 A-E and 3 A-E). But timing and length of pigment disappearance are strongly dependent on conditions, and a bad winter weather spell can appreciably delay the conversion of red-brown patterns to green ones. The effect of chilling is made apparent by the late *pid* peak at w. 13 in yr. 2018, compared to more normal winters showing *pid* peaks at w. 11-12 (yrs. 2019-2021) or even w. 10 (mild 2016 winter). In any case, the discoloration process was essentially completed in April at week 14 or w. 15 at the latest, except in 2016 (w. 13). Senescence and mass dieback of the *Erythronium* population will follow at the end of April.

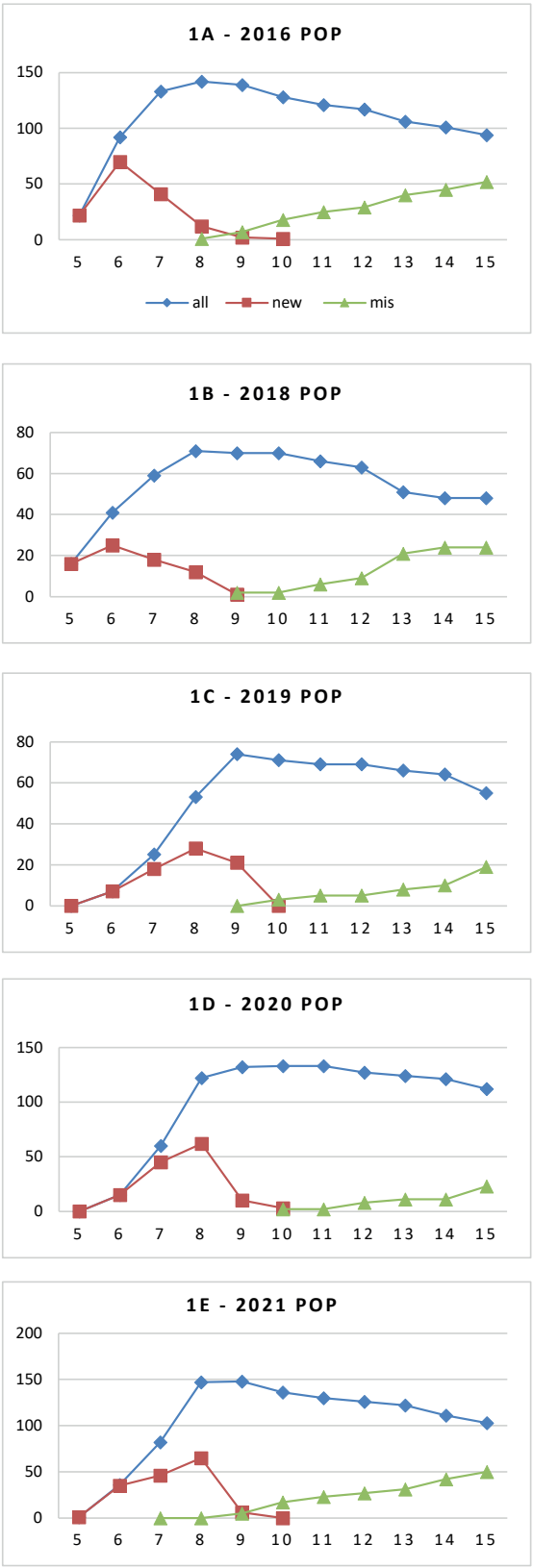
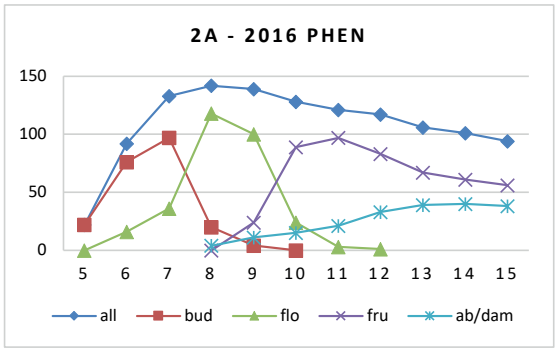
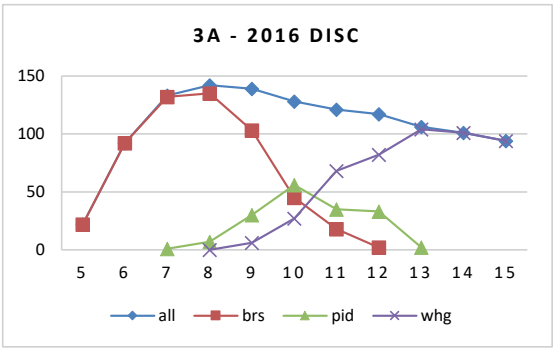
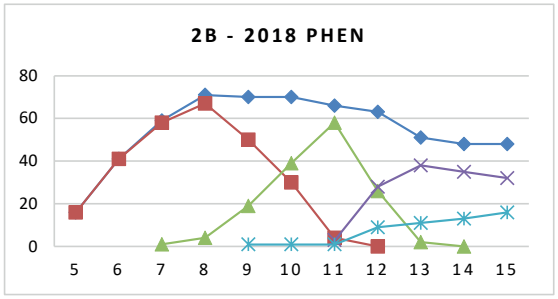
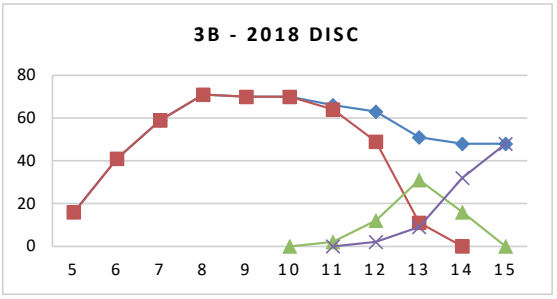


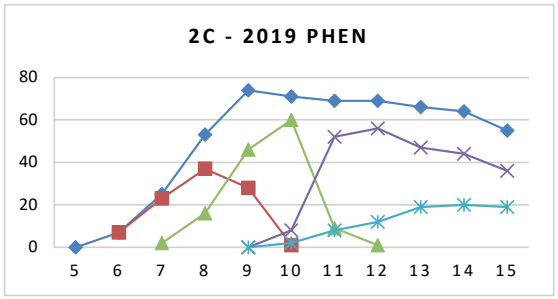
Fig. 1 (A-B-C-D-E). Trends of the flowering *Erythronium* population (POP) of Farneto-C site, 2016 to present (2017 omitted). Rhombus, total population (*all*); square, newly found plants (*new*); triangle, missing plants (*mis*).

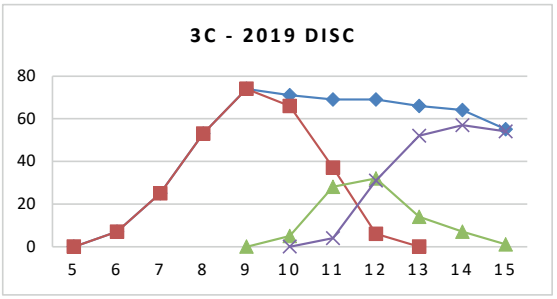


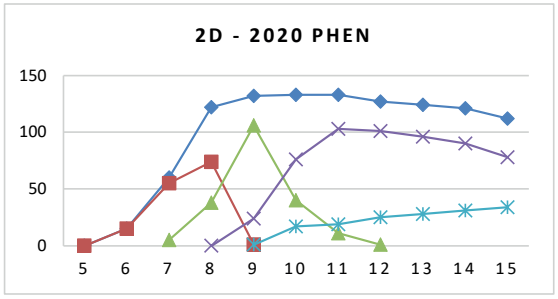


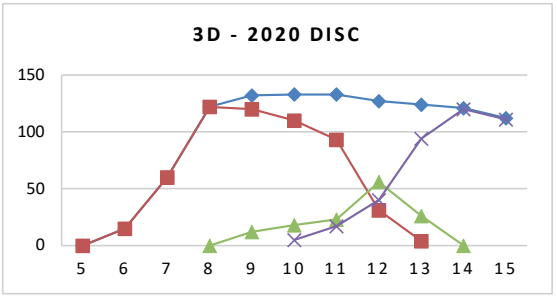


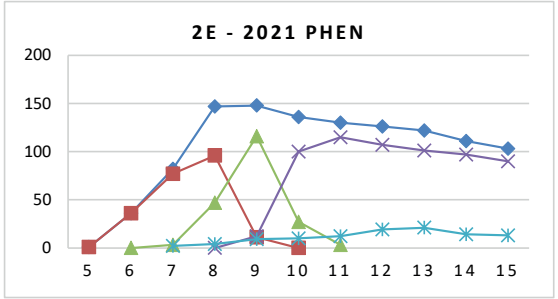












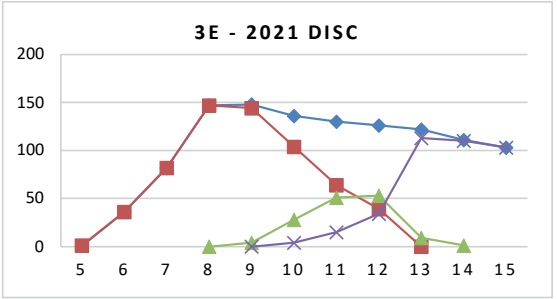


Fig. 2 (A-B-C-D-E). Flowering and fruiting phenology of *Erythronium* (PHEN) since 2016. Rhombus, total population (*all*); square, bud stages (*bud*); triangle, open flowers (*flo*); cross, fruit (*fru*); star, aborted plus damaged-detopped plants (*abo/dam*).

Fig. 3 (A-B-C-D-E). Leaf spot color and spot discoloration (DISC) in flowering *Erythronium* since 2016. Rhombus, total population (*all*); square, spots brown (*brs*); triangle, spots partially discolored (pigment disappearing, *pid*); cross, spots wholly converted to vivid green (*whg*).

The third group of observations focuses on the ground color of leaves, since a number of plants of the Farneto-C site fail to show the classic grey-green or silvery (*SLV*) leaf livery typical of the species, and are either full-green with brown spots (Fig. 4) or a mixed type (Pupillo & Astuti, 2017). In fact, a ~19% fraction of flowering erythroniums exhibit vivid green sectors on a grey background and brown spots and are named silvery-and-green (*S&G*), often with a chess-like pattern (Fig. 5B). A minor but stable fraction of flowering plants (about 3%) is full-green (Fig. 5C), while the large majority is regularly silvery (Fig. 5A). The percentage of the three types remained remarkably constant through years (Table 2).

Discussion

Erythronium dens-canis in the low Apennine heights near the city of Bologna is found in small and isolated locations in fresh deciduous woods. We are aware of four populations in the Parco dei Gessi Bolognesi, including Farneto-C where this study was carried out, but the species is absent from many sites that would seem suitable for it. Some concern for the future of this nice lily in the area is therefore justified, the more so as current climate changes in Italy (less snow and milder winters, hot and dry midsummer) open risk scenarios for spring ephemerals. The present study was undertaken to gain information on the phenology and survival ability of this relict species living at the edge of a densely inhabited region.

Phenology. The present overview of a flowering *Erythronium dens-canis* population for years 2016 to 2021 (except 2017) sheds light on several aspects of the lifecycle, and extends to a multi-year perspective an earlier report mainly based on non-flowering (i.e., young and nearly mature) plants (Pupillo & Astuti, 2017). A first conclusion emerging from this enlarged survey concerns the very early timing of plant sprouting during winter. The data confirm what appears to be a rule for this species, i.e. old-flowering plants are the first to emerge as a spike in late winter, while semimature-nonflowering plants as well as young plants and seedlings tend to flush later and later during early spring in an approximately inverse relationship of plant biological age *vs*. sprouting time (Pupillo & Astuti, 2017). This point is quite important to understand the status and population structure of the species, as the *Erythronium* population under study (and others in the park) are mainly constituted by relatively young, sexually incompetent individuals.

Year	*Stock*	*% SLV*	*% S&G*	*% GRN*
2016	146	75.3%	21.2%	3.4%
2018	72	81.9%	15.3%	2.8%
2019	74	80.2%	16.0%	4.1%
2020	135	77.8%	20.7%	1.5%
2021	153	78.4%	18.3%	3.3%
TOT	*580*	*78.1%*	*19.0%*	*2.9%*

Table 2. Leaf ground color frequency. SLV = silvery, S&G = silvery-&-green, GRN = full-green.

Fig. 4. Leaf variegation of *Erythronium dens-canis* at the Farneto-C site. Two mature, one-leaved nonflowering plants: right the common silvery type, left the full-green variant.

When an *Erythronium* species was defined "a true snow-borer" (Vézina & Grandtner, 1965) the definition originally referred to the yellow "trout lily" *E. americanum*, but it can equally apply to flowering *E. dens-canis* since both species are physiologically and anatomically well adapted to grow up through melting snow. For example, they are equipped with a drilling peg (often of a brilliant red color in the European species) on top of the emerging cylinder spike. Many studies report on effects of snowmelt on *Erythronium* sprouting and growth (e.g. Yoshie & Fukuda, 1994; Lapointe, 2001; Yamagishi *et al.,* 2005; Lambert *et al.,* 2010). But the exact moment of sprouting and flowering and other developmental events appear to be affected by current and past climate in a complex and somewhat intriguing way. An example is given by the retarded plant emergence, with an about 2 weeks' delay, in yrs. 2019-2020-2021 with respect to yrs. 2016 and 2018 (the latter recorded for an unusually cold late winter). By comparison, flowering time was approximately the same in yrs. 2016 and 2020-2021, peaking in each case at weeks 8-9 (late February), but was delayed by 1-2 weeks in colder yrs. 2018-2019. Interestingly, the progress of flowering (and fruiting) constantly parallels leaf spot discoloration, i.e. the loss of red anthocyanin of brown spots. The similar timing of flowering and discoloration in all five years of study, despite annual variations, suggests a dependence of both processes on ambient temperature, at variance with plant emergence which shows no obvious temperature sensitivity and seems to obey a different set of environmental factors. In other words, flowering/fruiting was promoted by milder spring periods suitable for pollination, as was noted by Schemske *et al.* (1978) for *E. albidum* and other ephemerals, and discoloration alike. This conclusion is also supported by the peculiar winter cycle of yr. 2018, marked by cold rain and blizzard during most of February, which was reflected into a delayed *flo* peak and a prolonged *brs* peak with no discoloration (Figs. 2B, 3B). Effects of cold weather on plant performance (Augspurger & Salk, 2017) and seed germination (Kondo *et al.,* 2002; Mondoni *et*

Fig. 5. Variegation variants of flowering *Erythronium*. A) A typical plant with the common silvery (*SLV*) leaf ground color and showy brown spots; B) a plant showing a chess-like alternance of silvery-and-green (*S&G*) sectors and brown spots; C) a specimen of the uncommon full-green (*GRN*) variant.

al., 2011) have been investigated in several *Erythronium* species, also with a view to gain insight into their resilience to climate change (Kim *et al.,* 2015; Thomson, 2019).
On the other hand, the surprising anticipation of plant emergence in yrs. 2016 and 2018 in comparison with more recent years is possibly related to harsh cold spells in late years 2015 and 2017. In fact, studies on *Erythronium* emergence as influenced by past temperatures clearly indicate that a cold acclimation in autumn (bulb stratification) can substantially accelerate *Erythronium* spring sprouting (Risser & Cottam, 1967; Yoshie & Fukuda, 1994; Kim *et al.,* 2014). This possible explanation is also a clue to future effects of long-term winter climate mitigation (Parmesan, 2018).

Reproductive success. In terms of the fraction of functional fruits (capsules) counted at end of April, *Erythronium dens-canis* is a fairly successful species despite grazing by ungulates (mainly roe- and fallow deer, less wildboar, all common in the park), without ruling out predation e.g by rodents. Loss of reproductive organs or whole plants was quantified on the basis of factual evidence such as bud or fruit removal (de-topping), or whole plant extirpation, and all "missing" individuals are in fact attributed to animal feeding in the present treatment. Missing plants amounted up to 34.2% of the population (yr. 2016), average 27.2% in five years. The number of damaged-detopped but still viable plants was distinctly less (7 to 18%, av. 10.2%) and comparable with that of abortions (av. 12.6%) which, however, was more variable from a year to next. In fact, a number of plants had infertile fruits as the likely consequence of prolonged periods of stormy weather preventing impollination, whereas others were late sprouting and often exposed to full sunlight, so in this case fruit failure was apparently related to springtime water stress.
Overall, the total of missing and of damaged-detopped specimens oscillated within a relatively narrow range (44% to 32%, av. 37.4%) with some tendency to decrease over several years. If cases of abortion are added up to those of missing fruits or plants, the total of unsuccessful fruiting rises to half of all plants under study (50.2% av.), though with a drooping trend from 62% in 2016 to 41% in 2021 (Table 1). This decrease may hint at recent milder winters and easier pollination service, but it may also be related to slightly decelerating ungulate pressure due to regular wolf presence in the park. The high percentage of reproductive failure, in any case, is in line with *Erythronium* literature (e.g. Muller, 1978; Kawano *et al,.* 1982; Sawada *et al.,* 1997). However, there is some expansion of fertile *Erythronium* plants in the site, both in terms of space occupied and of flowering percentage. The flower fraction in the study area amounts at present to about 11% (as a mean of various subsites), while it was 3.2% in an adjacent subsite in 2015 (Pupillo & Astuti, 2017). In any case, it is true today as before that flowering dog's tooth violet is likely to survive and reproduce only when plants are shielded by stones or bushes, but has little chance on open ground.

Green variant. In matter of plant risks, the presence of a "full-

green" variant at Farneto-C is remarkable. The normal grey-green or *silvery* (*SLV*) habit of the species is conferred by the light-reflecting effect of a thin intercellular space interposed between epidermis and mesophyll (Esteban *et al.*, 2008; La Rocca *et al.,* 2014). In this site of the Parco dei Gessi Bolognesi, the silvery type amounts to 78% (mean of five years), the tricolor *S&G* morph to 18% and the full green morph to 3%. This apparent balanced polymorphism (Pupillo & Astuti, 2017) is important, as no full-green leaf ground color has been reported to our knowledge throughout the vast distribution of *Erythronium dens-canis* in Europe. The persistence and resilience of the full-green variant through years at Farneto-C is reassuring, but some protection effort is in order since the flowering full-green plants can be esteemed to be a maximum of 20 per year in the whole Farneto-C site (about one hectare), and about half of them go destroyed every year through animals and landslides.

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