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Interpopulation variability on embryo growth, seed dormancy break, and germination in the endangered Iberian daffodil *Narcissus eugeniae* (Amaryllidaceae)

ELENA COPETE, JOSÉ Mª HERRANZ, MIGUEL ÁNGEL COPETE and PABLO FERRANDIS
Institute of Botany, University of Castilla-La Mancha, Albacete, Spain

Abstract

The main goal of the study was to assess germination requirements in a threatened daffodil to elaborate a detailed protocol for plant production from seeds, a key tool for conservation. Experiments were carried out both in the laboratory and outdoor conditions. In *Pseudonarcissi* section, endemic Iberian species of *Narcissus* studied heretofore have different levels of morphophysiological dormancy (MPD). Embryo length, radicle emergence, and shoot emergence were analyzed to determine the level of MPD. Both interpopulational variability and seed storage duration were also studied. Mean embryo length in fresh seeds was 1.32 mm and the embryo had to grow until it reached at least 2.00 mm to germinate. Embryo growth occurs during warm stratification, after which the radicle emerges when temperatures go down. Seed dormancy was broken in the laboratory at 28/14°C in darkness followed by 15/4°C, but the germination percentage varies depending on the population. In outdoor conditions, seed dispersal occurs in June, the embryo grows during the summer and then the radicle emerges in autumn. The radicle system continues to grow during the winter months, but the shoot does not emerge until the beginning of the spring because it is physiologically dormant and requires a cold period to break dormancy. Early cold temperatures interrupt embryo growth and induce dormancy in seeds with an advanced embryo development. Seeds of *N. eugeniae* have deep simple epicotyl MPD. In addition, we found that embryo growth and germination were improved by seed storage duration.

Keywords: epicotyl MPD, germination, phenology, secondary dormancy, shoot emergence.

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Introduction

Within the five classes accepted in the classification system for seed dormancy, morphophysiological dormancy (MPD) is considered one of the most difficult to overcome (Baskin & Baskin 1998, 2003) due to seeds with this class of dormancy having an underdeveloped embryo, with an additional physiological mechanism preventing embryo growth and germination.

Although many studies on germination ecology of seeds with MPD have been conducted in species of the Northern Hemisphere, studies for the Circum-Mediterranean region are still scarce (Baskin & Baskin 2004; Copete *et al*. 2011b). Therefore it is necessary to carry out studies focused on Mediterranean species, particularly if they belong to families such as the Amaryllidaceae, whose germination ecology is little known (Baskin & Baskin 1998).

Narcissus eugeniae Fern. Casas (Amaryllidaceae), *Pseudonarcissi* section, is a spring flowering, bulbous geophyte. It is endemic to eastern Spain with dispersed and fragmented populations in mountainous areas (> 1300 m a.s.l.) of the Iberian System (Mayoral & Gómez-Serrano 2004). Hence it has been catalogued as "vulnerable" (VU) in the Red List of Spanish Vascular Flora (Moreno 2008). For threatened plant species, information on seed germination is essential for *ex situ* plant production, since it allows maximization of genetic diversity (Fay 1992) and development of reinforcement programs of wild plant populations.

Correspondence: Elena Copete
Email: ecopete@hotmail.com

Preliminary analyses in the laboratory showed us that seeds of *N. eugeniae* have an underdeveloped embryo at dispersal time (embryo length = 1.32 ± 0.03 mm, seed length = 2.68 ± 0.06 mm, mean ± SE, n = 25). In addition, the embryo is physiologically dormant, since it does not germinate within 30 days under a wide range of temperature–light conditions simulating those which occur in the natural habitat of the species throughout the year (i.e., 5, 15/4, 20/7, 25/10, 28/14 and 32/18°C, in light and in darkness). Both records indicate that seeds of *N. eugeniae* have some level of MPD (unpubl. data).

Endemic Iberian species of *Narcissus* in Section *Pseudonarcissi* have different levels of MPD. For example, *Narcissus hispanicus*, which grows at low altitude (500–700 m a.s.l.) and near small streams with a certain summer water stress, has deep simple epicotyl MPD: radicle emergence takes place at cool temperatures in autumn following a previous warm period, but root-emerged seeds require cold stratification for shoot emergence in spring (Copete *et al*. 2011a). *Narcissus alcaracensis* (Herranz *et al*. 2013a) and *N. longispathus* ((Herranz *et al*., 2013b) are from mountainous areas (> 1200 m a.s.l.), living in habitats that are humid throughout the year. Both species have different levels of complex MPD. It means their seeds require a long period of cold stratification for dormancy break, so radicle and shoot emergence occur at late winter or early spring.

It is known that diverse evolutionary processes occur by adaptation to habitats with a different selection pressure, and consequently they produce a wide array of ecophysiological traits, such as the different types of seed dormancy observed among species (Walck & Hidayati 2004). In species with deep simple epicotyl MPD, the radicle emerges in autumn and develops during the winter months. The root system that developed during winter may provide an efficient supply of water and nutrients for shoot emergence at the beginning of spring, in contrast to those species with complex levels of MPD, whose root and shoot development occur at the same time (Baskin & Baskin 1985). Therefore, deep simple epicotyl MPD is considered an adaptation to overcome the summer water stress (Kondo *et al*. 2004), and in the same way, complex levels of MPD are considered an adaptation to mountainous areas, since seedling emergence is produced in early spring, avoiding winter strong frosts (Baskin *et al*. 2000). *Narcissus eugeniae* is a species from mountainous areas, but occurs in rocky ledges with a strong summer water stress due to shallow soils. In this work, we investigated whether the dormancy breaking requirements of *N. eugeniae* are more similar to those of *N. hispanicus*, supporting a prevalence of the microhabitat effect, or alternatively, closer to the requirements of *N. alcaracensis* or *N. longispathus*, indicating that general climatic conditions are predominant in modulating germination-response patterns.

Another fundamental aspect of this work is to analyze the interpopulation variability in germinative behavior, which is a very widespread phenomenon in species with developed embryos and different types of physiological dormancy (Beckstead *et al*. 1996; Pérez-García *et al*. 2003, etc.), but it is almost unknown in species with MPD (Herranz *et al*. 2010a; Chien *et al*., 2011).

Therefore, the general aim of this work was to determine the level of MPD in seeds of *N. eugeniae* and to analyze its relationship with the levels that have been documented in other species within the genus *Narcissus*. The specific aims were: (i) to describe the phenology of embryo growth, radicle emergence, and shoot emergence; (ii) to analyze the influence of several light and temperature conditions on embryo growth; (iii) to determine the effect of the seed storage duration on embryo growth and radicle emergence; (iv) to analyze if interpopulation variability affects radicle-emergence patterns; (v) to study the effect of stratification period on radicle emergence; and (vi) to determine if low temperatures are required for shoot emergence.

Material and methods

Source of seeds

Seeds were collected in mid-June 2007 and 2008 from capsules that turned yellow and began to open for seed dispersal. In each seed collection, seeds were collected from 200 to 220 healthy plants that were selected randomly. Each individual had a capsule with 46–52 seeds. Interpopulation variability was tested on seeds collected from two populations of the Iberian System center (eastern Spain, Cuenca province): (i) rocky cracks in the source of Chico River (Masegosa, 1460 m a.s.l.; CR hereafter), where annual and summer precipitations are 1095 and 130 mm, respectively; and (ii) Ranera Peak (Talayuelas, 1400 m a.s.l; RP hereafter), where annual and summer precipitations are 557 mm and 97 mm, respectively (Elías & Ruiz, 1981). Both populations had more than 2000 individuals, so enough seeds could be collected without any risk for the population viability. Collected fruits were spread in trays on a laboratory bench for gradual fruit opening. Seed age was considered 0 months on July 1. Seeds from CR and RP were collected in 2007 and 2008, respectively.

Non-heated shadehouse experiments: near-natural conditions

The aim of the experiments included in this section was to determine the different stages the seeds went through in

their natural habitat from dispersal time to seedling emergence. For this reason, these studies were conducted in a non-heated metal frame shadehouse, in which seeds were exposed to a near-natural temperature regime. A datalogger was installed to record air temperature throughout the study. In general, the water control system was programmed to water once a week, but it was reduced to twice a month in July and August to simulate summer drought that is common in the Mediterranean area. In addition, we did not water when the substrate was frozen during the winter.

Phenology of embryo growth and radicle emergence On July 1, 2007, five batches of 40 seeds from CR were mixed with sterilized fine sand, kept in nylon bags, and buried 5 cm deep in a pot placed in the shadehouse. One bag was exhumed each month: August, September, October, November, and December. A similar experiment was set up with seeds from RP on July 1, 2008. Seed and embryo lengths were measured from 25 seeds selected randomly each month from the bag exhumed. Embryos were excised with a razor blade from the seeds and their lengths measured with a binocular microscope equipped with an ocular micrometer. Embryos from seeds for which the radicle had emerged during burial were recorded as having a critical embryo length (2.62 ± 0.04, $n = 40$, range = 2.00–3.00 mm) (Hidayati *et al*. 2000). That parameter corresponded to the mean embryo length in 40 seeds with split coat, but before radicle emerged (Vandelook & Van Assche 2008). The E:S ratio is the quotient between embryo (E) and endosperm (S) lengths (Copete et al. 2011a,b). The critical E:S ratio (i.e., the E:S ratio for seeds measured in the calculation of the critical embryo length), ranged between 0.73–1.00, where 0.73 is the minimal E:S ratio recorded in germinated seeds ('threshold E:S ratio' hereafter).

Phenology of shoot emergence This experiment was initiated on July 1, 2007 and July 1, 2008 with seeds from CR and RP, respectively. For each population, 200 seeds were sown 3 mm deep in each of three drainage-holed trays (i.e., replicated), which were filled with cultivation medium composed of sterilized peat and sand (2:1 v/v). Emerged seedlings from both populations were counted and removed weekly until June 2009.

Laboratory experiments: controlled conditions

In this section, the main goal was to know which specific temperature–light requirements for embryo growth, radicle, and shoot emergence were necessary to overcome dormancy. The conditions were controlled by means of germination chambers (Ibercex model F-4, Madrid, Spain), equipped with a digital temperature and light control system (± 0.1°C, cool white fluorescent light, 25 μmol/m^2/s (1350 lux)). Regarding light conditions, the chambers were programmed with a 12-h daily photoperiod. Seeds were either exposed to this photoperiod (i.e., light treatment) or placed in Petri dishes wrapped with two layers of aluminium foil (i.e., darkness treatment) simulating dark seed burial conditions after dispersal. Experiments were conducted at constant temperatures of 5 and 10°C and at 12/12-h daily temperature regimes of 9/5, 15/4, 20/7, 25/10, 28/14 and 32/18°C. In the 12/12-h alternating treatments, the high temperature coincided with the light phase and the low temperature with darkness to simulate day/night conditions. These temperature regimes simulated mean maximum and mean minimum monthly temperatures that characterize the annual climate cycle in the natural habitat of the species (mountainous Iberian System in the central east of the Iberian Peninsula): 15/4°C, November and March; 20/7°C, October and April; 25/10°C, September and May; 28/14°C, August and June; and 32/18°C, July (Elías & Ruiz, 1981). The 5°C treatment simulated the mean temperature recorded during winter months: December, January, and February. The other low temperatures (9/5°C and 10°C) were chosen because they are within the effective temperature range for cold stratification, which is from around 0 to 10°C, being 5°C optimal for many species (Stokes 1965; Nikolaeva 1969).

Percentages of germination were computed based on the number of apparently viable seeds. Ungerminated seeds were checked for viability on the basis of embryo appearance, paying special attention to the colour and turgidity. Specifically, seeds were considered as viable if the embryo showed a white color and resistance to slight pressure with tweezers. More than 95% of the seeds were viable.

Embryo growth under cold or warm conditions The aim of this section was to determine the optimal temperature for embryo growth. Seeds were incubated for 90 days at six temperature regimes: 5, 15/4, 20/7, 25/10, 28/14 and 32/18°C, in darkness and in light, so 12 different conditions were tested (six temperatures by two light conditions). Therefore, 12 batches of 100 seeds each were placed on two sheets of filter paper moistened with distilled water in 9-cm Petri dishes. After 30, 60 and 90 days, embryos were measured after being excised from 25 healthy, randomly selected seeds. Mean length and standard error were calculated for each sample of 25 embryos. This experiment was started with 0-month-old seeds from both CR and RP.

Effect of light and seed storage duration on embryo growth at 20/7°C With this experiment we analyzed if dry seed storage promoted dormancy break when seeds were

incubated under moist conditions. Seeds from both populations were tested when they were 0 and 8 months old. In light conditions, four 100-seed lots (two seed storage durations by two seed sources) were stratified at 20/7°C for 90 days. Every month, 25 seeds were randomly extracted from each lot to measure their embryos and calculate the mean value throughout the experiment. In darkness, 12 lots (three 30-seed lots instead of one 100-seed lot per treatment) were tested. In this way, a lot was used each month while the rest of the Petri dishes were kept in darkness during the experiment.

Effect of cold and warm stratification followed by incubation at 15/4°C on embryo growth The purpose of this section was to determine the required duration of stratification period to complete embryo development and to break dormancy. We tested 15, 30, and 60 days of stratification at 5°C and at 28/14°C, both in light and in darkness. Two hundred seeds were placed in each of four 16-cm Petri dishes (two thermoperiods by two illumination conditions). After 15 days, 50 seeds were randomly extracted: the embryos of 25 seeds were excised and their length measured at that moment, and those in the other 25 seeds after being transferred to 15/4°C for 30 days. The complete procedure was repeated after 30 and 60 days of stratification at 5°C and at 28/14°C. Seeds from CR were tested at two seed dry storage periods (0 and 8 months), while those from RP were tested only at 0-month age. Manipulations/observations of seeds kept in darkness were conducted under a dim green light (Luna *et al*. 2004) to minimize the interruption of the continuous darkness.

Radicle emergence in seeds incubated at different conditions following a warm stratification The aim of this section was to determine the most favorable temperature–light conditions for germination in seeds with a fully developed embryo, because they were stratified previously at warm temperatures for 60 days (28/14°C + 25/10°C for 30 + 30 days). Both seed sources (CR and RP) were analyzed in this experiment, so two lots of 1100 seeds from each population were stratified in light and in darkness, respectively. Following the 60-day warm stratification period, 100 randomly selected seeds (four replicates of 25 seeds) were transferred to each incubation thermoperiod (5, 15/4, 20/7, 25/10 and 28/14°C) in light and in darkness for 30 days.

Effect of cold stratification on shoot emergence We analyzed whether the shoot was dormant and consequently required a cold stratification to emerge or, conversely, cold temperatures produced a slowing down in shoot development. Radicle-emerged seeds were obtained after being stratified in darkness at 28/14°C (60 days) and then incubated in darkness at 15/4°C (30 days). Seeds with emerged radicles 2–3 mm in length were exposed to 5°C for different periods of time (0, 4, 8 or 12 weeks), and then transferred to 15/4°C (mean maximum and minimum temperatures in March). The experiment was carried out in light, and both seed sources (CR and RP) were tested. In addition, four 25-seed lots from RP were incubated at 20/7°C with no previous 5°C cold stratification. Shoots were considered as developed when shoot-length reached 1 cm.

Induction of dormancy by cold temperatures in nondormant seeds The aim of this study was to check if low autumn temperatures can induce seeds to secondary PD during early overcoming-dormancy stages (i.e., when PD had been broken and embryos had begun to grow but were not fully developed yet). Three lots of 200 seeds each were placed in 9-cm Petri dishes in light to test the effect of two different sequences of stratification (warm plus cold and warm plus warm) on embryo growth and germination, using 12-month-old seeds from CR. First, the three lots were placed at 28/14°C for 60 days. Second, one of the lots was transferred to 5°C for 30 days, another one to 20/7°C for 30 days, and the third one to 25/10°C for 30 days. Finally, 4×25 seeds from each lot were used to test germination at 15/4°C in light for 30 days. Embryo growth was measured monthly throughout the stratification period and at the end of the incubation period. The critical embryo length (2.62 mm) was assigned to those seeds that had germinated during the experiment (Hidayati *et al*. 2000).

Statistical analysis

The effects of temperature, light conditions, duration of incubation, seed population, and seed storage duration on embryo growth were analyzed by multifactor ANOVAs. Seed germinability was evaluated by the final cumulative germination percentage, which was compared among treatments by multifactor ANOVAs. In the comparison of the germination percentage, the effects of four factors were analyzed: seed population (two levels), light conditions during stratification (two levels), light conditions during incubation (two levels), and incubation temperatures (five levels). When significant main effects existed, differences were detected by a multiple comparison Tukey test. In the case of significant interactions, differences were explored by contrasting confidence intervals. Prior to analyses, normality (Cochran test) and homoscedasticity (David test) of data were checked. Values of the final cumulative germination percentage were squared-root arcsine transformed for analyses.

Results

Non-heated shadehouse experiments: near-natural conditions

Phenology of embryo growth and of radicle emergence Embryo growth showed a similar pattern in seeds from both populations. In seeds from CR, mean embryo length was 1.32 ± 0.03 mm at the beginning of the experiment (July 1, 2007; Fig. 1B). This value increased to 2.10 ± 0.07 mm in seeds buried from July 1 to October 1, 2007. In this period, the mean minimum and maximum temperatures were 14 and 31°C, respectively. On October 1, 2007, 80% of the seeds had embryos exceeding the threshold E:S ratio.

In seeds from RP buried on July 1, 2008 (Fig. 1C), the initial embryo length was 1.33 ± 0.03 mm. Exhumed seeds on October 1, 2008 had a mean embryo length of 2.40 ± 0.06 mm, and 96% seeds had an E:S ratio longer than the threshold E:S ratio. The mean minimum and maximum temperatures from July 1 to October 1, 2008 were 14 and 31°C, respectively.

Radicle emergence did not begin simultaneously in both populations. In CR seeds, radicle emergence began in October but most of the buried seeds germinated during November (Fig. 1B), when the mean minimum and maximum temperatures were –2 and 15°C, respectively (Fig. 1A). In contrast, radicle emergence in RP seeds started somewhat earlier, in September, and then more abundantly during October (Fig. 1C), when the mean temperatures were 8 and 19°C. On December 1, radicle emergence was close to 100% in both seed populations.

Phenology of shoot emergence Shoot emergence in both CR and RP seeds buried in July occurred massively in the following February–May period. Shoot emergence in CR seeds (Fig. 1B) was 38 ± 6% at the end of February, and 80 ± 6% at the end of May. An additional shoot emergence (10%) was recorded in the following spring (2009). Shoot emergence in RP seeds (Fig. 1C) showed a very similar temporal pattern: 43 ± 3% shoots emerged after February, reaching 83 ± 3% in May.

Laboratory experiments: controlled conditions

Embryo growth under cold or warm conditions Embryos did not grow significantly in seeds incubated at 5°C regardless of light conditions or seed source (Table 1). Thus, embryo length values ranged between 1.38 ± 0.06 mm and 1.56 ± 0.04 mm, without reaching the threshold E:S ratio in any seed.

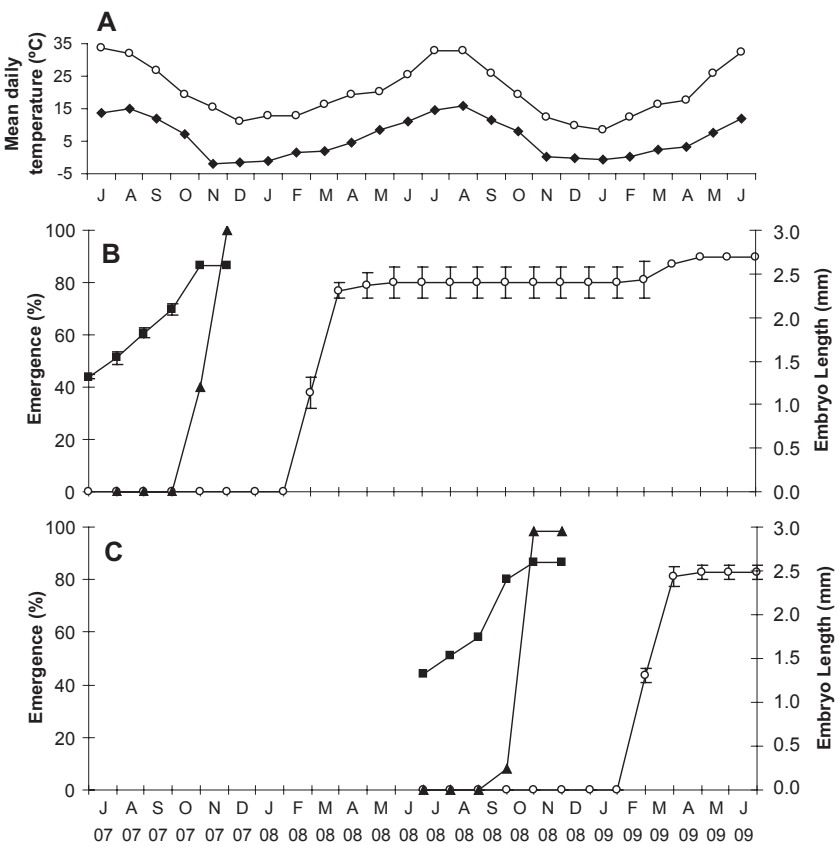

Fig. 1 (A) Minimum and maximum mean temperature, and phenology of embryo growth, radicle and shoot emergence in 0-month-old seeds from (B) CR buried on July 1, 2007 and (C) RP buried on July 1, 2008: error bars indicate SE. (A) —◆—, minimum; —○—, maximum; (B) —○—, shoot emergence (%); —▲—, radicle emergence (%); —■—, embryo length.

Table 1 Embryo growth (mean ± SE) in seeds of *Narcissus eugeniae* from two populations (CR and RP) stratified at 5, 15/4, 20/7, 25/10, 28/14 and 32/18°C in both light and darkness for 90 days

Temperature (°C)	Light/ darkness	CR			RP		
		30 days	60 days	90 days	30 days	60 days	90 days
5	Light	1.40 ± 0.05^{Aa} (0, 0)	1.38 ± 0.06^{Aa} (0, 0)	1.41 ± 0.07^{Aa} (0, 0)	1.49 ± 0.05^{ABa} (0, 0)	1.44 ± 0.04^{Aa} (0, 0)	1.45 ± 0.04^{Aa} (0, 0)
	Darkness	1.44 ± 0.06^{Aa} (0, 0)	1.39 ± 0.04^{Aa} (0, 0)	1.41 ± 0.03^{Aa} (0, 0)	1.42 ± 0.05^{Aa} (0, 0)	1.52 ± 0.04^{ABa} (0, 0)	1.56 ± 0.04^{ABa} (0, 0)
15/4	Light	1.49 ± 0.07^{ABa} (0, 0)	1.46 ± 0.05^{Aa} (0, 0)	1.56 ± 0.08^{Aa} (3, 10)	1.50 ± 0.04^{ABa} (0, 0)	1.57 ± 0.03^{ABCa} (0, 0)	1.64 ± 0.05^{ABCa} (0, 4)
	Darkness	1.66 ± 0.06^{ABa} (0, 5)	2.10 ± 0.10^{Cb} (16, 50)	2.10 ± 0.12^{BCb} (24, 50)	1.54 ± 0.05^{ABa} (0, 8)	1.58 ± 0.04^{ABCDa} (0, 4)	1.65 ± 0.04^{ABCa} (0, 8)
20/7	Light	1.64 ± 0.06^{ABa} (0, 10)	1.81 ± 0.06^{BCa} (0, 25)	2.13 ± 0.07^{BCb} (3, 65)	1.62 ± 0.05^{ABa} (0, 8)	1.62 ± 0.05^{ABCDa} (0, 4)	1.73 ± 0.04^{BCDa} (0, 8)
	Darkness	1.66 ± 0.07^{ABa} (0, 5)	1.94 ± 0.06^{BCbc} (0, 40)	2.28 ± 0.05^{Cd} (16, 95)	1.60 ± 0.05^{ABa} (0, 4)	1.76 ± 0.05^{CDEab} (0, 16)	2.13 ± 0.06^{Fcd} (4, 60)
25/10	Light	1.57 ± 0.05^{ABa} (0, 5)	1.66 ± 0.06^{ABa} (0, 10)	1.98 ± 0.07^{BCb} (0, 55)	1.61 ± 0.05^{ABa} (0, 0)	1.77 ± 0.05^{CDEFab} (0, 12)	1.77 ± 0.05^{BCDEab} (0, 4)
	Darkness	1.41 ± 0.05^{Aa} (0, 0)	1.90 ± 0.05^{BCcd} (0, 25)	2.10 ± 0.06^{BCd} (0, 85)	1.64 ± 0.05^{ABb} (0, 8)	1.78 ± 0.05^{DEFbc} (0, 16)	1.82 ± 0.06^{CDEbc} (0, 16)
28/14	Light	1.72 ± 0.05^{BCab} (0, 12)	1.94 ± 0.07^{BCbc} (0, 40)	2.03 ± 0.06^{BCc} (0, 60)	1.59 ± 0.05^{ABa} (0, 4)	1.72 ± 0.05^{BCDEab} (0, 12)	1.89 ± 0.05^{DEbc} (0, 20)
	Darkness	1.95 ± 0.05^{CDb} (0, 24)	2.02 ± 0.06^{Cb} (0, 44)	2.11 ± 0.07^{BCb} (0, 55)	1.70 ± 0.04^{Ba} (0, 4)	1.98 ± 0.04^{Fb} (0, 36)	1.94 ± 0.06^{DEFb} (0, 40)
32/18	Light	1.64 ± 0.05^{ABa} (0, 0)	1.83 ± 0.06^{BCab} (0, 40)	1.90 ± 0.07^{Bb} (0, 35)	1.66 ± 0.05^{Ba} (0, 8)	1.86 ± 0.04^{EFab} (0, 12)	1.99 ± 0.05^{EFb} (0, 28)
	Darkness	1.62 ± 0.08^{ABa} (0, 10)	1.95 ± 0.06^{BCcd} (0, 35)	2.11 ± 0.06^{BCd} (0, 70)	1.71 ± 0.04^{Bab} (0, 20)	1.78 ± 0.05^{DEFabc} (0, 20)	1.92 ± 0.04^{DEFbcd} (0, 20)

Values followed by different uppercase letters within a column, or different lowercase letters within a row are significantly different ($P < 0.05$). The first number between parentheses indicates the percentage of radicle emergence and the second is the percentage of seeds whose E:S ratio was > threshold E:S ratio (= 0.73).

In thermoperiods with 12/12 h of warm and cold stratification (i.e. 15/4, 20/7, and 25/10°C), embryo growth was in general higher in darkness than in light, becoming significantly different after 90 days at 20/7°C. In CR seeds, embryos grew substantially after being incubated at 15/4, 20/7, and 25/10°C for 90 days, a high proportion of embryos exceeded the threshold E:S ratio, and some seeds germinated. Embryos in RP seeds were smaller, so after 90 days at the most favorable conditions (20/7°C in darkness) embryo length was 2.13 ± 0.06 mm and 60% of the seeds exceeded the threshold E:S ratio. In warm thermoperiods, such as 28/14 and 32/18°C, embryos from both populations grew, reaching a length around 2 mm after 90 days of stratification, but no seed germinated.

Effect of light and seed storage duration on embryo growth at 20/7°C In 0-month-old CR seeds stratified 90 days at 20/7°C in light, the mean embryo length was 2.13 ± 0.07 mm and the radicle emergence 3%. In darkness, those parameters were 2.28 ± 0.05 mm and 16%, respectively (Fig. 2). After 8 months of dry storage, values were 2.37 ± 0.06 mm and 33% in light, and 2.48 ± 0.05 mm and 63% in darkness.

In 0-month-old RP seeds, the embryo length was 1.73 ± 0.04 mm when stratified 90 days at 20/7°C in light, and 2.13 ± 0.06 mm in darkness. Germination was negligible in both cases (Fig. 2). In 8-month-old seeds, the embryo measured 2.14 ± 0.07 mm and 2.28 ± 0.06 mm when stratified in light and darkness, respectively. The embryo growth was significantly influenced by all the main effects analyzed, being longer in the following levels: CR population, darkness stratification, 8 months of dry storage (Table 2).

Effect of cold and warm stratification followed by incubation at 15/4°C on embryo growth In light, embryo growth and radicle emergence increased with warm stratification length (Table 3). In darkness, embryo length in 0-month-old CR seeds was not affected by stratification length, but radicle emergence was higher after 30-day and 60-day periods (93 and 98, respectively) than after 15 days of

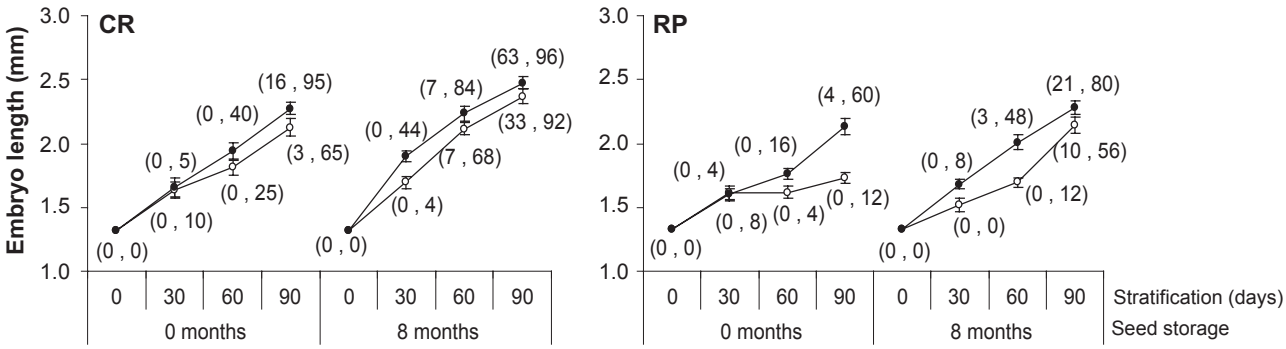

Fig. 2 Influence of the duration of the stratification period (0, 30, 60, or 90 days) at 20/7°C in light (open symbols) and in darkness (closed symbols) and seed storage duration (0 or 8 months) on embryo growth (mean + SE, SE > 2%) of *Narcissus eugeniae* seeds. Seed populations: CR and RP. The first number in parentheses is radicle emergence percentage and the second is the percentage of seeds whose E:S ratio was > threshold E:S ratio (0.73).

Factor	d.f.	*F*	*P*	Categories
Seed population	1	67.97	<0.001	PR < CR
Seed storage duration	1	55.45	<0.001	0 months < 8 months
Light conditions	1	40.18	<0.001	Light < darkness
Stratification length	3	398.57	<0.001	0^a, 30^b, 60^c, 90^d

Table 2 Summary of the effects of several factors on embryo length in seeds of *Narcissus eugeniae* incubated at 20/7°C

Main effects of seed population, seed storage duration, light conditions and stratification length on embryo growth in a multifactor analysis. Table shows degrees of freedom (d.f.), *F*-ratio values and the factor categories where differences were significant. Residual degrees of freedom: 139. Different letters denote significant differences ($P < 0.05$) between factor levels.

stratification (68%). Similar results were obtained with 8-month-old CR seeds. All factors significantly affected embryo growth (Table 4). However, embryos did not grow in seeds stratified at 5°C for 60 days and then incubated at 15/4°C, regardless of light conditions, and consequently radicle emergence was null (data not shown).

Radicle emergence in seeds incubated at different conditions following a warm stratification Radicle emergence was very low (< 20%) in both seed sources when incubated in light, regardless of light conditions during the stratification (Fig. 3). Although light conditions during the stratification phase did not immediately affect radicle emergence, they were determinant in the following experimental phase, when germination was higher in darkness than in light incubation (Fig. 3, Table 5). As for population factor, CR seeds germinated in a higher proportion than RP seeds. The optimal temperature condition for incubation was 15/4°C.

Effect of cold stratification on shoot emergence An initial 5°C stratification pretreatment reduced the time required for shoot development when seeds were transferred to 15/4°C, so slopes in shoot emergence curves increased with a rise in the number of previous cold weeks (Fig. 4). After 0, 4, 8, and 12 weeks at 5°C, the days required of incubation at 15/4°C for reaching 100% of shoot emergence were 181, 141, 93, and 28, respectively, in CR seeds. These values were 150, 114, and 52 days after 4, 8, and 12 weeks of cold stratification in RP seeds. The total time (days of stratification + days of incubation) required for reaching shoot emergence values close to 100% in CR seeds ranged between 112 days in seeds previously stratified at 5°C and 181 days in noncold-stratified seeds. In RP seeds, the total number of days was 180 when cold stratified for 4 weeks, and 144 with a 12-week cold stratification. Without any previous cold period, only 8% of the shoots emerged in RP seeds incubated at 15/4°C, and the remaining seeds finally decomposed after 10 weeks.

The shoot-emergence curve in radicle-emerged seeds incubated at 20/7°C with no cold pretreatment (Fig. 5) showed three stages: (i) no shoot developed for the first 55 days; (ii) emergence percentage increased slowly up to 12% during the following 63 days; and (iii) an inflection point occurred after 118 days, when the angle of the curve shifted upward because of a rise in emergence rate, so the proportion of seeds with a developed shoot grew from 12 to 92% in the last 70 days.

Table 3 Effect of seed source, stratification length, and light conditions on embryo length in seeds of *Narcissus eugeniae*

Seed storage duration (months)	Stratification length (days)	Light		Darkness	
		Stratification 28/14°C	Incubation 15/4°C	Stratification 28/14°C	Incubation 15/4°C
CR					
0	15	1.62 ± 0.07^{Aba} (0, 16)	1.68 ± 0.08^{ABCDa} (0, 8)	1.69 ± 0.04^{Aba} (0, 4)	2.50 ± 0.05^{Bb} (68, 92)
	30	1.72 ± 0.05^{Bca} (0, 12)	1.82 ± 0.05^{BCDab} (3, 16)	1.95 ± 0.05^{CDb} (0, 24)	2.60 ± 0.05^{Bc} (93, 100)
	60	1.94 ± 0.07^{Ca} (0, 40)	2.16 ± 0.07^{Eb} (8, 64)	2.02 ± 0.06^{Da} (0, 44)	2.60 ± 0.00^{Bc} (98, 100)
8	15	1.58 ± 0.05^{ABa} (0, 0)	1.63 ± 0.05^{ABCa} (0, 0)	1.62 ± 0.05^{ABa} (0, 4)	2.55 ± 0.03^{Bb} (85, 96)
	30	1.81 ± 0.05^{BCa} (0, 8)	1.90 ± 0.05^{Da} (3, 24)	1.76 ± 0.04^{BCa} (0, 20)	2.60 ± 0.00^{Bb} (96, 96)
	60	1.90 ± 0.04^{Ca} (0, 20)	2.28 ± 0.08^{Eb} (25, 72)	2.24 ± 0.06^{Eb} (0, 72)	2.60 ± 0.00^{Bc} (100, 100)
RP					
0	15	1.46 ± 0.05^{Aa} (0, 4)	1.56 ± 0.05^{Aa} (0, 0)	1.49 ± 0.04^{Aa} (0, 4)	1.92 ± 0.08^{Ab} (10, 44)
	30	1.59 ± 0.05^{ABa} (0, 4)	1.61 ± 0.04^{ABa} (0, 0)	1.70 ± 0.04^{ABa} (0, 4)	2.10 ± 0.09^{Ab} (31, 48)
	60	1.72 ± 0.05^{BCa} (0, 32)	1.86 ± 0.04^{CDab} (1, 12)	1.94 ± 0.06^{CDb} (0, 52)	2.52 ± 0.04^{Bc} (84, 96)

Values followed by different uppercase letters within a column, or different lowercase letters within a row are significantly different ($P < 0.05$). The first number between parentheses indicates the percentage of radicle emergence and the second is the percentage of embryos whose E:S ratio was > threshold E:S ratio (= 0.73).

Table 4 Summary of the effect of several factors on embryo growth in seeds of *Narcissus eugeniae*

Factor	d.f.	*F*	*P*	Categories
Seed population	1	63.00	<0.001	RP < CR
Seed storage duration	1	4.38	0.0383	0 months < 8 months
Stratification length	2	73.27	<0.001	15^{a}, 30^{ab}, 60^{b}
Light conditions (stratification)	1	11.37	<0.001	Light < darkness
Light conditions (incubation)	1	912.03	<0.001	Light < darkness

Main effects on embryo growth of locality, seed storage duration, stratification length, light conditions during incubation at 15/4°C and during stratification at 28/14°C in multifactor analysis of variance. The table shows degrees of freedom (d.f.), *F*-ratio values, and categories of factors where embryo-growth differences were significant. Residual degrees of freedom: 137. Different letters denote significant differences ($P < 0.05$) between factor levels.

Induction of dormancy by cold temperatures in nondormant seeds At the end of the incubation period at 15/4°C in light, the embryo length was significantly smaller in seeds subjected to warm + cold stratification than in warm stratified seeds. Radicle emergence after 30 days of incubation at 15/4°C was 40, 92, and 90% depending on stratification treatment (Table 6).

Discussion

Freshly matured seeds of *N. eugeniae* had a dormant (PD) and underdeveloped (MD) embryo, with an initial E:S ratio 0.49 ± 0.01, hence the embryo had to grow inside the seed before the radicle was able to emerge. Temperature requirements for embryo growth and dormancy break determine the level of MPD (Baskin & Baskin 1998). In the laboratory experiments, the embryo grew and the radicle emerged in more than 40% of the seeds stratified in darkness at warm temperatures for 60 days (30 days at 28/14°C + 30 days at 25/10°C) followed by an incubation period of 30 days at mild temperatures (at 20/7°C or at 15/4°C). So, seeds from CR exceeded 90% of radicle emergence after being incubated at 15/4°C (Fig. 3). This warm + cool temperature sequence occurs during

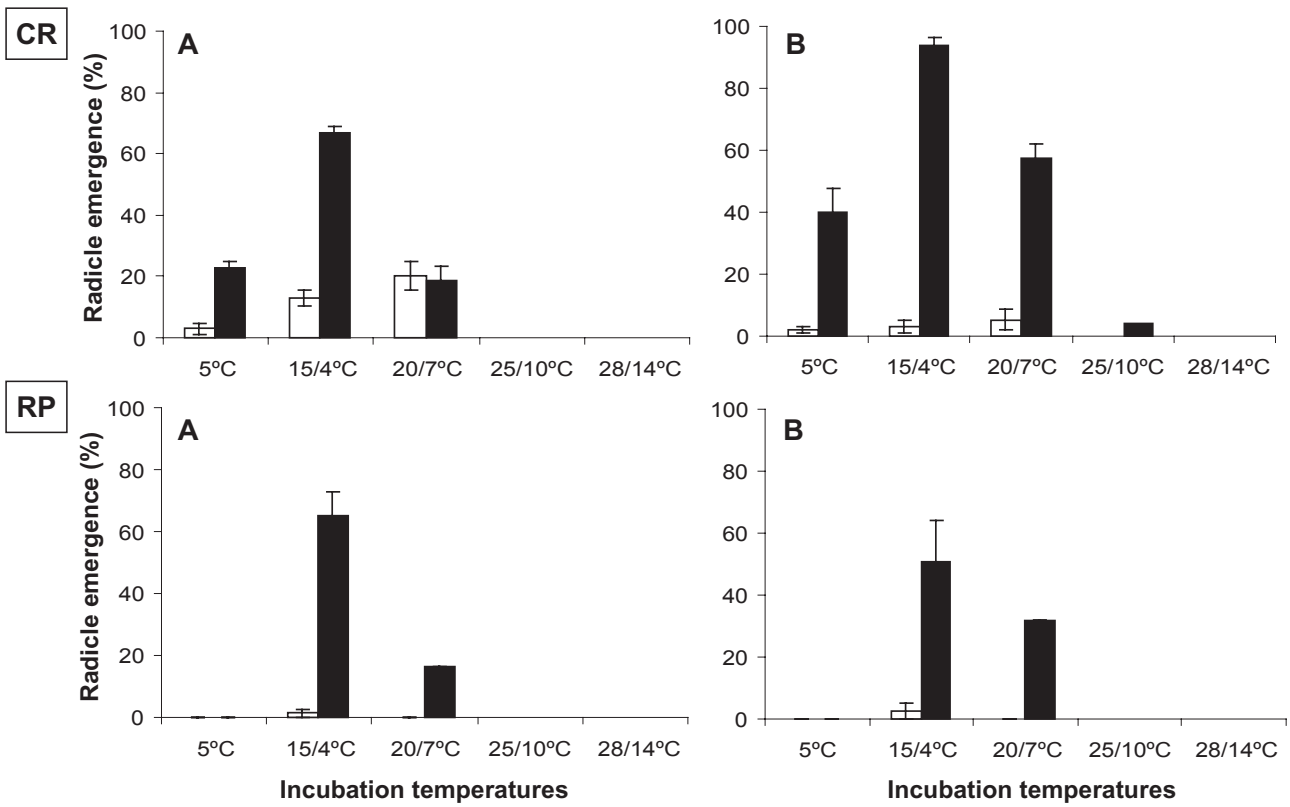

Fig. 3 Radicle emergence in *Narcissus eugeniae* seeds stratified at 28/14°C (30 days) + 25/10°C (30 days) in (A) light and (B) darkness (mean + SE), and then incubated at 5, 15/4, 20/7, 25/10, or 28/14°C for 30 days in both light (white columns) and darkness (black columns). Seed sources: CR and RP.

Table 5 Summary of the effect of several factors on radicle emergence in seeds of *Narcissus eugeniae* stratified at 28/14°C (30 days) + 25/10°C (30 days), and then incubated at 5, 15/4, 20/7, 25/10 and 28/14°C for 30 days

Factor	d.f.	*F*	*P*	Categories
Seed population	1	22.18	<0.001	PR < RC
Light conditions (stratification)	1	0.61	0.4346	
Light conditions (incubation)	1	76.86	<0.001	Light < darkness
Incubation temperature	4	44.73	<0.001	25/10[a], 28/14[a], 5[a], 20/7[b], 15/4[c]

Main effects on germination of locality, light conditions during incubation and during stratification, and incubation temperature in a multifactor analysis of variance. The table shows degrees of freedom (d.f.), *F*-ratio values, and categories of factors where germination differences were significant. Residual degrees of freedom: 152. Different letters denote significant differences ($P < 0.05$) between factor levels.

summer–autumn months in the natural habitat of the species. However, in those seeds incubated for 90 days only at warm temperatures (25/10, 28/14, and 32/18°C), the embryo grew but the radicle did not emerge. In addition, embryo growth was negligible (Table 1) at the mean temperature of winter months (i.e., 5°C). Therefore, embryo growth occurs during warm stratification (≥ 15°C), so *N. eugeniae* has one of the six known levels of simple MPD (Baskin & Baskin 1998; Baskin *et al.* 2008). Both dormancy components, PD and MD, were overcome simultaneously, since embryo growth began just when seeds were stratified at favorable temperatures. Thus, seeds of *N. eugeniae* do not have nondeep simple MPD, where PD is first broken and then the embryo grows quickly (MD). Radicles of *N. eugeniae* seeds have nondeep PD and require warm followed by cool temperatures.

The results obtained under laboratory conditions were highly consistent with those from phenology experiments under near-natural conditions (Fig. 1). Seeds sown outdoors in July had embryo growth in summer and early autumn, that is, during a warm stratification. Subsequently, embryo development was completed and the

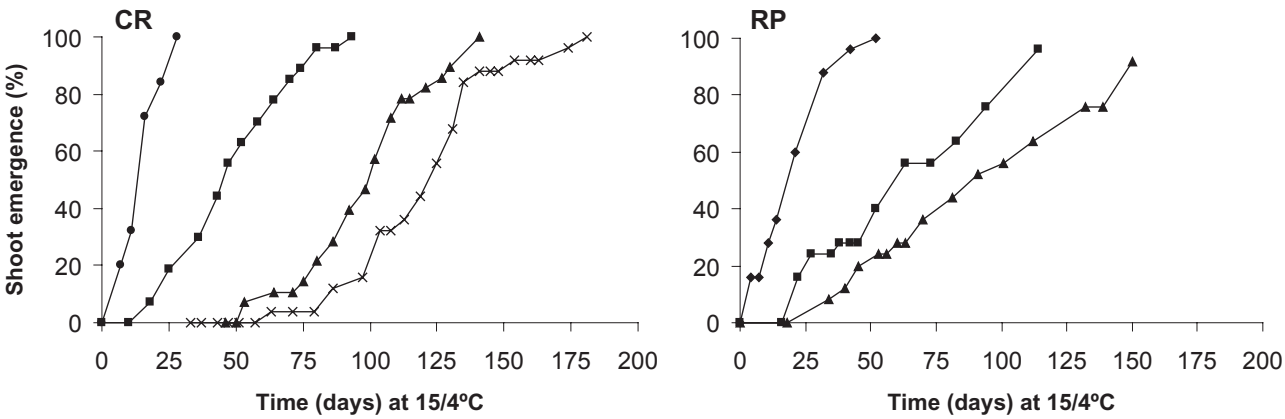

Fig. 4 Shoot emergence in germinated seeds of *Narcissus eugeniae* from CR and RP populations at 15/4°C following 0–12 weeks of cold treatment at 5°C. CR: —×—, 0 weeks at 5°C; —▲—, 4 weeks at 5°C; —■—, 8 weeks at 5°C; —●—, 12 weeks at 5°C. RP: —▲—, 4 weeks at 5°C; —■—, 8 weeks at 5°C; —◆—, 12 weeks at 5°C.

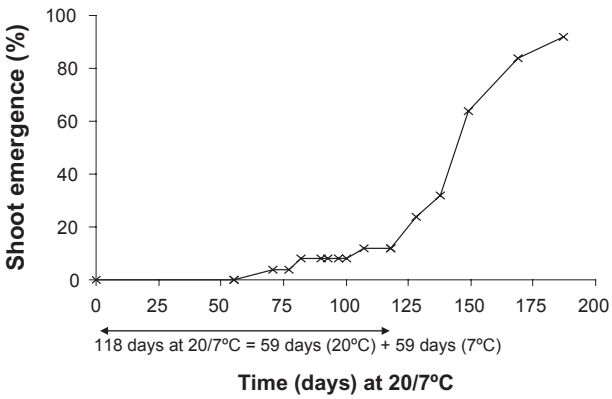

Fig. 5 Shoot emergence in germinated seeds of *Narcissus eugeniae* at 20/7°C without a previous cold treatment at 5°C.

radicle emerged in buried seeds when temperatures went down. In early October, seeds from RP had fully developed embryos while seeds from CR had embryos in an advanced stage of growth. In November, mean maximum and minimum temperatures were near to 15/4°C, which was the optimal temperature for radicle emergence in fully developed embryo seeds (Fig. 3, Table 5). Indeed, radicle emergence was 100% in early December in both seed populations.

However, shoots did not appear above the soil surface until March and April. Hence, radicle-emerged seeds stayed buried for more than 3 months, developing subterranean but not aerial parts (Fig. 1). Such a pattern is typical of species with deep simple epicotyl MPD. Species with deep simple double MPD also show a time lag between root and shoot emergence, but seeds in this dormancy group require two winters to emerge (Baskin & Baskin 1998; Kondo *et al*. 2011). In the same way, intermediate and deep simple MPD can be rejected because fully developed embryo seeds of *N. eugeniae* did not require cold temperatures to promote radicle emergence (Baskin & Baskin 1998).

Results in the phenology study were not conclusive enough to know the level of MPD in *N. eugeniae*, since the lapse between radicle and shoot emergence could be due to: (i) a dormant shoot which required a cold stratification to overcome physiological dormancy; or (ii) a nondormant shoot that grew in a slow but continuous way by cold winter temperatures (Vandelook & Van Assche 2008). However, a cold stratification at 5°C stimulated shoot emergence in radicle-emerged seeds, showing unequivocally that shoots were dormant. Thus, while shoot emergence was 12% in radicle-emerged seeds incubated at 15/4°C for 86 days (Fig. 4; RC), such a percentage was 42 in seeds incubated for 56 days at 5°C followed by 30 days at 15/4°C. Hence, the rate of shoot growth increased with the length of the cold stratification period. This pattern indicated that seeds of *N. eugeniae* have deep simple epicotyl MPD, a level also found in *N. hispanicus* (Copete *et al*. 2011a). Despite an epicotyl not being formed in seedlings of monocotyledon species such as *N. eugeniae* (Muller 1978; Vandelook & Van Assche 2008), a nomenclature change could be confusing (Copete *et al*. 2011a) since "epicotyl dormancy" has been widely used in reference to monocots: *Lilium auratum, L. canadense, L. japonicum, L. rubellum, L. superbum, L. szovitsianum* (Barton 1936; Crocker & Barton 1957), *L. polyphyllum* (Dhyani *et al*. 2013), *Fritillaria ussuriensis* (Liu *et al*. 1993), *Erythronium japonicum* (Kondo *et al*. 2002), and *Gagea lutea* (Kondo *et al*. 2004).

From an ecological point of view, this dormancy level is an effective adaptation to temperate regions with seasonal temperature and water-stress changes throughout the year (Baskin & Baskin 1985; Kondo *et al*. 2004). Seeds with an underdeveloped dormant embryo dispersed at

Previous common stratification at 28/14°C for 60 days	Subsequent different stratification temperatures (°C) for 30 days		Incubation at 15/4°C for 30 days
1.94 ± 0.07^{a} (0, 40)	5	2.16 ± 0.07^{Ab} (25, 44)	2.26 ± 0.06^{Ab} (40, 72)
	20/7	2.24 ± 0.07^{Ab} (13, 72)	2.54 ± 0.04^{Bc} (92, 96)
	25/10	2.09 ± 0.06^{Ab} (3, 56)	2.58 ± 0.02^{Bc} (90, 98)

Values followed by different uppercase letters within a column, or different lowercase letters within a row are significantly different ($P < 0.05$). The first number between parentheses is the percentage of radicle emergence, and the second is the percentage of embryos whose E:S ratio was > threshold E:S ratio (= 0.73).

Table 6 Effect on embryo growth (mean ± SE) of warm stratification (28/14°C) for 60 days followed by cold (5°C) or warm stratification (20/7°C or 25/10°C) in light for 30 days and then by incubation at 15/4°C in light for 30 days in 12-month-old seeds of *Narcissus eugeniae*. Seed source was CR

the end of the spring are prevented from germination due to eventual summer-rainfall episodes. Radicle emergence is delayed until autumn, when the water soil conditions are adequate for seedling survival in Mediterranean environments. Furthermore, the dormant shoot does not emerge during the winter period, avoiding exposure to cold temperatures. However, the root grows during winter months beneath the soil surface where temperatures are mild. Such a strategy provides seedlings with an early well-developed root system enhancing juvenile survival (Kondo *et al*. 2004). Definitive seedling establishment occurs at spring, the most favourable season for plant recruitment in European forest (Grime 2001), when shoot growth is triggered by previous cold winter effect.

Approximately, 10% seedlings in the phenology experiments did not emerge up to 20 months after seed sown (i.e., from July 2007 to March 2009; Fig. 1b). Such a long delay may be explained by induction of dormancy in seeds. Our experiments demonstrated that growing-embryo seeds can respond to cold temperatures by reentering into a dormancy stage prior to reaching the threshold E:S ratio. Dormancy-induction experiments have been undertaken on some species with MPD, such as *Frasera caroliniensis* (Threadgill *et al*. 1981) and *Delphinium fissum* subsp. *sordidum* (Herranz *et al*. 2010b), both with complex MPD levels; *Chaerophyllum tainturieri* (Baskin & Baskin 1990) and *Torilis japonica* (Vandelook *et al*. 2008) with nondeep simple MPD; and *N. hispanicus* (Copete *et al*. 2011a) with deep simple epicotyl dormancy. However, many of those dormancy-induction studies only analyzed germinative responses. Here we report the interruption of embryo growth by the effect of environmental factors.

With regard to seed storage duration, its influence on embryo growth and radicle emergence was significant (Fig. 2, Table 2), so old seeds required a shorter stratification period than fresh ones. The improvement of germinative ability with seed dry storage or after-ripening is a quite common phenomenon in seeds with developed embryos and with nondeep physiological dormancy (Baskin & Baskin 1998; Copete *et al*. 2005), but this topic has been little studied in species with MPD: *Delphinium fissum* subsp. *sordidum* (Herranz *et al*. 2010b), *Aconitum napellus* subsp. *castellanum* (Herranz *et al*. 2010a), *Narcissus alcaracensis* (Herranz *et al*. 2013a), *N. hispanicus* (Copete *et al*. 2011a), and *Merendera montana* (Copete *et al*. 2011b). Therefore, such a positive effect of dry storage points to a nondeep physiological component in the MPD level of *N. eugeniae*. In the natural habitat, seeds of *N. eugeniae* could experience an after-ripening process during drought summer periods. Embryo growth would be interrupted in dry conditions. Our results, however, show that embryo growth would be stimulated after autumn rains, substantially promoting radicle emergence and seedling establishment.

Phenology experiments showed a similar response in both seed populations (Fig. 1B,C), but dormancy breaking occurred faster in RP than in CR seeds. Thus, embryo growth in seeds from RP was completed and radicles began to emerge in October, one month before those from CR. Although caution should be applied when interpreting germination differences between seeds collected in different years (2007 for CR, 2008 for CP), the high interannual homogeneity in weather summer and autumn conditions for that period, lead us to suggest that differences detected in phenology should have an important interpopulation basis. Such differences may reflect local, environmental adaptations to habitat, and/or may be the result of the environmental conditions at each site during seed development (Scholten *et al*. 2009). A reduction in the required stratification length may be a good adaptation to habitats such as RP, where soil moisture persistence is shorter than in CR (see Source of seeds section), and consequently also the period of effective warm stratification during the summer.

The knowledge of classes and levels of dormancy in *Narcissus* contributes to understanding evolutionary processes and current phylogeny within the genus. In *N. eugeniae*, the adaptation to a microhabitat with summer water stress has been more decisive in the modulation of the MPD level than the cold-winter mountain climate characterizing the habitat. In addition, researching specific environmental conditions that trigger dormancy break of *N. eugeniae*, a threatened taxon, is a key factor in designing *ex situ* propagation protocols to reinforce wild plant populations.

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References

Barton L. V. (1936) Germination and seedling production in *Lilium* sp[p]. *Contributions from Boyce Thompson Institute* **8**: 297–309.

Baskin C. C. & Baskin J. M. (1998) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego, CA.

Baskin C. C., Chien C. T., Chen S. Y. & Baskin J. M. (2008) Germination of *Viburnum odoratissimum* seeds: a new level of morphophysiological dormancy. *Seed Science Research* **18**: 179–184.

Baskin C. C., Milberg P., Andersson L. & Baskin J. M. (2000) Deep complex morphophysiological dormancy in seeds of *Anthriscus sylvestris* (Apiaceae). *Flora* **195**: 245–251.

Baskin J. M. & Baskin C. C. (1985) Epicotyl dormancy in seeds of *Cimicifuga racemosa* and *Hepatica acutiloba*. *Bulletin of the Torrey Botanical Club* **112**: 253–257.

Baskin J. M. & Baskin C. C. (1990) Germination ecophysiology of seeds of the winter annual *Chaerophyllum tainturieri*: a new type of morphophysiological dormancy. *Journal of Ecology* **78**: 993–1004.

Baskin J. M. & Baskin C. C. (2003) Classification, biography and phylogenetic relationships of seed dormancy. In: Smith R. D., Dickie J. B., Linigton S. H., Pritchard H. W. & Probert R. J. (eds). *Seed Conservation: Turning Science into Practice*. The Royal Botanic Gardens, Kew, London, pp. 519–542.

Baskin J. M. & Baskin C. C. (2004) A classification system for seed dormancy. *Seed Science Research* **14**: 1–16.

Beckstead J., Meyer S. E. & Allen P. S. (1996) *Bromus tectorum* seed germination: between-population and between-year variation. *Canadian Journal of Botany* **74**: 875–882.

Chien C.-T., Chen S.-Y., Tsai C.-C., Baskin J. M., Baskin C. C. & Kuo-Huang L.-L. (2011) Deep simple epicotyl morphophysiological dormancy in seeds of two *Viburnum* species, with special reference to shoot growth and development inside the seed. *Annals of Botany* **108**: 13–22.

Copete E., Herranz J. M., Copete M. A., Baskin J. M. & Baskin C. C. (2011b) Non-deep complex morphophysiological dormancy in seeds of the Iberian Peninsula endemic geophyte *Merendera montana* (Colchicaceae). *Seed Science Research* **21**: 267–281.

Copete E., Herranz J. M., Ferrandis P., Baskin C. C. & Baskin J. M. (2011a) Physiology, morphology and phenology of seed dormancy-break and germination in the endemic Iberian species *Narcissus hispanicus* (Amaryllidaceae). *Annals of Botany* **107**: 1003–1016.

Copete M. A., Herranz J. M. & Ferrandis P. (2005) Seed dormancy and germination in threatened Iberian *Coincya* (Brassicaceae) taxa. *Ecoscience* **12**: 257–266.

Crocker W. & Barton L. V. (1957) *Physiology of Seeds*. Chronica Botanica, Waltham, MA.

Dhyani A., Phartyal S. S., Nautiyal B. P. & Nautiyal M. C. (2013) Epicotyl morphophysiological dormancy in seeds of *Lilium polyphyllum* (Liliaceae). *Journal of Bioscience* **38**: 13–19.

Elías F., Ruiz L. (1981) *Estudio agroclimático de la región de Castilla-La Mancha. Consejería de Agricultura*. Junta de Comunidades de Castilla-La Mancha, Toledo, 247 pp.

Fay M. F. (1992) Conservation of rare and endangered plants using *in vitro* methods. *In Vitro Cellular Developmental Biology-Plant* **28**: 1–4.

Grime J. P. (2001) *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd edn. John Wiley & Sons Ltd, Chichester.

Herranz J. M., Copete E. & Ferrandis P. (2013b) Nondeep complex morphophysiological dormancy in *Narcissus longispathus* (Amaryllidaceae): implications for evolution of dormancy levels within section *Pseudonarcissi*. *Seed Science Research* **23**: 141–155.

Herranz J. M., Copete M. A. & Ferrandis P. (2013) Environmental regulation of embryo growth, dormancy break and germination of *Narcissus alcaracensis* (Amaryllidaceae), a threatened daffodil endemic iberic. *American Midland Naturalist* **169**: 147–167.

Herranz J. M., Copete M. A., Ferrandis P. & Copete E. (2010a) Intermediate complex morphophysiological dormancy in the endemic Iberian *Aconitum napellus* subsp. *castellanum* (Ranunculaceae). *Seed Science Research* **20**: 109–121.

Herranz J. M., Ferrandis P. & Martínez-Duro E. (2010b) Seed germination ecology of the threatened endemic Iberian *Delphinium fissum* subsp. *sordidum* (Ranunculaceae). *Plant Ecology* **211**: 89–106.

Hidayati S. N., Baskin J. M. & Baskin C. C. (2000) Dormancy-breaking and germination requirements of seeds of four *Lonicera* species (Caprifoliaceae) with underdeveloped spatulate embryos. *Seed Science Research* **10**: 459–469.

Kondo T., Mikubo M., Yamada K., Walck J. & Hidayati S. N. (2011) Seed dormancy in *Trillium camschatcense* (Melanthiaceae) and the possible roles of light and temperature requirements for seed germination in forests. *American Journal of Botany* **98**: 215–226.

Kondo T., Miura T., Okubo N., Shimada M., Baskin C. C. & Baskin J. M. (2004) Ecophysiology of deep simple epicotyl morphophysiological dormancy in seeds of *Gagea lutea* (Liliaceae). *Seed Science Research* **14**: 371–378.

Kondo T., Okubo N., Miura T., Honda K. & Ishikawa Y. (2002) Ecophysiology of seed germination in *Erythronium japonicum* (Liliaceae) with underdeveloped embryos. *American Journal of Botany* **89**: 1779–1784.

Liu M., Li R.-J. & Liu M.-Y. (1993) Adaptive responses of roots and root systems to seasonal changes. *Environmental and Experimental Botany* **33**: 175–188.

Luna B., Pérez B., Fernández-González F. & Moreno J. M. (2004) Sensitivity to green safelight of 12 Mediterranean species. *Seed Science and Technology* **32**: 113–117.

Mayoral O. & Gómez-Serrano M. A. (2004) Sobre la distribución de *Narcissus eugeniae* Fern. Casas (Amaryllidaceae) en Castilla-La Mancha. *Flora Montiberica* **28**: 83–89.

Moreno J. C. (coord.). (2008) *Lista Roja 2008 de la flora vascular española*. Dirección General de Medio Natural y Política Forestal y Sociedad Española de Biología de Conservación de plantas. Madrid. 86 pp.

Muller F. M. (1978) *Seedlings of the North-Western European Lowland. A Flora of Seedlings*. Dr W. Junk B.V. Publishers, The Hague.

Nikolaeva M. G. (1969) *Physiology of Deep Dormancy in Seeds*. Izdatel'stvo "Nauka", Leningrad.

Pérez-García F., Hornero J. & González-Benito M. E. (2003) Interpopulation variation in seed germination of five Mediterranean Labiatae shrubby species. *Israel Journal of Plant Species* **51**: 117–124.

Scholten M., Donahue J., Shaw N. L. & Serpe M. D. (2009) Environmental regulation of dormancy loss in seeds of *Lomantium dissectum* (Apiaceae). *Annals of Botany* **103**: 1091–1101.

Stokes P. (1965) Temperature and seed dormancy. In: Ruhland W. (ed.). *Encyclopaedia of Plant Physiology*. Vol. 15/2: Springer-Verlag, New York, pp. 746–803.

Threadgill P. F., Baskin J. M. & Baskin C. C. (1981) Dormancy in seeds of *Frasera caroliniensis* (Gentianaceae). *American Journal of Botany* **68**: 80–86.

Vandelook F., Bolle N. & Van Asche J. A. (2008) Seasonal dormancy cycles in the biennial *Torilis japonica* (Apicaceae), a species with morphophysiological dormancy. *Seed Science Research* **18**: 161–171.

Vandelook F. & Van Assche J. A. (2008) Temperature requirements for seed germination and seedling development determine timing of seedling emergence of three monocotyledonous temperate forest spring geophytes. *Annals of Botany* **102**: 865–875.

Walck J. L. & Hidayati S. N. (2004) Germination ecophysiology of the western North American species *Osmorhiza depauperata* (Apiaceae): implications of preadaptation and phylogenetic niche conservatism in seed dormancy evolution. *Seed Science Research* **14**: 387–394.