

Short Communication

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Two closely related *Tulipa* species with different ploidy levels show distinct germination rates

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Abstract

In the tetraploid *Tulipa sylvestris* we found larger and heavier seeds that germinated around 28 days earlier than those of the diploid ancestor *Tulipa pumila*. The faster germination of the tetraploid species is linked to the faster growth of embryos, which reached their final length much earlier than the embryos of the diploid species. In conclusion, we argue a cautionary approach when dealing with comparative studies on ploidy level and germination to avoid misinterpretation of results when set against the natural conditions.

Introduction

Many polyploid taxa produce larger and/or heavier seeds as compared to related diploids, a feature that may favour speciation, diversification and geographical expansion on the short term (Maceira et al., 1993; Bretagnolle et al., 1995; Hoya et al., 2007; Urwin et al., 2007; Eliášová and Münzbergová, 2017). Indeed, the gigas effect on seeds has been found to positively affect the germination capacity, both in final percentage and in rate (Bretagnolle et al., 1995; Hoya et al., 2007; Eliášová and Münzbergová, 2014) and the production of larger seedlings (Moles and Westoby, 2004), which in turn may enhance plant establishment.

Plant recruitment from seeds is usually studied to investigate the environmental drivers shaping the ecological niche in both seed germination and seedling establishment (Poschlod et al., 2013). For instance, these drivers include several physical and biotic cues (reviewed by Donohue et al., 2010; Baskin and Baskin, 2014). On the contrary, there are only a few studies addressing the effect of ploidy level on germination (Bretagnolle et al., 1995; Hoya et al., 2007; Broadhurst et al., 2012; Carta et al., 2014; Eliášová and Münzbergová, 2014), and in none of them embryo growth was taken into account. In this context, the role of the relative embryo size (usually expressed as the ratio of the embryo to seed length) is crucial from the ecological point of view (see Nikolaeva, 1977; Vandeloek et al., 2012; Carta et al., 2014; Blandino et al., 2019). The importance of embryo growth, for instance, makes sense especially in those species that produce underdeveloped embryos at dispersal, and which are only able to germinate when the embryo reaches a critical length (Nikolaeva, 1977). This seed property has been documented in several phylogenetically unrelated angiosperm families, from Ranunculaceae to Apiaceae (Baskin and Baskin, 2014), although the absence of growth of the embryo prior to germination is also reported (Vandeloek et al., 2019). For instance, many Eurasian geophytes possess underdeveloped embryos, including many Liliaceae (Carasso et al., 2011; Mondoni et al., 2012; Baskin and Baskin, 2014), such as representatives of *Tulipa* (Martin, 1946; Tang et al., 2009; Baskin and Baskin, 2014). This genus includes several species inhabiting open habitats, among which the only ones native to western Europe, namely *Tulipa pumila* Moench and *Tulipa sylvestris* L.; the latter presumably originated from the former via autopolyploidy (Cesca, 1986). This species reproduces sexually, and populations with functionally male flowers have been reported (Peruzzi, 2012), sharing this feature with the diploid ancestor *T. pumila* (Astuti et al., 2020).

As many other bulbous geophytes in the Mediterranean area (e.g. Doussi and Thanos, 2002; Carta et al., 2016, 2017), tulips are reported to preferentially germinate at cool temperature and in the dark (Tang et al., 2009; Baskin and Baskin, 2014), except the desert species *Tulipa systola* Stapf, which seems insensitive to light exposure (Boeken and Gutterman, 1990).

Considering the alleged gigas effect displayed by polyploids, we compared seed mass and size in *T. pumila* and *T. sylvestris*. Then, we monitored embryo growth and radicle protrusion of both species under laboratory conditions to evaluate putative differences in germinability in terms of both percentage and rate.

Materials and methods

We sampled seeds and bulbs of *T. pumila* from one population growing in open dry habitats at Doccino (43°23'54"N, 10°37'19"E) (Riparbella, Pisa; Tuscany, central Italy). Seeds and bulbs

of *T. sylvestris* were collected from one population growing in olive groves at Le Rose (43°43'15"N, 11°13'40"E) (Impruneta, Firenze; Tuscany, central Italy). Fruits of both species were collected in the field on June 2012 from around 50 individuals per species and processed in the laboratory. Empty or not fully developed seeds were discarded. Seed sowing was started within 1 month after collection, to avoid conservation procedures that may affect dormancy (Tang et al., 2009) and longevity (Baskin and Baskin, 2014).

For seed mass, we weighed five batches of 30 randomly selected seeds for each species (accuracy of 1×10^{-4} g). Then, we measured seed size (length, width and thickness of 30 randomly chosen seeds for each species (resolution of 5×10^{-5} m)). For seed mass and size, a *t*-Student was applied.

Seeds used for monitoring embryo growth and radicle protrusion were sown in Petri dishes with 1% agar placed under the best temperature (7°C) and light (in the dark) conditions of germination according to previous studies (Van Tuyl and Van Creijl, 2006; Carta et al., 2017) and our preliminary results (Fig. 1).

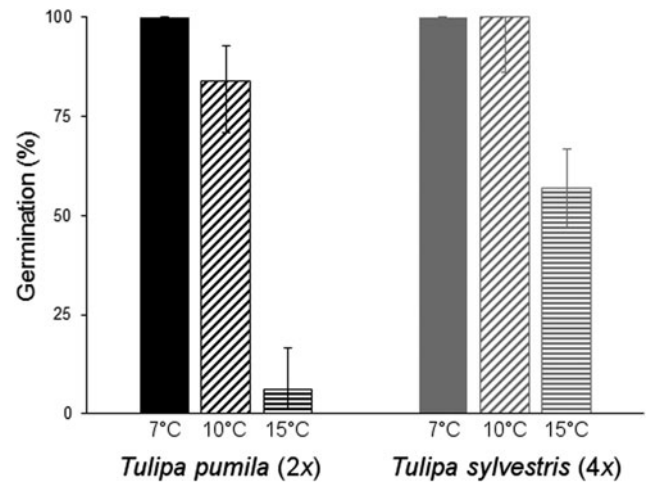


Fig. 1. Germination proportion ($\pm 95\%$ CI) at different temperatures in *T. pumila* and *T. sylvestris*.



Fig. 2. Embryo growth progress in seeds of *T. pumila*. From left to right: at incubation, after 30 days of incubation and after 80 days of incubation. Bar = 1 mm.

We measured, by means of a hand calliper, the embryo and seed length every week in ten seeds for each species (Fig. 2). Then, we calculated the embryo length to seed length ratio (E:S hereafter). For calculating the mean E:S at radicle protrusion, we measured the embryo length at radicle protrusion in each seed, and we calculated the mean critical E:S for each species.

For seed germination, we performed tests using 100 seeds for each species. Seeds were considered germinated only when the radicle reached 1 mm in length. We calculated the mean germination time (MGT hereafter) according to the formula:

$$\text{MGT} = \sum \frac{n_i t_i}{N}$$

where n_i is the number of seeds germinated in the specific interval of time, t_i is the time elapsed since the start of the test to the end of the specific interval of measurement and N is the total number of germinated seeds. The number of germinated seeds was recorded approximately every week. At the end of the germination test, we performed a cut test on those seeds that did not germinate to evaluate the number of not viable seeds.

In order to check the ploidy level of the studied populations, root tips from potted bulbs were pretreated with 0.4% colchicine and stained with leuco-basic fuchsin after hydrolysis in HCl 1 N at 60°C for 7 min. For chromosome counting, stained root tips were squashed on a drop of acetic orcein and observed under a light microscope.

Table 1. Seed mass and size comparison between *T. pumila* and *T. sylvestris*

Seed features	<i>T. pumila</i>	<i>T. sylvestris</i>
Mass (30 seeds) [mg]	4.24 \pm 0.23	5.96 \pm 0.28
Length [mm]	4.73 \pm 0.40	5.06 \pm 0.47
Width [mm]	3.99 \pm 0.40	4.10 \pm 0.41
Thickness [mm]	0.38 \pm 0.07	0.46 \pm 0.11

For each feature the mean and the standard deviation are given.

Results

Mean weight, length, width and thickness of seeds are reported in Table 1. Seeds of both species are flattened but distinctly three-dimensional, light-coloured and smooth, with copious endosperm and a well-distinct underdeveloped embryo at the acute apex with an E:S value at the dispersal of ca. 0.25 in both species. Seeds of *T. sylvestris* were significantly heavier ($t = 10.58$, $df = 4$, $P < 0.01$) and larger (length: $t = 2.92$, $df = 29$, $P < 0.01$; thickness: $t = 3.30$, $df = 29$, $P < 0.01$; width: $t = 0.95$, $df = 29$, $P > 0.05$) than those of *T. pumila*.

Progress of embryo growth is shown in Fig. 3. Although the mean E:S at germination was similar and not significantly different ($t = 1.995$, $df = 9$, $P > 0.05$) in the two species (ca. 0.91 in *T. pumila*, 0.87 in *T. sylvestris*), the embryo growth of *T. sylvestris* showed a unimodal progress and its rate was faster (ca. 48 days) as compared to *T. pumila* (ca. 97 days). In the latter species,

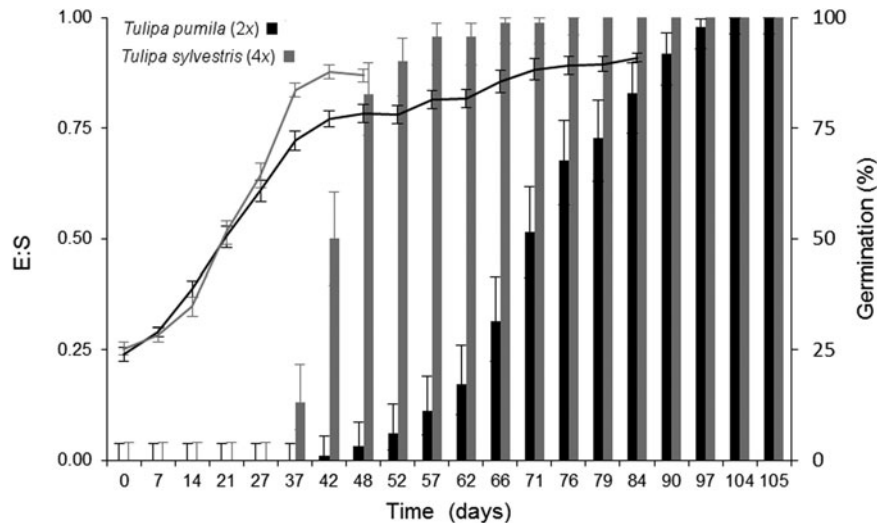


Fig. 3. Embryo growth curves (\pm SE) and cumulative germination (\pm 95% CI), in the diploid *T. pumila* and tetraploid *T. sylvestris*.

instead, the embryos grew at two different rates (bimodal progress), increasing rapidly in the first 28 days, after which they slowly reached their critical length.

The radicle protrusion curves of both species are shown in Fig. 3. Radicle protrusion in *T. sylvestris* was completed in 76 days, with MGT = 46.03 days. On the contrary, *T. pumila* germination was much more lagged, with MGT = 74.20 days, and was completed after 104 days. Interestingly, while 50% of germination was reached much more earlier in *T. sylvestris*, E:S of 0.5 was reached roughly at the same time in both the species. Nevertheless, final germination was close to 100% in both the species.

From the karyological analysis, we counted $2n = 48$ chromosomes for *T. sylvestris* (Fig. 4) and $2n = 24$ chromosomes for *T. pumila* (not shown).

Discussion

Seeds of both the species germinated completely, without the need for a preliminary disruption of a physiological block by a cold stratification period. Hence, the cold period stimulating embryo



Fig. 4. Metaphasic plate of *T. sylvestris* from Le Rose ($2n = 4x = 48$). Red dots indicate the centromeres. Scale bar = 10 μ m.

growth suggests that seeds cannot be considered physiologically dormant, but rather as morphologically dormant according to the classification proposed by Baskin and Baskin (2004). Particularly, for our *Tulipa* species we could draw the same conclusion made by Carasso et al. (2011) for a *Fritillaria* species (Liliaceae): seeds simply germinate slowly under a range of low temperatures without any lag between embryo growth, radicle protrusion and cotyledon emergence, without physiological and epicotyl dormancy.

Tulipa sylvestris and *T. pumila* were confirmed to be tetraploid and diploid, respectively, also in our studied populations. *Tulipa sylvestris* has larger and heavier seeds than *T. pumila*. Indeed, polyploids have a larger genome size, which is considered one of the main drivers in yielding divergence in seed size among angiosperms (Beaulieu et al., 2007). Here, larger and heavier seeds are paralleled by faster embryo growth and germination rates in *T. sylvestris*, as found in other polyploids (Bretagnolle et al., 1995; Hoya et al., 2007; Broadhurst et al., 2012; Eliášová and Münzbergová, 2014). In *T. sylvestris*, indeed, the germination was significantly faster, with an anticipation of ca. 28 days as compared to *T. pumila*. Two main explanations have been provided for the faster germination of polyploids: (1) they are better in mobilizing seed reserves (von Well and Fossey, 1998) or (2) larger seeds induce faster germination (Thompson, 1990). However, although the seeds of the two species are different in size and weight, they share similar starting E:S and mean E:S at germination, meaning that ploidy level likely does not affect the contribution of endosperm and/or integuments in controlling the whole seed structure. Furthermore, we neither found a higher proportion of germination in polyploids, as found in other genera such as *Dactylis* (Bretagnolle et al., 1995), *Taraxacum* (Hoya et al., 2007) and *Vicia* (Eliášová and Münzbergová, 2014), nor a significant germination decrease as reported by Cohen et al. (2013) and Hosseini et al. (2013).

As seeds from both the species showed high viability, we did not find evidence of different performance in germination caused by polyploidy. Instead, we have shown that *T. pumila* and *T. sylvestris* show a different seed germination rate, and cytotype variation may be considered the reason for these germination differences as previously detected in other species (Hoya et al.,

2007; Broadhurst et al., 2012; Eliášová and Münzbergová, 2014). It is possible, however, that the faster germination of the polyploid species represents a local adaptation to a new environment (Donohue et al., 2010) different from that inhabited by the diploid ancestors. Indeed, as also observed in our target populations, the two species show a peculiar ecological shift in habitat preference, which makes it difficult to disentangle to which extent habitat conditions rather than ploidy level contribute to the difference found here, especially concerning seed mass. It is possible that the poorer soils typically colonized by *T. pumila* negatively affect its seed size. In conclusion, it should be remarked that the germination at dispersal does not necessarily reflect the fitness potential of seeds in the natural environment, a discrepancy already highlighted in studies dealing with pollination and mating systems (Carta et al., 2015; Baskin and Baskin, 2019). Hence, we recommend a more cautionary approach when dealing with studies on ploidy level and germination to avoid misinterpretation of the results when tested on natural conditions.

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References

- Astuti G, Pratesi S, Carta A and Peruzzi L (2020) Male flowers in *Tulipa pumila* Moench (Liliaceae) potentially originate from gender diphasy. *Plant Species Biology* 1–8. doi:10.1111/1442-1984.12267.
- Baskin CC and Baskin JM (2004) A classification system for seed dormancy. *Seed Science Research* 14, 1–16.
- Baskin CC and Baskin JM (2014) *Seeds: ecology, biogeography, and evolution of dormancy and germination*. Amsterdam, Elsevier.
- Baskin JM and Baskin CC (2015) Inbreeding depression and the cost of inbreeding on seed germination. *Seed Science Research* 25, 355–385.
- Baskin JM and Baskin CC (2019) Effect of selective abortion on seed germination and post-germination performance of offspring. *Seed Science Research* 29, 210–214.
- Beaulieu JM, Leitch IJ and Knight CA (2007) Genome size evolution in relation to leaf strategy and metabolic rates revisited. *Annals of Botany* 99, 495–505.
- Blandino C, Fernández-Pascual E, Marin M, Vernet A and Pritchard HW (2019) Seed ecology of the geophyte *Conopodium majus* (Apiaceae), indicator species of ancient woodland understories and oligotrophic meadows. *Plant Biology* 21, 487–497.
- Boeken B and Gutterman Y (1990) The effect of temperature on seed germination in three common bulbous plants of different habitats in the Central Negev Desert of Israel. *Journal of Arid Environments* 18, 175–184.
- Bretagnolle F, Thompson JD and Lumaret R (1995) The influence of seed size variation on seed germination and seedling vigour in diploid and tetraploid *Dactylis glomerata* L. *Annals of Botany* 76, 607–615.
- Broadhurst LM, Murray BG, Forrester R and Young AG (2012) Cryptic genetic variability in *Swainsona sericea* (A.Lee) H.Eichler (Fabaceae): lessons for restoration. *Australian Journal of Botany* 60, 429–438.
- Carasso V, Hay FR, Probert RJ and Mucciarelli M (2011) Temperature control of seed germination in *Fritillaria tubiformis* subsp. *moggridgei* (Liliaceae) a rare endemic of the South-west Alps. *Seed Science Research* 21, 33–38.
- Carta A, Probert R, Moretti M, Peruzzi L and Bedini G (2014) Seed dormancy and germination in three *Crocus* ser. *Verni* species (Iridaceae): implications for evolution of dormancy within the genus. *Plant Biology* 16, 1065–1074.
- Carta A, Bedini G, Giannotti A, Savio L and Peruzzi L (2015) Mating system modulates degree of seed dormancy in *Hypericum elodes* L. (Hypericaceae). *Seed Science Research* 25, 299–305.
- Carta A, Hanson S and Müller JV (2016) Plant regeneration from seeds responds to phylogenetic relatedness and local adaptation in Mediterranean *Romulea* (Iridaceae) species. *Ecology and Evolution* 6, 4166–4178.
- Carta A, Skourti E, Mattana E, Vandeloos F and Thanos K (2017) Photoinhibition of seed germination: occurrence, ecology and phylogeny. *Seed Science Research* 27, 131–153.
- Cesca G (1986) Note fitogeografiche e citotassonomiche su *Adoxa moschatellina* L., *Tulipa sylvestris* L., *Fritillaria tenella* Bieb. *Biogeographia* 10, 109–141.
- Cohen H, Fait A and Tel-Zur N (2013) Morphological, cytological and metabolic consequences of autopolyploidization in *Hylocereus* (Cactaceae) species. *BMC Plant Biology* 13, 173.
- Donohue K, de Casas RR, Burghardt L, Kovach K and Willis CG (2010) Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution and Systematics* 41, 293–319.
- Doussi MA and Thanos CA (2002) Ecophysiology of seed germination in Mediterranean geophytes. 1. *Muscari* spp. *Seed Science Research* 12, 193–201.
- Hosseini HR, Chehrizi M, Sorestani MM and Ahmadi DN (2013) Polyploidy and comparison of diploid and autotetraploid seedling of Madagascar periwinkle (*Catharanthus roseus* cv. alba). *International Research Journal of Applied and Basic Sciences* 4, 402–406.
- Eliášová A and Münzbergová Z (2014) Higher seed size and germination rate may favour autotetraploids of *Vicia cracca* L. (Fabaceae). *Botanical Journal of the Linnean Society* 113, 57–73.
- Eliášová A and Münzbergová Z (2017) Factors influencing distribution and local coexistence of diploids and tetraploids of *Vicia cracca*: inferences from a common garden experiment. *Journal of Plant Research* 130, 677–687.
- Hoya A, Shibaike H, Morita T and Ito M (2007) Germination characteristics of native Japanese dandelion autopolyploids and their putative diploid parent species. *Journal of Plant Research* 120, 139–147.
- Maceira NO, Jacquard P and Lumaret R (1993) Competition between diploid and derivative autotetraploid *Dactylis glomerata* L. from Galicia. Implications for the establishment of novel polyploid populations. *New Phytologist* 124, 321–328.
- Martin AC (1946) The comparative internal morphology of seeds. *American Midland Naturalist* 36, 513–660.
- Moles AT and Westoby M (2004) Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* 92, 372–383.
- Mondoni A, Rossi G and Probert RJ (2012) Temperature controls seed germination and dormancy in the European woodland herbaceous perennial *Erythronium dens-canis* (Liliaceae). *Plant Biology* 14, 475–480.
- Nikolaeva MG (1977) Factors controlling the seed dormancy pattern, pp. 51–74 in Khan AA (Ed.) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, Elsevier.
- Peruzzi L (2012) Male flowers in Liliaceae are more frequent than previously thought. *Bocconea* 24, 301–304.
- Poschold P, Mehdi A, Bartelheimer M, Drobnik J, Rosbakh S and Saatkamp A (2013) Seed ecology and assembly rules in plant communities, pp. 164–202 in Van der Maarel E; Franklin J (Eds.) *Vegetation ecology*. Malden, Wiley-Blackwell.
- Tang A, Tian M and Long C (2009) Seed dormancy and germination of three herbaceous perennial desert ephemerals from the Junggar Basin, China. *Seed Science Research* 19, 183–189.
- Thompson K (1990) Genome size, seed size and germination temperature in herbaceous angiosperms. *Evolutionary Trends in Plants* 4, 113–116.
- Urwin NAR, Horsnell J and Moon T (2007) Generation and characterisation of colchicine-induced autotetraploid *Lavandula angustifolia*. *Euphytica* 156, 257–266.
- Vandeloos F, Janssens SB and Probert RJ (2012) Relative embryo length as an adaptation to habitat and life cycle in Apiaceae. *New Phytologist* 195, 479–487.
- Vandeloos F, Van de Vyver A and Carta A (2019) Three phylogenetically distant shade-tolerant temperate forest herbs have similar seed germination syndromes. *Folia Geobotanica* 54, 73–84.
- Van Tuyl JM and Van Creijl MGM (2006). Tulip, pp. 623–641 in Anderson N (Ed.) *Flower breeding and genetics*. Dordrecht, Springer.
- Von Well E and Fossey A (1998) A comparative investigation of seed germination, metabolism and seedling growth between two polyploid *Triticum* species. *Euphytica* 101, 83–89.