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Research article

Evolutionary ecology of fast seed germination—A case study in Amaranthaceae/Chenopodiaceae

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ABSTRACT

Germination is a vulnerable and risky step in a plant's life cycle. Particularly under harsh environmental conditions, where time windows favourable for seedling establishment and survival are short or unpredictable, germination speed might play a highly adaptive role. We investigated the germination speed of 107 Amaranthaceae s.l. at two different temperatures and related the results to various plant and habitat traits taking into account the molecular phylogenetic relatedness of the species sampled.

Germination speed is a fast evolving trait in Amaranthaceae s.l. It evolves towards significantly faster optima in C_4 and halophyte lineages, albeit for different reasons. While C_4 photosynthesis and fast germination are simply traits beneficial under the same environmental conditions, saline habitats, especially in subtropical regions, seem to select for fast germination. Heterospermy is probably much more common than currently known in Amaranthaceae s.l. and likely evolved as a bet-hedging strategy in lineages with fast seed germination. The evolution of germination speed is neither related to seed mass, an evolutionary stable trait, nor dependent on plant longevity and plant height. Correlation analyses of climate variables and germination speed suggest that there is an indirect effect of climate which is dependent on the ecological niche of the species.

1. Introduction

Angiosperm seeds germinate at widely different speeds. The time that passes between the start of imbibition and the emergence of the radicle or shoot from the seed coat can vary between minutes and months or sometimes even years (Baskin and Baskin, 2014). The speed of germination is influenced by seed morphology, physiology, gene regulation and the environment to which seeds are exposed (Nonogaki et al., 2010). Germination is an irreversible process and its speed determines the point in time when an individual enters the ecosystem and its struggle for existence begins. Therefore, it is closely associated with a species' ecology and is under high selection pressure. Based on their seed internal morphology, early angiosperms are believed to have been slow germinators (Forbis et al., 2002; Friis et al., 2015). Seeds of the most basal angiosperms are dispersed with copious nutritive tissue surrounding small embryos (Fogliani et al., 2010). The underdeveloped embryo in these "primitive" seeds typically has to complete development inside the seed prior to radicle emergence, hence making germination a slow process (Vandelook et al., 2007; Vandelook and Van Assche, 2008). Evolution towards mature seeds with little or no nutritive tissue only enabled seeds to germinate faster (Vivrette, 1995; Vandelook et al., 2012a). As such, seeds with completely developed embryos at dispersal are able to germinate faster when they "sense" appropriate environmental conditions for seedling establishment.

Germination speed, mediated by seed morphology, is expected to be adapted to habitat conditions, life-cycle strategy and local climate. Vandelook et al. (2012a) found that Apiaceae with high relative embryo length and faster germination were typically short-lived and growing in open habitats in dry climates. Similarly, Vandelook et al. (2012b) found that angiosperms with large embryos and little nutritive tissue were overrepresented in dry calcareous grasslands compared with more mesic habitat. Faster germination may be especially advantageous for species that experience only short spells of suitable climatic or biotic conditions for germination and seedling establishment. This is the case for species growing in open and dry habitats (Vivrette, 1995), and for short-lived species with a persistent seed bank and only a small window of opportunity to germinate after a disturbance event (Grime et al., 1981; Vranckx and Vandelook, 2012).

Evolution towards faster germination has been driven to the extreme in very fast germinating seeds, i.e. seed germinating within one

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day (Parsons, 2012). The majority of species that germinate very quickly are members of the order Caryophyllales, belonging to Amaranthaceae s.l., Cactaceae and Tamaricaceae (Parsons et al., 2014). From the scarce literature available it is already clear that fast germination is mainly found in stressful, highly dynamic and pioneer habitats where it reduces chances of seedling mortality (Karrenberg et al., 2002; Gutterman, 2003). Of the 49 species listed by Parsons (2012) and Parsons et al. (2014) as very fast germinating species, 27 belong to Amaranthaceae s.l. [including Chenopodiaceae according to Stevens (2001)]. Several species, especially those of the subfamily Salsoloideae which all possess a spirally-coiled embryo, are able to germinate even within one hour, e.g. *Anabasis aretioides* (10 min), *Haloxylon stocksii* (25 min), *Salsola tragus* (29 min) and *Haloxylon salicornicum* (45 min) (Parsons, 2012 and ref. therein). In particular, the fast germinating seeds of species belonging to Salsoloideae and Suaedoideae are interpreted as cryptoviviparous-like due to a number of traits they share with truly viviparous species, such as little or no nutritive tissue, fully developed and chlorophyllous embryos and very fast germination (Liu et al., 2013). Several of the very fast germinating species are also known for a prominent seed dimorphism [heterospermy; e.g. *Chenopodium album* L., *Halogeton glomeratus* (M. Bieb.) Mey., *Suaeda acuminata* L and *Suaeda aralocaspica* (Bunge) Freitag & Schulze] (Baskin et al., 2014 and ref. therein). These species usually develop seed morphs that germinate rapidly and seed morphs that show a physiological dormancy and the ability to accumulate in a soil seed bank (e.g., Cao et al., 2012). Heterospermy is known from at least nine genera in Chenopodiaceae belonging to four different subfamilies (Imbert, 2002). Another trait surprisingly common among very fast germinating species, especially from Amaranthaceae s.l., is C_4 photosynthesis. Of the 27 species that show very fast germination, 22 are C_4 species.

From the occurrence of very fast seed germination in several phylogenetically distant groups of Amaranthaceae s.l. it is obvious that this character likely evolved several times in the family. Also, Amaranthaceae s.l. comprise a broad variety of life forms growing in hot, dry and saline environments as well as in coastal, humid temperate and tropical regions. Therefore, they are an ideal group to study the evolution and adaptive nature of fast seed germination. The main hypotheses we test here are the following: 1. Fast germination is an adaptation to stressful habitat conditions with short and/or irregular phases of favourable conditions (e.g. deserts and saline habitats). 2. Fast germination is advantageous for short-lived species and pioneer species, which generally form a soil seed bank to reduce the impact of environmental variability (Venable and Brown, 1988). 3. Due to the presence of a very large number of C_4-plants in Amaranthaceae s.l. and the observation that C_4 lineages are concentrated in hot and dry climates and in saline habitats (Kadereit et al., 2012), we expect that greater germination speed has co-evolved with C_4 photosynthesis in this particular plant family. 4. The multiple origin of heterospermy might be tightly connected to fast seed germination as a bet-hedging trait to reduce the risk of fast seed germination of all seeds.

2. Material and methods

A total of 107 Amaranthaceae s.l. species were included in the analyses (Table S1), representing all major clades of the Chenopodiaceae and Amaranthaceae s.s. subfamilies, all habitat types, climatic regions and ecological niches in the family.

Seeds of Amaranthaceae species stored at the Millennium Seed Bank (MSB), Kew, were used in this study. Collections at the MSB are dried to equilibrium with 15% RH and 18 °C and then stored at −20 °C. Seeds were removed from cold storage (–20 °C) and allowed to equilibrate to room temperature for 24 h before containers were opened. Germination tests typically comprised two replicates of about 50 intact seeds; however, in some species where seeds were limited, this was reduced to 10 seeds, while in other species the number of seeds sown was increased to account for limited viability (Table S1). Seeds were sown on 1% agar in 9 cm Petri dishes and placed into temperature controlled incubators at 20 °C and 25 °C illuminated laterally by 30 W cool white light with a 12 h photoperiod. These two temperature conditions were chosen, because they are close to optimal for most species studied and to account for variation in optimum temperature between species from different climate regions. The number of germinated seeds were recorded and removed daily during the first week and twice weekly thereafter until no further germination occurred over a 4 week period. Seeds which had not germinated were dissected and their viability assessed. Empty or infested seeds or seeds without an embryo were excluded from the total number of seeds sown, as they would never have been able to germinate. Firm seeds with an intact embryo were considered dormant while soft or discoloured seeds were considered non-viable. Mean time to germination ($MTG = \Sigma n_i t_i / \Sigma n_i$, with t_i time from start of the experiment and n_i the number of germinated seeds in the i^{th} time) and final germination percentage were calculated for both temperature conditions.

For all species a data set was compiled for a number of continuous and categorical traits describing the species' ecological niche (Table S1). Seed mass data were obtained by weighing at least 50 air dried dispersal units (referred to as seeds). In the case of *Beta vulgaris*, the dispersal units usually contained several seeds, but it was impossible to separate them. Data on maximum plant height, C_3 or C_4 photosynthesis, saline habitat (yes/no), coastal habitat (yes/no), ruderal (yes/no) and adult longevity (annual, biennial, perennial) were retrieved from literature and local floras. Based on the geographical coordinates at the collection sites, all species were classified into one of four Köppen climate groups (A: tropical; B: dry; C: temperate and D: continental). The geographical coordinates were also used to derive data on the altitude of the collection site, and 19 climate variables through WorldClim version 1.3 (Hijmans et al., 2005), accessed through DIVA-GIS version 7.1.7 (Hijmans et al., 2001). These 19 climate variables were reduced to two non-correlated climate variables (MDS1 and MDS2) using non-metric multidimensional scaling (NMDS) in the "vegan" Package (Oksanen et al., 2016) in R version 3.3.1 (R Development Core Team, 2016). MDS1 correlated mainly with temperature and precipitation variables, with low values for MDS1 indicating hot and dry climate conditions (Table S2). A strong negative correlation was observed between MDS2 and temperature seasonality and temperature annual range, suggesting that low values for MDS2 coincide with little variation in temperature throughout the year and high minimum temperatures (i.e. tropical climates).

2.1. Phylogenetic tree

The phylogenetic tree was constructed with DNA extracted from silica gel dried seedlings or young plants from the germination trials (Table S1) using the DNeasy Plant Mini Kit (QIAGEN, Germany). DNA quality was excellent for most samples and PCRs for four variable markers (*atpB-rbcL* spacer, *matK-trnK* intron, *ndhF-rpL*32 spacer and *rps*16 intron) of the chloroplast genome were carried out in T-Professional or T-Gradient Thermocycler (Biometra, Germany; (Table S3)). After PCR product purification using the NucleoSpin® Gel or PCR clean-up-Kit (Macherey-Nagel, Germany) a sequencing reaction using the Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the respective PCR primers was carried out followed by product purification using IllustraTM SephadexTM G-50 Fine DNA Grade (GE Healthcare, UK). Sequencing followed the Sanger method and was performed on a 3130xI Genetic Analyzer (Applied Biosystems Inc., USA). Forward and reverse chromatograms were checked in Sequencher 4.1.4 (Gene Codes Corporation, USA) and clean sequences were combined to a consensus sequence. In total we generated 411 new sequences which are deposited in Genbank (compare Table S1 for accession numbers KY884333–KY884636, and for missing data in a few cases). The alignment was first done automatically using MAFFT vers. 7 (Katoh and Standley, 2013) and then manually checked and corrected