

GLOBAL SYNTHESIS OF ALPINE GERMINATION – COMMENTS TO PRELIMINARY REPORT

Eduardo Fernández Pascual: Thanks to all who contributed to this discussion. I now close this document. At this point, the database is closed, following the improvements discussed below. We will now do the final analyses. Once they are done, I will divide the writing in several specific sections, so we can divide the work and write in groups.

DATASET

Data quality check

Eduardo Fernández Pascual: I also include a list with the species and the classification we have made of them as generalist or strict alpine (depending on the regional elevation of the treeline, and the species elevation ranges). Please check the species for your region and give me your input.

Angelino Carta: I do not find the file

Veronica Briceño: I can't access the link below. I get lost in the discussion of generalist vs alpine over the emails. Can you explain the classification please Eduardo?.

Eduardo Fernández Pascual: I have uploaded the list to Google Docs so everyone can see and edit it:

<https://drive.google.com/file/d/1lmlb4BCrHjaTyWWU68HI7ptByfmLTYtH/view?usp=sharing>

Borja: We just finished with the assessment of 3333 species living in European alpine vegetation (checked by regional experts) following the approach used in GLORA, so we can use it to verify the classification. By the way, I would use another term: specialist, alpine...

Eduardo Fernández Pascual: We have revised the list of species, we will use a categorical trait (strict or generalist) which is based on a first automated classification using elevations (from Floras) and a double-check by experts.

Light requirements

Adrienne Nicotra If we want light requirement data we have that for the Hoyle et al Frontiers dataset for the Australian alpine species.

Angelino Carta: this is of course an important factor, it is a shame we do not have so many data on this; my analyses on the alpine species stored in ENSCOBASE show that there is a significant positive effect of light on germination, but this effect was particularly evident in the Monocots clade only, while Ranunculales, Rosids and Asterids do not show this effect. Altogether, I believe that my results are not so strong because also in ENSCOBASE there are not so many tests in darkness for the Alpine species, but at the same time I will not be surprised if using a better dataset it will be revealed that Alpine species have a peculiar light response compared with traditional temperate species (or lowland as Arne are calling them below). See also my comment about constant temperatures below.

Eduardo Fernández Pascual: When revising the database I have found that some of the Chinese tests had light information. **I now CLOSE the germination database, I will not be adding any more germination data to it.**

Geographical coverage

Lydia Guja: Does everyone feel comfortable that this is a good representation of global alpine flora? Are there really critical gaps where we should put some effort into finding contributors (e.g. I know you've tried for the USA)? If we can confidently say that alpine germination research has not been done in key regions then that could form part of the future directions or recommendations discussed in the paper.

Eduardo Fernández Pascual: I don't think it is, there are at least several instances I know of which are missing: old articles with alpine plants of the Rockies, alpine species from Japan (Hokkaido and Mt Fuji), all the work of the Erschbamer group in Austria, who was not interested in joining (but this is less important because the true Alps are well covered). The massive paramo ecosystem of the tropical Andes is missing, and there is also the question of all the articles done with Arctic tundra species, which we could debate if they fall within our scope. **But this is not a methodical list of everything which is missing**, to have that, we should do a systematic search of the literature for alpine germination. This search might be part of the manuscript, but I am not myself able to dedicate the time it would require doing it. If there is any volunteer to do that, I would be happy to discuss the details with him.

Susanna Venn - My PhD student is currently working on a systematic literature review of just that - about alpine seeds and seedlings in relation to drought.

Arne: Let me suggest to define "alpine" somewhere as "temperate mountains above treeline" where high constant temperatures (and as you show here nicely!) are an effective signal to detect low risk of late frost; in tropical (without thermal seasons) mountains this might be completely different, and I would expect lower germination temperatures compared to this data-set and no need of cold (but may be dry) stratification, also floristically very different groups are involved so this might make sense

Veronica Briceño: Was the scope of the paper to have a good representation of the germination of global alpine flora?. If so then trying to pursue data from places we have gaps seems like a good idea. I know there is information on seed germination of Páramo species (mostly in Spanish and mostly in small local journals). There is actually a seed Bank

in Bogota that has that information too. Happy to help with finding this data. What Arne mentioned is interesting, comparing seed germination of tropical mountains and temperate ones is an interesting question but perhaps out of the scope of this ms. The idea of focusing on temperate mountains is a good alternative too.

Sergey: I think it is fine to 'skip' other regions for now. After all, this is the very first attempt to describe germination patterns/strategies in 'alpine' species. In a couple of years, when more data are generated, we could re-do the analysis with more data from the missing regions. Moreover, lack of data from some corners of the world is an important results itself - we could briefly discuss the future directions of research on this topic (missing regions, taxa, traits).

Veronica Briceño: In our case it seems that there is information on germination in other areas (Rockies, Paramo) but it's harder to access

Angelino Carta: I also think we can "skip" this issue for now, but a stronger (and shorter) justification should be raised.

Susanna Venn Back to the issue of data from the USA - Tara Forbis worked on alpine seed years ago at Niwot Ridge (but I can't find her now on Google), and there's got to be something from the Baskin's??

Forbis, T. A. (2003). Seedling demography in an alpine ecosystem. *American Journal of Botany*, 90(8), 1197-1206.

Forbis, T. A., & Doak, D. F. (2004). Seedling establishment and life history trade-offs in alpine plants. *American Journal of Botany*, 91(7), 1147-1153.

Borja: I also think we should rely on the data we have. I have been exploring this since the trait workshop in Perth, and Julie Larson was searching for data in the USA for a while. Unfortunately, she concluded that there is nothing available about germination data. Of course there should be something somewhere, but I'm afraid we are not able to collect in the short/middle term. Regarding the Paramo, I am in contact with Gwendoline Peyre from the University of Los Andes (Bogotá) and we have a plan for germination experiments using one biological station she has just established there. But it will need a few years to have data :(

Veronica: There is a Seed Bank in Bogota (only one in Colombia) that might be willing to share some data if we want to compare temperate and tropical seed germination strategies. I have a friend who works there, if people want this data I can ask.

Annisa: I met Shyam Partyal in Bonn last autumn, he indicated that he has some data for Eastern Himalaya sp. Maybe worth pursuing?

Lydia Guja: I support Arne's suggestion to define as temperate alpine. I think this is a useful definition as it can inform our hypotheses

Eduardo Fernández Pascual: We have been looking for alpine germination contributors for several years now. Some of the people discussed here was contacted but did not provide data. **I now CLOSE the germination database, I will not be adding any more germination data to it.**

Kew and EnscoBASE data

Lydia Guja: Is it really true that the ENSCOBASE data don't have collection location of the accessions used!? I thought Kew's data always contained this information.

Eduardo Fernández Pascual: Let me clarify, there are two separate databases which I considered but excluded: (A) The Kew germination database keeps records of germination tests at the Millennium Seed Bank. I believe those records do have precise geographical information, but although I asked for it, it wasn't provided. However, the utility of this database is low, because most of the records are not comparable, i.e. they have information **on only one set of experimental conditions**. (B) The ENSCOBASE keeps records of germination tests in seed banks belonging to ENSCONET (European Native Seed Conservation Network). In this case, the only geographical information available (Angelino correct me if I am wrong) is the country of origin. Overall, I believe it is better to stick to a highly curated dataset (where we know precisely the alpine character of the seed lots because we collected them) rather than adding noise with datasets about which we know less.

Angelino Carta: in general I agree with Eduardo, unfortunately only the country of collection is indicated in ENSCOBASE, so it would be good to remove most of these data; however, also the seed bank collector and accession number is reported. Hence, for a given species we can reasonably say that it was collected in alpine environments, this is particularly true for those species that we know to live only in alpine or mountains while of course for e.g. *Anthoxanthum odoratum* and many other species I fully agree we should remove them. In conclusion, we should focus on the accession not on the species and discuss whether some data can be included or not.

Borja: I agree with Eduardo and Angelino

Lydia Guja: Thanks for answering my question. Agree to stick with highly curated dataset.

Eduardo Fernández Pascual: We will be using the additional alpine species from ENSCOBASE, their character as alpine has been double checked by Angelino and me.

Field data

Lydia Guja: Might need to explain in more detail why the field results from China were excluded. Is it because all other data were lab based and the field data didn't have all the data required e.g. germination temperature?

Eduardo Fernández Pascual: I excluded that dataset because it didn't have required data, specifically, experimental germination conditions. It did have temperature values in the field, but that is uncontrolled, and we cannot compare it with the laboratory-set temperatures used in the rest of the datasets.

Susanna Venn: Do you think there would be sufficient field data from other regions to add to the Chinese data? This would be really useful for modelling and predicting future germination/regeneration opportunities for alpine plants in the field under natural

conditions I have some snowgum (*Eucalyptus pauciflora*) data from Australia - its the alpine treeline tree species - so perhaps not a suitable growth form for this data set

Andrea Mondoni: I do not think there are many data on field germination experiments in alpine environments. This is mostly due to the fact it requires a lot of efforts in terms of both time and funds, i.e. to reach the remote places, set up and follow the experiments. So, perhaps it's a pity to discard field data from China, as they may add further value to our lab conclusions; is there a way to consider them separately? I can provide only few data (10-15 species) on field studies from the Alps.

Borja: Agree with Andrea

Veronica: The Chinese species are a good portion of the data, It might be a good idea to consider this data separately given that field data is rare, and it is perhaps more realistic than our lab data? can we compare field versus lab data?. If the Chinese data includes temperature then we can extract the temp at peak germination or we can see if species need cold stratification or not (e.g if most of them germinate after winter). I just put those ideas there because field data is always good.

Sergey Rosbakh: There is a PhD project on field seed germination at the University of Innsbruck, Austria. As far as I know, the data are not published yet and they might not be willing to share them.

Lydia Guja: Agree with excluding field results and I understand the justification but thought we might need to mention it in methods since the dataset is mentioned but then not used. Maybe just don't mention it at all. There is still lots of lab data for Chinese species. Agree with Andrea and others that a separate consideration of field dataset, even if it is small, might be a useful addition to support/extend beyond lab data.

Sergey : Although field seed germination data would be a valuable addition, I am afraid, it made the paper even more complex, as field and lab data are not really comparable. I would leave it for another paper.

Eduardo Fernández Pascual: The field data is very different from the lab data, and we have it only for very few places. I agree with Sergey. **Field data will not be considered in this manuscript.**

Seed dormancy

Sergey Rosbakh: Dormancy. I think we should add dormancy to the analysis. It would be interesting to see whether 1) alpine species have dormant seeds indeed (most likely, PD) and whether 2) dormancy pattern is different between strict alpine species and generalists. I believe that each contributor "knows" her/his species better and could also provide these data to you. Plus, we could use literature to infer this information.

Eduardo Fernández Pascual: We do have the Baskins dormancy types dataset provided by Willis, and it is true that we could complete it for the rest of the species. However, I have

several arguments not to do it: (1) I believe the proportion of non-dormant species will be so low as to make the analysis undoable; (2) I do not trust an expert-based system to assign the ND type: it will depend too much on the person's opinion on the eternal debate about "what is dormancy" and on the specific set of experimental conditions he has employed; this limitation could dissipate somewhat if we were managing huge numbers of species but it is not the case; and (3) I think the response to cold stratification (for which we have proportion data) is a better proxy for that than a categorical trait.

Arne: I find the approach of the useful stratification type excellent! This transforms the dormancy classification system (which to me as such has no functional meaning), and gives the focus back to what actual adaptation is characterizing alpine plants; would be interesting if underdeveloped embryos (MD) and permeable seed coats (PY) are also higher in alpine plants? this also sheds the question on how to compare, most evidently I also would be interested in having a lowland comparison for related taxa

Sergey: The main reason why I brought dormancy up is that our study contains a very few findings so far, which, in my opinion, are 1) not entirely new and 2) not 'big' enough to be a part of a global synthesis.

I agree with both of you data dormancy kind after Baskin and Baskin is not a very informative trait (even more some believe that this is not a real trait) and has known disadvantages. Nevertheless, information on dormancy is still an important part of seed germination strategy in any habitat. It has been said that a typical alpine species has dormant seeds, but, what I can see (at least my study region) that there are different types of dormancy even among alpine-nival species, due to extremely high environmental heterogeneity in alpine habitats, including those, where species should have non-dormant seeds (e.g. ruderals on rocky screes). It would be nice to formally test it.

What is, however, even more interesting to see, whether certain types of dormancy show some regional differences. For example, in the Northern Alps, there are just a very few species with PY (usually Fabaceae), but their proportion should be higher in mountains with Mediterranean climates. Next, are there any species with MPD dormancy in alpine environments? I would say no, as the seeds ripe and disperse at the end of the growing season and have no time for embryo development (compare to spring geophytes in forests). Again, a very simple thesis, which could be tested formally.

I am sceptical about the stratification times included in the data set. I guess not many of us have tested the stratification times in a full-factorial design (at least I did not), thus it will be almost impossible to find out after how many days/weeks seeds dormancy is broken.

Eduardo Fernández Pascual: The stratification times are disparate but I believe we can simplify the trait to stratification yes/no for comparison.

Angelino Carta: we should indeed focus on something functional and easily interpretable, in my opinion the dormancy classification do not meet these criteria, in addition it can be strongly skewed for some categories leading to strongly biased results especially because for any analyses these are treated as categorical factors. However, it should be indeed noted that PY and MD are two important features that need to be explored perhaps separately as footnotes of the overall analysis especially if the latter is based on stratification type.

Veronica: i agree with Angelino, the classification is hard to use

Eduardo Fernández Pascual: I agree that it would be very interesting to test the prevalence of MD and PY. **Can we all complete these categories for our species, if they are not in the Baskin/Willis list?**

Sergey: First of all, some data on our species can be found here: https://www.researchgate.net/publication/340382910_Nikolaeva_et_al's_reference_book_on_seed_dormancy_and_germination. I wrote this paper together with the Baskins, so the data are more or less compatible. Second, PY has been shown to be limited to 6 families only, thus, this kind of dormancy has a very clear phylogenetic nature. The M(P)D can be estimated by presence of undeveloped embryo at the time of dispersal and thus can be based on seed anatomy data.

Filip Vandeloock: Instead of assigning MD or PD it would be better to assign a value for the amount of extra-embryonic reserve tissue or embryo size. MD (and I'm not even convinced it exists) also implies that the embryo grows, which cannot be derived purely from anatomy. Also a small embryo may also have other reasons for existing, for example there is a theory that the embryo is smaller when the period for seed maturation is shorter, which may be the case in alpine habitats. Or if predation is less in alpine regions, perhaps the embryo is larger. Looking at the species list, it seems to me there is a considerable number of species with small embryos (ranunculaceae, apiaceae, amaryllidaceae...). I'm currently compiling a list of embryo sizes that maybe be useful to assign classes.

Andrea Mondoni: I know that dormancy presence/absence is not an exciting novel output but it is part of the knowledge base of germination studies, so it should be included. The cold-stratification approach sounds perfect to me to detect PD, as well as anatomy data for the other dormancy types.

Borja: Nice discussion... I support getting the dormancy data, even if I share the concerns about their limitations. But we can use the results of the patterns (or no-patterns) on dormancy to discuss the same point you are discussing here, i.e. whether the data about dormancy is meaningful or not, but testing it with real data. Personally, my impression is that dormancy might not be a distinct trait of alpine versus non-alpine species, because other (temperate) montane species are also physiologically dormant. This could be a message from the preliminary results on cold stratification (and maybe alternating temperatures).

Annisa: This is quite interesting. If alpine and non alpine (temperate) species share (M)PD feature, what (if any) makes their germination traits different? Can we see adaptation to more variable climate variable in alpine seeds (asynchronous germination) compared to non-alpine? Does the minT required for germination is indeed higher in alpine sp?

Eduardo Fernández Pascual: We have incorporated the available information on seed dormancy from the Baskin book and the Nikolaeva book translated by Sergey.

Seed quality

Sergey Rosbakh: Seed quality. There is an opinion that seed production is impaired by the harsh climate at high elevations. If you have data on seed filling rates, that is the number of viable seeds/total number of seeds, then we could see whether alpine species produce low-quality seeds.

Eduardo Fernández Pascual: Unfortunately, we don't have this data recorded for the whole dataset, but can I try to assess this question with the subset that does have it recorded.

Arne: maximum final germination percentage or percentage of viable seeds if recorded might be a proxy here

Angelino Carta: maximum final germination percentage can be a rough proxy; percentage of viable seeds would be ideal but of course the problem is "if it was recorded"

Andrea: hope germination test here have all recorded the number of empty/dead seeds at the end of them, otherwise their final percentage might be misleading (e.g. if based on no. of seed sown, but when many of them were dead).

Annisa: Arne is right, the data I contributed includes fill (viable seed) as well as final germination taken into account viability

Eduardo Fernández Pascual: I have kept the filled seeds info, when available.

Functional traits

Sergey Rosbakh: Seed traits. What about other seed traits, like seed weight, shape, number, endosperm presence/absence, etc.? Can't the contributors provide such data? I have it for my species. I am analyzing now data on soil seed bank persistence along elevational gradient and found out that species with small, round-shaped seeds without endosperm build up soil seed banks in alpine soils.

Lydia Guja: Is it feasible to add data on some (likely) functional seed traits to see if they correspond to some of the patterns?

Eduardo Fernández Pascual: The problem to do this (and do a "seed ecological spectrum" type of analysis) is that the dataset is asymmetrical: not all species were tested in the same conditions, far from it. So, we do not have a large enough dataset with complete sets of cases for several traits (for instance, we may have response to temperature for species A, response to alternating for species B, and cold stratification for species C). One possibility to address this would be to go for the experimental combination that is more common (germination at 15 alternating with fresh seeds) and compare that germination proportion with other plant traits. But does the germination proportion at one specific condition have any ecological functionality to interpret? At least I don't think so. This would also depend on the research question and hypotheses that we want to address with the manuscript (see below), i.e. what research question would we be addressing by adding other plant traits?

Arne: an easy thing is to add seed mass, because it is known for so many species and easy to access via kew, and, there are straightforward hypotheses how it might relate to altitude: lower seed size in general in alpine compared to lowland species, since disturbances and light availability are higher in alpine environments, but according to the Stöcklin work (citation needed) this did not work in within species comparisons, but you have a wonderful data set so you might revisit their hypotheses here; also seed size is related to higher germination temperature at a global scale (Arene et al. SSR) but exactly this relation might be inversed in temperature high mountain (“alpine” not “andine”) gradients due to the specific role temperature plays as a cue here; a similar argumentation holds for fluctuating temperatures: in alpine environments open soils and bare ground are frequent as are fluctuating temperatures, but the highest fitness might be on stable soils and moister soils with comparatively lower temperature fluctuations does this make sense?

Sergey: Once again, we need to extend the paper to make it a real synthesis. Seed mass and endosperm presence/absence are very easy to obtain.

Another two traits, which can be easily calculated, are seed germination speed and synchrony. We know from the analysis optimal temperatures for germination in both groups of species. If the contributors can provide germination data with counts at different periods of times (daily, weekly basis) for the ‘optimal’ temperatures, I can do it within a day or two.

Angelino Carta: seed mass should be considered, however we also know how much it can vary within the same species, within the same population, between years ecc. Any values we will use here would be a very approximate value, especially for the non-strict alpine species because we likely find seed mass values obtained from populations of the non alpine environment.

Eduardo Fernández Pascual: I agree with incorporating seed mass and endosperm presence, **but I will need help collecting these traits.**

Angelino Carta: I will try to get the seed mass data; another trait we can consider is the life form: we may focus on the differences between geophytes, hemicryptophytes and chamaephytes but we need to define specific hypotheses.

Susanna Venn Seed mass trait data is probably the most important trait to have in the dataset, even if there are gaps in the data, because this trait can be combined with other plant traits for many subsequent analyses relating to plant growth, seedling success, dispersal etc etc

Borja: I agree we should include seed mass

Annisa: As Angelino said, because seed mass can vary substantially across collections/seed lots, it would be more informative to use the seed mass of the corresponding seed lots that we use in the experiment to produce germination data (if present) instead of using any seed mass data from the database. We can rely on the database for endosperm presence/absence as it is a stable trait.

Veronica: I agree with everyone, we should include seed mass and use data for the particular batch of seeds used in our experiments instead of Kew data to start with. It will be easy to include endospermic and non endospermic class too

Lydia Guja: Some great discussion here. Could we all agree on the method to use for mass data before we continue? That is, will we use measurements from the accession germinated, and if they don't exist then use data for the same species but derived from a different accession? I suspect we will need to do this to reduce gaps in the data set. also, if we have >1 value for a species should we average? I just wanted to check these things so we can be consistent across the combined data.

Eduardo Fernández Pascual: We will use the **seed mass** data from the SID. When not available for the species, we will use genus averages. Sergey is calculating the **timing traits** from the available information. We will also use the **endosperm** trait provided by Filip and the **life forms** that are being recorded. **This closes the inclusion of new traits.**

HYPOTHESIS & STATISTICAL ANALYSIS

Hypotheses, main research questions

Lydia Guja: We should write out some hypotheses sooner than later. Even if they are really general. What did we expect to find when combining datasets and looking for global trends (and why)? E.g. responses to cold strat likely. What else?

Lydia Guja: Do we conclude alpine species around the globe have common germination requirements/behaviours? Should we also try to investigate some of the seed/plant traits associated with this?

Eduardo Fernández Pascual: **This is a central topic.** I believe the main research question to address with the dataset is the one of the “alpine germination syndrome”. The hypothesis would be that the alpine environment filters a specific set of germination characteristics. We could test two predictions derived from that: that (a) the generalists and the strict alpinists will have different germination niches; and (b) the germination niche is homogeneous across regions. In terms of statistical tests, it would mean a significant interaction between strict/generalist and the experimental treatments, and a non-significant interaction between the region and the experimental treatments. Please provide your opinions on this.

Veronica: I like the idea of looking for the “alpine germination syndrome/strategy”. Hoyle et al 2015 (Seed germination strategies: An evolutionary trajectory independent of vegetative functional traits) shows that there are several germination strategies for her Australian species and I'm finding the same with my data in Chile, however most of the species germinate after spring like conditions. Is this an universal pattern?, it will be nice to test that...

Annisa: Re specialist vs generalist, I think we need to be aware that some alpine sp is known to shift the rear edge c 480 m downhill within several decades (e.g. *Globularia nudicaulis*) (see Rumpf et al 2019). Further, several species (e.g. *Carex mucronata*, *Cerastium carianthiacum*) are suggested to expand their elevation range and could likely “become generalist”? Maybe irrelevant for this global alpine study, but worth to note I think.

Veronica: I agree with Annisa. Also, is elevation something we will consider to see how the patterns we are finding vary with elevation?

Borja: fully agree with Eduardon on this, but I am biased, we have discussed about it several times in recent times...

Arne: First I am tempted to ask the alpine-lowland question but this is not the focus here but will allways show up in some way; several hypotheses on how alpine species behave to lowland or generalists are out there, e.g. (1) alpine species should germinate at higher temperatures compared to lowland/generalists (this is well known so may be not the main emphasis of the present work?) I did not realise earlier the possible functional relationship between fluctuating temperatures (which are extremely fluctuating in alpine environments) and germination in alpine species: correct me but I think that this is really new! this would mean that constant temperatures indicate favorable conditions for germination in alpine environments, where in other environments often fluctuating temps are “better”, if these two are the only strong predictors than a two way graph with fluctuating temps on one axis and temperature on the other axis separating two clouds of points (alpine vs generalists) might be interesting and give your alpine germination syndrome;

Adrienne Nicotra: I think there are several suggestions here for interesting moderating factors to consider in the meta-analysis. Continent and MAT or mean min / max etc temps could be interesting, as well as the generalist/strict division. Dormancy type, embryo type, could also be good. Some of these are discrete and so pretty straight forward analytically. the continuous ones might be more challenging.

Sergey: I imagine this paper as a paper testing several suggestions on alpine plant germination. Seedling recruitment in the alpine environments is very risky, therefore alpine plants should have following adaptations:

1. Have dormant seeds as it prevents from germination in autumn. The main type of dormancy is PD with some regional differences.
2. Germinate better under constant temperatures as litter and established vegetation protect seedlings from radiation frost.
3. Have germination optimum under higher temperatures that correspond to temperatures in the middle of the vegetation period, when frost probability is low.
4. Germinate with comparatively slow speed to spread the risk of being killed by frost.
5. Germinate in asynchronous way to spread the risk of being killed by frost.

Annisa: I think testing synchrony of germination timing is quite novel re alpine species strategy in temporally fluctuating environment. For temporal spread of Australian alpine seed germination, see Hoyle et al 2015. A major portion of species (21/50) show synchronous spring germination, 17/50 species exhibit synchronous autumn germination and the remaining species stagger (asynchronous) germination overtime. The same level of substantial variation in germination timing is also known to occur within a species (among populations). Would be nice to demonstrate for the first time the universality of this pattern across alpine seeds globally.

Veronica: Can we actually answer the asynchronous question with lab data? or is it something we need to test with field data like the Chinese one?. Also, I like the questions that Sergey mentioned above, they will be a good start

Sergey:

Additional hypothesis not directly related to germination:

1. Seed quality is generally low in alpine environments, due to the negative effects on pollination, fertilization and seed/fruit maturation.
2. Seed mass - the old, good seed mass-seed number trade-off in the alpine environments.
3. Endosperm presence/absence could be used as a proxy for seed longevity (seeds without endosperm tend to live longer) - I would expect more species without endosperm in alpine environments.

Annisa:

- how to set the category for quality? are we going to compare seed fill alpine vs non-alpine? or set a standard < 50% fill is low quality? seed quality can vary much across populations but also across collection years but we could note that down the line. Sergey: For the beginning, we could compare seed filling rates in generalist vs. specialist. Then, we could take a look at variation in this trait among different mountain 'systems'.

- in our study, we did not find a clear association between seed longevity and endospermy across Australian alpine seeds Sergey: In a soil seed bank study we did in the Alps, there was a clear relationship between ability to build a persistent seed bank and endospermy.

Angelino Carta: my opinion is in line with Eduardo who proposed an elegant and broad overarching question. Then of course specific questions/hypotheses proposed by Arne and especially Sergey should be considered either to describe our specific aims but also to drive our analyses and discussion.

Sergey: Another thing we could try, once we have more data, is to look for functional types in alpine species as related to their regeneration by seed. The formal question here is whether there are different strategies in seed germination and it could be tested by applying clustering techniques (with or without phylogeny).

Annisa: Sergey, not sure what you meant. Functional type refers to annual (seed dependent) vs perennial (tend to do vegetative reproduction)?

Sergey: Annisa, I meant a kind of cluster analysis, to look for possible seed germination strategies in the alpine species.

Phylogeny

Sergey Rosbakh: Phylogeny. One possible (though not elegant) solution would be to make a number of assumptions, to make it possible to include phylogeny into analysis: - Use quantitative (binary) traits instead of responses (e.g. light requirement for germination: 1 – yes, 0 – no) - Assume that the binary trait measurements were made under similar conditions (like vegetative traits) - “Alpine” conditions are similar in all study mountain range. With such assumptions you could use phyloglms (<https://cran.r->

project.org/web/packages/phyloilm/index.html) to analyse the data. Of course, this is a very simple, even a primitive approach, but it would be a simple and easy solution.

Lydia Guja: Accounting for phylogeny - what if the models had genus or family as random (rather than species)?

Eduardo Fernández Pascual: Angelino Carta will deal with the phylogenetic part of the analysis once we settle for a definitive analysis approach.

Veronica: I'm trying to include phylogeny in my study and I'm using the MCMCglmm R package that allows you to include phylogeny as a random factor in your analysis.

Arne: I agree many useful phylogenies are out there you just need to clip in your species at genus or family level and some sophisticated phylogenetically controlled (pgls...) or explicit (OU, lambda) analysis would fit nicely here but I guess measuring phylogenetic signal is not the focus.

Sergey: Of course, this is not the main focus of this paper, but we should account for that to meet the model assumptions. With phylogeny included the results might differ strongly from the present ones.

Angelino Carta: measuring phylogenetic signal is indeed not the focus but a phylogenetic comparative approach could be adopted because not accounting for phylogeny tend to overestimate the importance of species traits and other predictors while violating the assumption of independence among data points. The phylogenetic comparative approach consider the shared evolutionary history as a random effect or covariate, this is only marginally the same as using family or genus as random effects because in latter cases the whole phylogenetic relatedness (especially branch lengths) will not be considered. The kind of random effect supported by the phylogeny here would be also helpful to partially cover the fact that we have unbalanced data: that is to say that the lack of data for a given species at a given condition is partially covered by the response of a related species.

In addition, we could also split our dataset and reanalyse it separately for each main APGIV clades (Monocots, Rosids, Asterids ecc.. or simply Monocots vs Eudicots if the data are not enough) looking at similar (convergence) or different responses/strategies within different lineages.

Borja: I think it is important to account for phylogenies, mostly to know at what extent the patterns we observe are (or not) driven by phylogenetic conservatism

INTERPRETATION OF RESULTS

Lower germination of strictly alpine species

Lydia Guja: The lower germination of strictly alpine species compared to generalists is a nice result

Arne: so this mixes two aspects: lower viability and lower final germination percentage higher level of "dormancy" = higher level of bet hedging against unpredictable years, can we desintricate this?

Angelino Carta: interesting and indeed difficult to disentangle, another difficulty is also the large interannual variability especially in such extreme environment, and the alternative ways used by the plants to set seeds apomixis, selfing....

Borja: The pattern is interesting. Probably we have to list alternative hypotheses for explaining this finding (dormancy is not that clear, if we attend to the results of cold stratification)

Annisa: did the figure reflect the final germination results adjusted to the seed fill? in a perfect world we should have the number of germinating seeds vs number of filled seed vs number of alive dormant seed

Alternating temperatures

Sergey Rosbakh: Alternating temperatures. This is a very interesting finding. It seems that the generalist need gaps for establishment, whereas strict alpine species prefer to germinate under protection of mother plant/in established vegetation (lower temperature fluctuations).

Veronica: Perhaps those alternating temperatures happen in the field when snow starts to melt. I have measured soil temperature in the field and when the soil (and seeds) are covered by the snow, the soil temp is very constant (around 0C), once the snow starts to melt, then you get huge variation in soil temp. I guess for seeds is very “dangerous” to germinate when the snow start to melt and there is a risk of freezing damage so perhaps constant temperatures are an indication of a “good timing to germinate” and a trigger for germination?

Lydia Guja: Constant v alternating seems like it might be a false positive? Data don't look that significant but then again not sure what kind of error bars we're seeing? Or is it a confidence interval of some kind?

Eduardo Fernández Pascual: The brackets are the 95% binomial confidence interval. They are huge because each bar is composed of dozens or hundreds of species, each one of which has its own germination proportion which can be from 0 to 1. It is true that it might be a type I error, does anyone have any suggestions about how to control for this?

Arne: I think this is the main result! there can be a functional sound interpretation (see my comments above), bare soil and openings are often available in alpine environments but moments and places where conditions are stable are rare and may be indicate the better places and times for establishment, this argumentation can be worked out better, Sergey gave a nice cue how to do so; I don't feel as if it would be a false positive

Sergey: First, we need to use box-plots instead of bar plots, to show the data distribution. Second, the estimates will be different when a) phylogeny and b) measurement errors are included. Third, it is a common pattern in large data sets including many species that difference is significant, even with very small effect sizes.

Angelino Carta: results of mean temperature and alternating temperature are in line with my results presented in Regensburg (and not yet published), especially interesting is the negative effect of alternating in the strict alpine, this is indeed one of the core result useful to describe the “sindrome”. I originally interpreted this as a tendency for some species to

germinate under the snow cover however considering the relatively warm optimum temperature, the snow cover is perhaps not fully right; at the same time however I have some doubt that the established vegetation (herbaceous and short) could adequately buffer daily alternating conditions, that is to say that I will expect constant temperature in the soil but not within herbaceous vegetation where in addition much light should penetrate triggering germination. Altogether the interpretation of the positive effect of constant temperature is not as straight as it appear, an alternative explanation could be that the seeds germinate immediately after the melt of snow and here the combination of waterlogged soils and established vegetation may be capable to buffer daily alternating temperatures...

Concerning figure 2,3,4 an option to generate the columns and still representing a binomial structure of the data (as Eduardo was doing) could be to merge data of all species together summing all germinated seeds and all sown seed per each germination condition, then using these data to get the proportion and the CI interval. Alternatively you can run the model and then predicting the values and their CI intervals. The option to use boxplots suggested by Sergey is however also important, of course not to show the results but to show what is the internal variability of the data (perhaps as Suppl.Material).

In general however, I need to understand better the structure of the data and the analyses done until now.

Eduardo Fernández Pascual: Regarding the need for constant temperatures, I like the potential explanations. We need to remember that the “alpine environment” is a mosaic of microniches, but still I find logic for a need for constant in different microsites. In the case of snow patches, constant + low temperatures is reasonable. In the case of exposed, windswept sites, cryoturbated sites, constant may also mean being deeper into crevices. In regards to that, see Arslan H., Kirmizi S., G€ulery€uz G., Sakar F. (2011) Germination requirements of *Androsace villosa* L.(Primulaceae). *Acta Biologica Cracoviensia Series Botanica*, 53, 32–36., where they interpret the need for darkness as a crevice-detecting mechanism in *Androsace villosa*.

Filip Vandeloos: Perhaps true alpine species have a more strict temperature range in which they germinate? They want to avoid germination at too low temperatures, which seems to be confirmed by figure 2. Therefore perhaps when testing in alternating temperatures, conditions for germination may be suboptimal part of the time. Would it be useful to have a look also to the lower limit or the mean of the alternating temperature. I agree with Angelino that you have to look at several potential explanations (and also non adaptive effects).

Andrea Mondoni: in my field experiments seeds of alpine plants germinate as soon as snow melts in spring (which may be July in nival environments...), when water availability is high, as well as temperature (relatively high, in this latter case). Therefore, Angelino’s speculations might be right! Do field data of temperature and water potential might help to support the results observed here? I know these are very site-specific...

Sergey: Correct me if I am wrong, Andrea, but there is a big difference between temperate and Mediterranean mts: former are not usually water limited, whereas latter are. Therefore, seeds should have different temperature requirements for germination (low in dry regions, high in wetter climates).

Borja: yes, there is a clear difference in water limitation between temperate and mediterranean mountains, but the relationship with temperature germination is not clear. The drought is influencing the middle of the season rather than the spring, and mediterranean “alpine” habitats may have long snow cover in winter. There is also a strong effect of topographical micro-niches in both systems. For the alpine species germinating under snow, alternate temperature is obviously not needed. For alpine species germinating after snowmelt, I don't see alternate temperatures as a critical factor because the soil temperature in alpine soils has a strong daily variation anyway (the cushion/facilitation effect seems a very specific event for few species/seeds and habitats, and the real buffering of temperature also may be variable). In any case, I agree it is necessary to complement the results with effect sizes to see the magnitude of the factors we are evaluating.

Lydia Guja: agree constant v alternating is interesting and important to include, my question was aimed at understanding the data and analysis in more detail. Sorry if it sounded like the result should be removed that was not my intention. And the great discussion here suggests it should indeed be included.

Cold stratification

Lydia Guja: Cold strat result is nice support for seed ecology applying around the world

Eduardo Fernández Pascual: Sorry Lydia can you elaborate? I don't understand your meaning.

Arne: this only makes sense in temperate mountains with thermal seasons, for this reason I also expect hard seed coats to be rarer; if ever there are tropical mountains in the dataset (doesn't seem so) they should be analysed separately

Borja: I think the main point here is that cold stratification increases germination of mountain species (at least from non-tropical regions - i.e. our data) but this is not exclusive of alpine species. Maybe the comment of Lydia was in this line.

Lydia Guja: I was thinking of the general seed ecology idea that environment/biogeography is related to seed regeneration patterns and can drive the cues seeds respond to, e.g. cold strat for temperate alpine. So that's a simple story we can tell. But then, there's Borhja's good comment about this not being exclusive to alpine and all the discussion relating to dormancy class that shows how it can be much more complicated and interesting. I wonder in structuring the paper whether we could reflect this e.g. with a structure that compares general biogeography/ecology type hypotheses versus the trait hypotheses to see which, or perhaps both, are related to the patterns we see.

PUBLICATION

Tables, figures and supplementary

Lydia Guja: Figure 1 will probably be useful for the publication too. It would be nice if it was possible to map the geographic range of alpine regions in a different colour e.g. dark grey, then have the circles for the datasets overlaid.

Eduardo Fernández Pascual: Yes, I will use the shapefile in Fig 1 of Testolin et al. 2020 (<https://onlinelibrary.wiley.com/doi/full/10.1111/ecog.05012>) which the authors provide as supplementary material.

Lydia Guja: Sources, some of the data are from published sources but were provided directly rather than extracted from the papers. Nonetheless it would be good to cite the publications in an extra column in Table 1

Eduardo Fernández Pascual: I agree, all published sourced will be cited in the manuscript.

Arne: I suggested to make a figure using optimal temperature and response to fluctuating temperatures to highlight the differences again, but just a suggestion,

Open access to the dataset

Eduardo Fernández Pascual: I believe it would be very useful to make a clean version of the dataset freely available in a data repository, but because the data belongs to each of you, every contributor should make a decision about their part of the data. **Please comment on this.**

Sergey: I am OK with that.

Andrea: Fine to me

Veronica: I have not published my data yet, so let me think about this for a bit please..

Borja: Agree

Eduardo Fernández Pascual: I propose that we include a version of the database with the datasets that are already published. We can keep this online, and update it after other datasets are published.

Manuscript timeline and submissions

Lydia Guja: Note below our new conference dates. Maybe we could aim to have this paper ready in time and someone present?

Eduardo Fernández Pascual: I agree with this proposal, especially because it would give as a timeline to finish the manuscript. **But it would require a commitment by the coauthors to provide feedback in a timely manner.** Right now, the schedule is this: finish receiving reports to the preliminary report, write and circulate first draft, receive records on the first draft, write second draft which hopefully will be almost publication-ready.

Arne: giving a sequence of several small deadlines works best for me, or assigning specific topics per person, if I can give more input would be fun!

Sergey: I can squeeze the paper into my schedule given that at least some of us will contribute to the writing process.

