**Title:** Transgenerational plasticity in an *Plantago patagonica*, an arid land annual plant and priority restoration species

**Introduction**

Ecosystems around the world are facing rapid and unpredictable climatic changes (IPCC 2021). Over the past 50 years, many regions have faced decreased precipitation, increased temperatures, and longer durations between precipitation events (Spinoni et al., 2019). Increased number and a higher severity of droughts is expected across many landscapes in the coming decade (Dai, 2011). These drought events, driven by climate change, pose a significant problem for plant populations and communities. In response to these changes, most organisms are expected to (1) migrate, (2) undergo adaptive genetic changes, and/or (3) exhibit phenotypic plasticity (Hoffmann & Sgrò, 2011). However, as sessile organisms it is unlikely that most plant populations will be able to keep pace with these climatic changes on an evolutionary scale and may not migrate fast enough to cope (Song et al., 2021). Phenotypic plasticity, however, allows plants to rapidly respond to fluctuations in environmental variation. Phenotypic plasticity is generally considered when expressed by individuals in response to their immediate environment, with the range of expression limited by evolution and genetic factors. However, there is increasing evidence that environmental conditions experienced by parent plants can influence the phenotype and degree of plasticity in offspring generations (Bonduriansky, 2021; Herman & Sultan, 2011; Uller, 2008). Termed “transgenerational plasticity (TGP)”, this mechanism may represent an additional mechanism by which species cope with a shifting climate.

This form on non-genetic inheritance may serve as a source of phenotypic variation with significant evolutionary consequences, particularly if it influences offspring fitness (Donelson et al., 2018; Herman & Sultan, 2011). In many cases, exposure to environmental stress across multiple generations can have a positive anticipatory effect, improving offspring performance under similar stressful conditions (Bonduriansky, 2021; J. Marshall & Uller, 2007; Yin et al., 2019). Adaptive TGP occurs when parental exposure to an environmental stressor enhances offspring fitness in response to the same environmental stressor (Bell & Hellmann, 2019; Colicchio & Herman, 2020; Donelson et al., 2018; Engqvist & Reinhold, 2016). Because adaptive TGP can induce beneficial phenotypic changes within just a single generation and affect many offspring, it may enhance population persistence in stressful environments that might otherwise reduce fitness (Herman & Sultan, 2011).

However, in the past two decades, it has become clear that the effects of TGP are diverse, not necessarily always adaptive, and complex (reviewed in Bonduriansky, 2021; Holeski et al., 2012; Mousseau & Fox, 1998; Uller, 2008; Yin et al., 2019). For example, when exposed to two generations of drought treatments, offspring of the perennial grass Secale sylvestre exhibited higher aboveground biomass and higher seed production compared to offspring of control (non-droughted) parental plants (Mojzes et al., 2021). This adaptive TGP effect is not always consistent, however. When the annual leguminous herb Lupinus angustifolius was exposed to two generations of drought, offspring from this treatment exhibited significantly reduced seed mass and lower reproductive biomass (Matesanz et al., 2022). While the number of studies investigating transgenerational plasticity in the past two decades has risen exponentially, no clear patterns have emerged as to the adaptivity of transgenerational effects, and several published meta-analyses have reached opposite conclusions on the perceived benefits of transgenerational plasticity (Sánchez-Tójar et al., 2020; Uller et al., 2013; Yin et al., 2019).

Transgenerational plasticity is more likely to arise within a population environmental fluctuations occur predictably across generations, these is little or no cost to a plastic response, and when environmental cues are reliable indicators of future conditions (Colicchio & Herman, 2020; Hoyle & Ezard, 2012; Räsänen & Kruuk, 2007; Reed et al., 2010; Uller, 2008). TGP is particularly favored when parental and offspring environments are correlated or fluctuate predictably over time, allowing parents to adjust offspring phenotypes in ways that can enhance both their own fitness and offspring fitness, making TGP adaptive (Burgess & Marshall, 2014; J. Marshall & Uller, 2007; Kuijper & Hoyle, 2015). However, TGP may also manifest in different offspring reactions depending on environmental predictability. When future conditions are uncertain—when environments fluctuate more unpredictably—parent plants may prioritize their own fitness over their offspring, or may produce more diverse offspring phenotypes to reduce fitness variability (bet hedging) (Fischer et al., 2011; J. Marshall & Uller, 2007). While TGP can enhance offspring fitness, its expression depends on the environmental and ecological context of a population or species’ home site.

Fewer studies have considered how environmental conditions influence the degree of transgenerational plasticity across populations of species (Groot et al., 2017; Lampei et al., 2017; Riginos et al., 2023; Wadgymar et al., 2018), and among genotypes within populations (Galloway, 2001; Holeski, 2007; Latzel et al., 2014). Despite the large number of studies examining the effects of TGP, less is known about how environmental conditions influence the degree of transgenerational plasticity across populations of species. Different factors, including local climate variables and patterns of environmental heterogeneity across years, may results in populations expressing transgenerational effects differently, or not at all. Experiments designed to include climatically distinct populations may offer valuable insight on the role of local climate regimes on the evolution of TGP between populations (Groot et al., 2017).

Because the effect of TGP on trait expression depends on the environmental and ecological context of a population’s home site, quantification of this variation is imperative to understanding parental effects on fitness and performance. This is one confounding factor that may account for obscure results in transgenerational studies as mentioned above—variance in parental effects on populations collected from environmentally distinct sites, or lack of variance in plants collected from the same genetic pool (Nicotra et al., 2010; Sultan, 1987). For example, Latzel et al. found strong evidence for parental effects in Arabidopsis thaliana in response to a wide variety of abiotic stressors, with the effects being strongly dependent on genotype and often acting in different directions and magnitudes depending on the genotype (Latzel et al., 2023). Wadgymar et al. found that in populations of Boechera stricta distributed across an elevational gradient, low elevation populations had greater transgenerational plasticity than within generational plasticity in germination success than higher-elevation populations (Wadgymar et al., 2018). Transgenerational and within generation plasticity likely mediate fitness and performance across genetically different populations. Understanding how climatically distinct populations influence parental effects between populations may offer valuable insights to understanding how plant populations might respond to a changing climate.

One key environmental factor influencing plant performance, fitness, and the potential for TGP is water availability, which strongly impacts plant performance, driving major plant species distributions across the world (Bartlett et al., 2012; Cornwell & Ackerly, 2009; Louthan et al., 2015). As climate change progresses, increasing aridity poses a significant concern, potentially shifting plant distributions or leading local extinction events. Aridity is associated with higher temperatures and drier conditions, both of which act together to increase physiological stress and affect plant growth and reproduction. One measure of this physiological stress is vapor pressure deficit (VPD), or the difference in the amount of moisture in the air vs. fully saturated air, combining the effect of precipitation and rainfall into one metric. With increasing VPD, transpiration increases, increasing the water demand needed to maintain turgor pressure and generally negatively impacting plant survival, growth, and reproduction. As the frequency and severity of droughts continues to rise (Dai, 2011), understanding how the multi-year drought patterns affect potential transgenerational plasticity in performance and fitness traits is imperative for predicting plant population response to climate change.

*Plantago patagonica* (woolly plantain) is a small statured, fully self-pollinating annual forb (due to parenthesis cleistogamy; Sharma et al., 1992) with a broad biogeographic extending across North America, into northern Mexico, and occurring in parts of South America. *P. patagonica* is characterized by a basal cluster of hairy, grass like leaves with dense spikes of white and green flowers. In North America, *P. patagonica* is a common winter and spring annual that often forms dense mats (SEINet Portal Network, 2024). *P. patagonica* is a fitting plant to test the occurrence of transgenerational effects due to its wide natural range and reliable inbreeding. Within the southwestern portion of North America, *P. patagonica* has been identified as a priority restoration species by the Bureau of Land Management’s Colorado Platea Native Plant Program due to its high likelihood for establishment in large scale projects (Wood et al., 2015), high germination probability (Gremer & Venable, 2014), its ability to seed bank (Haight et al., 2019), and its potential role in promoting perennial establishment (Barak et al., 2015).

This project proposes to investigate the environmental and evolutionary mechanisms driving transgenerational plasticity (TGP) in the annual plant *Plantago patagonica*, with a focus on drought-induced effects across multiple generations. The central objectives of the proposed research are: **(1) to evaluate whether water limitation induces TGP in performance and fitness-related traits in *P. patagonica*, (2) to determine whether the climate at the seed source site predicts the magnitude and direction of TGP responses, and (3) to assess whether such transgenerational responses are adaptive.** These objectives will contribute to a growing body of research investigating non-genetic inheritance as a rapid-response mechanism to environmental stress, which is critical to understanding plant population dynamics under accelerating climate change. This work will build on prior work indicating that *P. patagonica* exhibits substantial within-generation plasticity to drought and shows population-specific performance responses based on seed origin.

**Experimental Approach**

The research plan involves a two-generation, fully factorial greenhouse experiment. Seeds collected from 12 populations of *P. patagonica* across a climatic gradient in the southwestern U.S. were first grown under ambient and drought conditions in a common garden (F1 generation) (Klein and Mitchell 2023). Seeds from these plants will then be used in a fully factorial greenhouse experiment (F2 generation), where they will be again subjected to control or drought watering regimes. This will result in four treatment combinations: CC (control parent/control offspring), CD (control parent/drought offspring), DC (drought parent/control offspring), and DD (drought parent/drought offspring) (Figure 1) (n = 1980; 495 plants per treatment x 4 = 1980)

Parental plants will be grown under drought and well-watered watering regimes. To maximize differences between our two water treatments, plants in the control treatment will receive water equal to the 30-year mean spring (March-June) rainfall amounts for the wettest seed source location in our study (60ml/week). Beginning on day 14 of the experiment, plants in the dry treatment group will be watered at a rate of 50% of the 30-year mean spring rainfall amount for the driest location in our study (15ml/week) (PRISM Climate Group).

**(1) Evaluate whether water limitation induces TGP in performance and fitness-related traits in *P. patagonica*:** Leveraging the fully factorial experimental design, I will compare differences between the four groups (CC, DC, CD, DD) in phenotypic expression of functional and life history traits, and whether these expressions differ between populations. To investigate the transgenerational effects of drought in *P. patagonica*, I will quantify the F2 generation’s germination rates, growth rate, mortality, time to flowering, biomass allocation (above and belowground), SLA, LDMC, seed number, and seed weight in response to multigenerational dry conditions. I will use mixed effect models to assess TGP presence (trait ~ offspring treatment + parental treatment + offspring\*parental + (1|population).

**(2) Determine whether the climate at the seed source site predicts the magnitude and direction of TGP responses:** In addition to the fully factorial design, 12 populations will be included in the study. In addition to comparing the trait differences between the four groups listed above, I will ask if they are different based on climatic characteristics of seed source locations. Potential climate characteristics that could influence the presence or capacity of TGP in response to drought include growing season temperature, precipitation, VPD and variability (CV % of spring precipitation, temperature, VPD). I will used mixed effect models to assess the effects of seed source climate characteristics on TGP (trait ~ OT \* PT \* spring temp \* spring precip \* spring VPD \* variability of each variable) and select the best model using AIC and backward steps.

**(3) Assess whether such transgenerational responses are adaptive:** I will calculate the extent of plasticity for any traits that have a signal of TGP using a plasticity index (RDPI, rdpi function in the plasticity package (Valladares et al., 2006, Ameztegui 2017)). This metric calculates pairwise distances among individuals within each population whose parents were grown under different environments (here, between CC – DD). The average distance within a population provides an estimate of the degree of TGP, ranging from 0 (no plasticity) to 1 (high plasticity). I will then calculate Pearson correlations between these trait plasticity values, and three traits related to fitness (seed number, mortality proportion, and flowering proportion).

A map with red arrows and numbers

AI-generated content may be incorrect.Figure 1. a) Collection locations and b) growing season climate characteristics for each population of *Plantago patagonica*. Spring climate characteristics were averaged across 30 years (1989 – 2019, April – June).

**Trait Data Collection**

Shoot biomass, root biomass, total biomass, R:S ratio: Destructive measurements that will be collected at the end of the experiment (August 2023). Plants will be collected, roots will be washed, roots and shoots will be separated and dried in the oven at 75 C for three days. The weight will then be measured.

Max height and relative growth rate: Height will be measured in cm every 7 days.

SLA and LDMC: To categorize *P. patagonica* on the leaf economics spectrum, which characterizes a species capacity for stress tolerance vs. resource acquisition (Wright et al., 2004). On day 60 (roughly 3/4ths of the way through the experiment), On day 60, we will collect one to five mature, healthy leaves per individual to measure SLA and LDMC. Collected leaves will be stored in zip lock bags with one water-saturated paper towel sheet and placed overnight in cool, dark conditions (35° F). After 12-14 hours, leaves will be weighed with a microbalance (1-μg precision; Mettler Toledo) to obtain saturated weight. The leaves will then scanned using a LI-COR LI-3100C leaf scanner to quantify leaf area. Leaves will be dried for 48 hours in an oven at 60° C and then weighed again using the Mettler Toledo microbalance. Specific leaf area (SLA) is calculated as the area of fresh leaf divided by the oven dried mass. Leaf dry matter content is calculated as the leaf dry weight divided by the saturated leaf weight.

Mortality: Mortality will be assessed once a day for the entirety of the experiment.

Days to flower: The date of first flowering will be recorded. Plants will be checked daily.

Number of flowering structures: Number of reproductive structures will be recorded until the end of the experiment (August 2024, or when the plant senesces).

Seed number and seed weight: Seed heads will be collected, dried, and cleaned. Seeds will be weighed and counted per plant.

**Broader Impacts**

Diagram of a plant that is drought

AI-generated content may be incorrect.Understanding TGP in wild species informs predictions of how populations will respond to future climatic variability. This research contributes to conservation biology by identifying populations with traits that enhance resilience. Moreover, *P. patagonica* is a candidate for restoration in arid lands, and findings could inform seed sourcing decisions for practitioners and guidelines on seed grow out methods.

**Figure 2.** Experimental design for F1 and F2 generations.

**EXTRA PLANNING THINGS:**

**Timeline and Tasks**

April 2023:

* Rent greenhouse
  + Greenhouse key request, service form
* Purchase conetainers, trays, soil
* Organize seeds
* Sterilize conetainers
* Plan out greenhouse layout
* Make datasheets

May 2023:

* Start experiment
  + Fill conetainers with soil mix
  + Plant seeds
  + Set up greenhouse layout
  + 14 days of well watered treatment
  + May 25: start experiment

June 2023: Continue experiment; measure traits as specified above

July 2023: Continue experiment; measure traits as specified above

* July 30th: SLA + LDMC leaf sampling

August 2023:

* August 10th: Start destructive measurements (biomass)
* August 31st: Done with experiment in the greenhouse
  + Clean greenhouse
  + Sanitize trays and cones

September 2023:

* Seed drying, cleaning, weighing, counting
* Measure biomass after drying

Greenhouse layout:

**A screenshot of a computer screen

AI-generated content may be incorrect.**

**Greenhouse Methods**

1. Scarify seeds with 150-grit sandpaper, rubbing seed between two pieces in circular motion for 10 seconds (Christie et al. 2022).
2. Soak seeds in tap water for 12 hours.
3. Plant one seed per cone-tainer.
   1. Soil mixture is a 50:50 mix of generic potting soil and sterilized sand. No fertilizer, no pesticides.
   2. Depending on source, cone-tainers should be sterilized with a 10% bleach solution and tap water rinse.
   3. Cotton ball in bottom of cone-tainer to prevent soil loss?
   4. Fill cone-tainer with soil mixture, saturate with water, allow soil mixture to settle, and top off with soil mixture.
   5. Create ~0.5 m indent in each cone-tainer.
   6. Using tweezers, transfer one seed per cone-tainer and top with a layer of soil.
   7. Top water to moisten seed and soil.
   8. Label cones with appropriate info (hidden? If labeling is necessary?), enter into data sheet, randomize according to greenhouse layout above.
      1. Population # – P:D/C (parent:drought/control) – F1:D/C
4. Maintain greenhouse around 77-79° F with ambient light conditions (accounting for average increase in temperatures across the US Southwest region, Gonzalez et al. 2018).
5. Water each cone-tainer to saturation daily for 10 days to ensure germination.
6. On day 10, begin drought and control watering treatments.
   1. Control treatments will be the 30-year mean spring rainfall amount for wettest seed source location in the populations (60mm/week) (PRISM Climate Group).
   2. Drought treatments will be 25% of the 30-year mean spring rainfall amount for the driest source location in the populations (6mm/week) (PRISM Climate Group).
7. Water twice a week: 3mm to drought treatment, 30mm to control treatment (Tuesday/Friday).
8. Measure plant height at day 7, 14, and 21. After day 21, measure plant height every one week.
9. Measure mortality rate, presence of reproductive structures, and number of reproductive structures once a day.
10. On day 65, collect one leaf per individual to measure SLA.
11. A screenshot of a greenhouse table

    AI-generated content may be incorrect.On day 80, measure above-ground, below-ground and total plant biomass.

**Supply List**

* 150-grit sandpaper **(in box!)**
* Petri dishes (to soak seeds) **(get from lab)**
* Paper towels – grocery store
* 1,980 cone-tainers
* 30 cone-tainer trays (hopefully the ones with 72 or 98 holders)
* Generic potting soil
* Sterilized sand
* 10% bleach solution in spray bottle – grocery store
* Cotton balls? – grocery store
* Tweezers **(somewhere in lab)**
* Tap water spray bottle – grocery store
* Popsicle sticks for labeling? Or duct tape? – grocery store
* Sharpie
* Syringe x 2 (order this online? Maybe see what kind Zoë used)
* Ruler
* Ruler – grocery store
* Service agreement form
* Greenhouse key request

**Greenhouse rates example:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Roger Rd** | **GH #** | **Sq Ft** | **Months** | **Rate** | **Totals** |
| Farm | 2078-4 | 1,152.00 | 1.00 | $   0.48 | $552.96 |

**Greenhouse watering protocols**

Bring lots of water, tank tops/shorts, maybe some electrolytes!

Door code: 1474#

1. First, switch 6, 7, 8 can get switched from ‘auto’ to ‘on’ to force the fans on and get the air flowing.
2. Set up any podcast or any entertainment you need ☺
3. Hose will be on. Check the water temperature with your hand before you fill up the water cup thingy.
4. See schematic—the greenhouse side closer to the parking lot area is the drought side. Each plant gets **15 mL** of water.
5. All cones with plants—even if they look super dead!—get watered. If there is no plant in it don’t worry about watering it.
6. **Check the temp of the water each time you fill up the little watering thingy.** It gets hot in the hose super quick.
7. Be careful not to create a hole in the soil with the force of the water from the syringe. I usually go slow and water in a circle around the cone top. If you do make a hole, no worries, just push the dirt back with your finger.
8. The side of the greenhouse closer to the rest of the greenhouses is the control side. All of the plants on this side of the aisle gets **30 mL** of water. Be more careful of the holes on this side, since it’s more water it’s way easier to create the holes.
9. Once you’re finishing up, just set the syringe, bottle and hose aside.
10. Turn switch 6, 7, 8 and back to AUTO. Make sure it’s on auto, not off or on!!!
11. Leave! And have a nice day. The door will close after you in 30 seconds.