Intra-individual variation in *Galium odoratum* is partly genetically based and is affected by experimental drought and shading

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Running title: Intra-individual variation under experimental drought and shading

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Abstract

Background and aims

Climate-change induced warmer spring temperatures advance tree leaf-out and result in earlier shading of the forest floor. Climate change also leads to more frequent droughts. Forest understorey herbs may respond to these environmental changes by varying functional traits at different hierarchical levels of organisation. While trait variation at the intra-specific level is well-studied, little is known about how variation at the intra-individual level responds to environmental changes.

Methods

We sampled genets of the forest understorey herb *Galium odoratum* from 21 populations in three regions in Germany, varying in microclimatic conditions. The genets were transplanted into a common garden, where we applied shading and drought treatments. We measured plant height, leaf length and width, and calculated the coefficient of variation (CV) at different hierarchical levels: intra-population, intra-genet, intra-clone and intra-ramet, the latter two representing intra-individual variation.

Key results

Variance partitioning showed that intra-ramet CV explained most of the total variation, followed by intra-clone CV. We found significant variation in CV of plant height and leaf width among populations of origin, indicating that CV is at least partly genetically based. Mean soil temperature at population origins correlated negatively with CV in plant height, suggesting adaptation to local conditions. Furthermore, we observed that early shade led to increased intra-clone CV in leaf length and drought reduced intra-ramet CV in leaf width. Finally, intra-ramet mean leaf width and CV were independent under control conditions but became correlated under drought.

Conclusions

Our experimental results reveal genetically based patterns in CV and correlations with soil temperature, indicating that intra-individual variation can evolve and may be adaptive. Intra-individual variation responded plastically to drought and shading, suggesting functional changes to improve light capture and reduce evapotranspiration. In conclusion, intra-individual variation makes up the majority of total trait variation and can play a key role in plant adaptation to climatic change.

Keywords

Clonal plant, common-garden experiment, forest understorey herbs, *Galium odoratum*, genetic differentiation, intra-individual variation, intra-specific trait variation, microclimate, phenotypic plasticity, sub-individual variation

Introduction

Intra-specific trait variation, *i.e.,* the phenotypic variation among individuals of the same species, can have important ecological impacts from individual functioning to population dynamics and species distributions, and from species interactions (Westerband *et al.*, 2021) to co-evolutionary dynamics and community assembly (Violle *et al.*, 2012; Westerband *et al.*, 2021). Intra-specific trait variation is generated through genetic variation and phenotypic plasticity (Albert *et al.*, 2010; Des Roches *et al.*, 2018; Westerband *et al.*, 2021), and it has repeatedly been shown that intra-specific trait variation can be comparable in magnitude to inter-specific variation (Kinnison and Hairston, 2007; Siefert *et al.*, 2015; Govaert *et al.*, 2016).

In clonal plant species, one genet can produce several ramets (*i.e*., a potentially independent individual derived by vegetative production from a single parent plant) that can vary phenotypically, although arising from the same genotype (Sobral *et al.*, 2013). Such phenotypic variation among repeated homologous structures within the same individual is known as intra-individual variation (Herrera, 2009). In clonal plants, the intra-individual hierarchical level upwards from the ramet is the clone, followed by the intra-specific levels; genet and population (Fig. 1). Increasing evidence shows that intra-individual variation may have effects on the overall individual fitness and underlying functional mechanisms and may affect population-level variation (Herrera *et al.*, 2015; Herrera, 2017; March-Salas *et al.*, 2021). Due to the modular nature of plants, the majority of variation in homologous structures of plants (e.g., fruits, leaves and seeds) may in fact be explained at the intra-individual level (Herrera, 2017). For instance, Herrera et al. (2015) found that the intra-ramet level explained approximately 50% of both leaf length and leaf width, while the intra-specific level explained less than 40% for both traits, and the intra-clone level explained 5-10%. Therefore, if intra-individual variation is not considered, large fractions of functional variation and their effects on fitness could potentially be overlooked.

The part of phenotypic variation that is genetically based can be the result of mutation, genetic drift, gene flow, or natural selection, with only the latter causing adaptive evolution (Hedrick, 2009). Clonal plants can experience natural selection on traits at several levels – genet, clone and ramet – rendering measurements of natural selection and predictions of evolutionary responses considerably complex (Fischer and van Kleunen, 2001). Moreover, previous studies have shown that intra-individual variation can be subject to selection within each of these levels (Sobral *et al.*, 2013; Alonso *et al.*, 2018; March-Salas *et al.*, 2021). This is important because a genetic basis for intra-individual variation would be the first step to prove that intra-individual variation can have a functional role. However, the vast majority of studies on intra-individual variation has been conducted under natural conditions, impeding the assessment of the genetic basis of intra-individual variation. A commonly used and powerful approach to test for genetic inheritance of traits is to grow plants in a common garden (Turesson, 1922; De Frenne *et al.*, 2013; Scheepens *et al.*, 2013). Although this approach commonly focusses on mean trait values at the population level and is increasingly being used to study genetic differentiation in the strength of phenotypic plasticity to experimental treatments (West-Eberhard, 2003; Forsman, 2015), the common garden approach can also be used to measure genetic variation in intra-individual variation and, likewise, in the plastic responses of intra-individual variation to any applied treatments.

While genetic adaptation is important for population survival on the long run, plastic responses are crucial for plant survival to rapid changes in environmental conditions, including those provoked by climate change, although they have their limits. In Concord, North America, tree species have significantly advanced their leaf-out with nearly two weeks since the 1850s in response to increasing spring temperatures, whereas forest understorey herbs were much less responsive (Heberling *et al.*, 2019). This phenological mismatch between overstorey trees and understorey herbs is expected to increase with further warming (Chen *et al.*, 2019; Vitasse *et al.*, 2022), suggesting that plastic responses may not be sufficient over time and genetic adaptation in trait means and/or plasticity is needed. Additionally, droughts are becoming more frequent with global climate change (Dai, 2013; Büntgen *et al.*, 2021), also in temperate forests (Trenberth *et al.*, 2014; Millar and Stephenson, 2015; Dai *et al.*, 2018), adding yet another stressful condition for understorey plants to overcome. Plants have evolved different ways to cope with drought: they can either escape, avoid or tolerate drought stress (Fang and Xiong, 2015). In response to drought conditions, plants can accelerate their reproductive cycle (escape strategy) (Franks *et al.*, 2007), reduce their mean leaf size and increase specific leaf area to lower their transpiration rate (avoidance strategy) (Esau, 1960) or improve water use efficiency (tolerance strategy) (Marron *et al.*, 2003; Liu and Stützel, 2004; Wellstein *et al.*, 2017).

In the above examples, population responses may not only harbour changes in trait means but also in intra-individual variation, for instance in leaf morphology. Larger variation in leaf characteristics and architecture may allow plants to thrive under variable light and drought conditions. Indeed, capturing different light intensities may help plants to acclimate to seasonal variation in light conditions, as occurs in the deciduous forest understorey (Martinez and Fridley, 2018). Thus, functional variation within the same individual, such as physiological and morphological variation among leaves, may help plants to adapt to fine-grained environmental changes and ultimately favour population performance (Winn, 1996a). Nevertheless, experiments addressing the function of intra-individual level are to our knowledge absent.

Here we examine whether and how inter-individual and intra-individual variation is determined by genetic variation, correlates with microclimatic conditions, and responds to experimental environmental manipulations, *i.e.*, drought and shading treatments. We used the understory clonal herb *Galium odoratum* (L.) Scop. as study species to 1) compare variation within various hierarchical levels of organisation (population, genet, clone and ramet), measured in three functional vegetative traits: plant height, and leaf length and width; 2) to test whether intra-specific and intra-individual trait variation has a genetic basis; 3) to test if forest management and related micro-environmental factors at the site of origin may have affected genetic variation in individual trait variation; and lastly, 4) to investigate how experimentally induced drought and earlier shading drive plastic responses in individual trait variation and trait means. By answering these questions, this study provides insightful knowledge on the variation and evolutionary causes of intra-individual variation in the face of future climatic conditions.

Material and Methods

Study species and experimental system

Galium odoratum (L.) Scop. (Rubiaceae) is a perennial forest understorey herb, reaching a height of 10-30 centimetres. The leaves are lancet-shaped, widest in or just above the middle, and mostly appearing eight at a time arranged in whorls. *Galium odoratum* can reproduce sexually via seeds (outcrossing), but also heavily relies on vegetative spread through stolons (Frederiksen and Rasmussen, 2006).

We sampled plants from forest plots (100 m × 100 m) in the Biodiversity Exploratories (www.biodiversity-exploratories.de), a large-scale platform for ecological research in Germany (Fischer *et al.*, 2010). In May 2020, we sampled individuals of *G. odoratum* in three different regions across Germany: Schwäbische Alb (ALB) characterized by calcareous bedrock with an elevation up to 860 m, an annual mean temperature of 6-7 °C, and a mean annual precipitation of 700-1000 mm; Hainich-Dün (HAI) also consisting of calcareous bedrock, with a maximum elevation of 550 m, mean annual temperature of 6.5-8 °C, and a mean annual precipitation of 500-800 mm; and Schorfheide-Chorin (SCH) which is a young glacial landscape with a maximum elevation of 140 m, annual mean temperature of 8-8.5 °C, and mean annual precipitation of 500-600 mm (Fischer *et al.*, 2010). An index for silvicultural management intensity (SMI) has been developed to capture forest management intensity (Schall and Ammer, 2013, 2014). We chose nine plots along the SMI gradient from low to high management intensity in each region. In each plot, we sampled five individuals with a minimum inter-individual distance of 10 meters to ensure they were genetically different (hereafter referred to as genets). Each genet was separated into four ramets and planted into multitrays (51.5 cm width, 33.5 cm length, 5.5 cm deep, 54 cells per tray; potted with potting soil "CL T torffrei", Einheitserde, Sinntal-Altengronau, Germany) for establishment and growth until November 2020, when all individuals were transferred into 1.5 L pots with potting soil ("Typ T, Struktur 1B", Hawita, Vechta, Germany). In spring 2021, all pots were relocated to a foil tunnel allowing us to apply watering and shading treatments. Mortality in early spring caused reduced numbers before the start of the experiment (see Table S1 for final numbers).

The ability of *G. odoratum* to spread vegetatively allows us to study numerous hierarchical levels of organisation: Population/Genet/Clone/Ramet (Fig. 1). We define ramet as a single shoot, and thus a potentially independent plant unit (usually multiple in a pot), clone as all connected ramets (*i.e.*, in a single pot), and genet as all plants which are genetically identical (*i.e.*, individuals that were sampled from the populations and subsequently divided into four different clones, each planted in a single pot).

Shading and drought treatments

To simulate the shaded forest understorey environment, we applied shading cloth over the foil tunnel in two layers (45% shading for each layer resulting in 90% total shading). Each layer of shading cloth was applied one week apart. To simulate future tree leaf-out conditions, the first layer was applied on 12 Apr. 2021, approximately two weeks before the anticipated leaf out of beech and oak trees in the surrounding area. Control shading cloth was applied when the natural leaf out of surrounding trees were observed in the area (Frankfurt am Main, Germany) on 30 Apr. 2021, likewise applied in two stages, one week apart.

We applied the drought treatment at the flowering start of the first plant that flowered (7 May 2021). The drought treatment was applied as a single event, in which all watering was ceased until substantial wilting (50% of all pots) was observed. This drought treatment lasted for two weeks until watering of all pots was resumed. Control plants received water by irrigation from above *ad libitum* during the whole experiment. The shading and drought treatments were applied in a full-factorial design (Control, N = 30 pots; Early shading, N = 39; Drought, N = 37, Early shading + Drought, N = 29).

Measured traits and their variation

We measured plant height, leaf length and leaf width after all plants finished flowering (end of June). Plant height was measured to the nearest 0.5 cm for all ramets in each pot. Leaf length and leaf width were measured with callipers to the nearest 0.1 mm precision on one randomly chosen leaf per whorl on up to five randomly chosen ramets per pot.

The coefficient of variation (CV) – calculated as the standard deviation divided by the mean of a specific trait – was used to estimate variation in each trait at each hierarchical level of organisation: intra-population (CV_{IP}), intra-genet (CV_{IG}), intra-clone (CV_{IC}) and intra-ramet (CV_{IR}) (Fig. 1). The CV_{IR} was not calculated for plant height since one ramet has only a single height value and thus no CV can be calculated.

Data analyses

Firstly, to test which hierarchical level of organisation explains the most variation in our measured traits, we performed variance partitioning on the traits using the R package ‘cati’ and the function ‘partvar’ (Taudiere and Violle, 2016). Two different variance partitioning models were performed. The variance partitioning for plant height only included the nested structure of intra-population, intra-genet, and intra-clone. The variance partitioning for leaf length and leaf width included all hierarchical levels.

Secondly, we ran linear mixed-effect models (LMM) for CV_{IG}, $CV_{IC,}$ and CV_{IR} of each trait with treatments as fixed factor for intra-clone and intra-ramet level. We used the hierarchical levels of organisation as nested random factors, where the region and population level reflect genetic differentiation among populations, and the genet level is reflecting genetic differences among individuals. For intra-genet level we had no fixed factors and defined the intercept as +1. To test for the genetic basis of intra-individual variation, we performed Akaike information criterion (AIC) model selection. Each level of the nested random factor was removed one at a time, allowing us to compare the AIC of the various models. If the difference in the goodness of fit between two models exceeds an absolute value of 2 (Vrieze, 2012), we interpret the models as being significantly different and the hierarchical level of organization in the model with the lowest AIC value having importance for explaining CV in the trait and, since the data originate from plants grown under controlled conditions, is at least partly genetically based.

Thirdly, we tested for treatment effects on CV using LMMs with microclimatic variables and the SMI from the origin of each population as fixed effects, including shade, drought, and the interaction between the two treatments on the intra-clone and intra-ramet level. Hierarchical levels of organisation were used as random nested structure. LMMs were run using the function “lmer” from the package “lme4” (Bates *et al.*, 2007) and model test results were obtained by applying the function “Anova” from the package “car” (Fox *et al.*, 2012). To investigate significant differences between treatments with more than two levels or significant interactions, a post-hoc Tukey test was applied using the function ‘lsmeans’ from the package ‘lsmeans’ (Lenth, 2016).

Fourthly, in the same mixed-effects models, we tested for relationships between CV and environmental variables at the sites of origin. To avoid multicollinearity of environmental variables in the mixed-effects models, we applied a principal component analysis (PCA) from the package ‘factoextra’ on the following microclimate variables: mean annual soil temperature, mean annual air temperature, mean annual humidity, and mean annual precipitation. The PCA showed that PC1 explained 63.8% of the variation in microclimatic variables, and the primary variable (>36%) for PC1 was mean annual soil temperature (Figure S1). Therefore, besides SMI, mean annual soil temperature was included in the models.

Lastly, we investigated the relationship between trait means and CV to test whether CV varies independently from trait means. At population and genet level, we ran LMMs with the trait mean as the response variable, CV and quadratic CV of the trait as fixed effects, and nested hierarchical levels as random effect. At clone and ramet level, shade and drought treatments, along with all possible interactions with CV and quadratic CV of the traits included as fixed effects, with region, population, and genet as nested random effect for clone level, and the fully nested hierarchical levels of organisation (*i.e.*, region, population, genet, and clone) as nested random effect for ramet level.

All statistical analyses were conducted with R version 4.1.2 (R Core Team, 2021). Shapiro-Wilk and Bartlett tests were used to assess whether the assumptions of normality and homoscedasticity of model residuals were met, respectively. When at least one of these assumptions was violated, the response variable was transformed. Stepwise backward model selection was applied in all models (Pearce and Ferrier, 2000).

Results

We measured a total of 2,304 leaves, from a total of 517 ramets, arising from 135 clones, originating from 71 genets, sampled from 21 different populations across the three different regions (ALB, HAI, and SCH; Table S1). Height was measured on all 517 ramets.

Variance partitioning on hierarchical levels of organisation

Partitions of trait variation into hierarchically nested levels of organisation revealed that the variation within ramets (CV_{IR}) was the main source of variation in leaf width and length, accounting for 92.5% and 85.8% of the total variation, respectively (Fig. 2). For leaf width, 7.5% was explained by the variation at intra-clone level, whereas for leaf length the remaining variation, 11.2%, was mainly explained by the variation at intra-clone level, and 2.8% by population, and the remaining 0.2% was unexplained variation (Fig. 2). Variation in height was primarily explained by clone level with 71.4%, genet level explained 17.9%, population explained 3.8%, and the remaining 6.9% was unexplained variation (Fig. 2).

Genetic basis of CV

The hierarchical level of organisation with the best goodness of fit, *i.e.*, that best explained CV, varied among traits. At the intra-genet level (reflecting a combination of genetic and plastic effects), AIC values showed that region best explained CV_{IG} in leaf width, and population best explained CV_{IG} in plant height, whereas no differences (≥2) between AIC in models were found for CV_{IG} in leaf length (Table 1). At the intra-clone level (reflecting genetic effects), none of the hierarchical levels of organisation had an effect on the CV_{IC} in height, leaf length, or leaf width (Table 1). Finally, on the intra-ramet level (reflecting genetic effects), region had the lowest AIC values, and therefore best explained CV_{IR}, in leaf width, whereas population and genet best explained CV_{IR} in leaf length.

Microclimate and treatment effects on CV

CV_{IP}, CV_{IG}, and CV_{IC} in height were all significantly affected by mean annual soil temperature (Table 2): CV decreased with increasing mean annual soil temperature (Fig. 3). Additionally, CV_{IP} in leaf length was also significantly negatively affected by mean annual soil temperature. No significant effect of mean annual soil temperature was found on CV in leaf width. No significant effect of SMI was found on CV in any of the vegetative traits.

The early shading treatment significantly increased CV_{IC} in leaf length compared to control shading (Table 2; Fig. 4A). The two-way interaction between shade and drought was significant for CV_{IR} in leaf width (Table 2), with the combination of drought treatment and control shading resulting in lower CV_{IR} compared to the other treatment combinations (Fig. 4B). No significant treatment effects were found in CV in height in any of the hierarchical levels (Table 2).

Trait mean and CV relationship

Mean height at intra-genet and intra-clone level was significantly affected by the quadratic terms of CV_{IG} and CV_{IC} in height (Table 3). Mean leaf length was significantly affected by the quadratic terms of CV_{IG}, CV_{IC}, and CV_{IR}. Mean leaf width was significantly affected by the CV_{IG}, CV_{IC}, and CV_{IR} of leaf width. Furthermore, our results show a significant interaction for mean leaf length on the intra-clone level between quadratic CV_{IC} and drought treatment (Table 3). Specifically, mean leaf length and quadratic CV_{IC} of leaf length are related under the drought treatment, but they are independent under the control treatment (Fig. 5).

Discussion

This experimental study using *G. odoratum* as model species revealed (1) that intra-ramet variation, which is the lowest hierarchical level in our system, explains the vast majority of overall leaf trait variation in the populations, followed by intra-clone variation. These two levels represent intra-individual variation, and our results thus confirm that intra-individual variation can exceed inter-individual variation (*i.e.*, intra-population and inter-genet variation), as previously observed (Herrera 2017); (2) that intra-specific and intra-individual trait variation at different scales is partly genetically based and (3) that this variation may have been the result of selection by microclimatic conditions in the populations of origin. Furthermore, (4) intra-ramet and intra-clone variation in leaf traits vary under induced drought and early shading, possibly as a functional response to changes in water and light conditions. Finally, (5) drought also led to a dependent relationship between mean leaf size and intra-individual variation in leaf size, as a plastic response that may promote population stability in stressful conditions (Nicotra *et al.*, 2010).

Variance partitioning across hierarchical levels of biological organisation

Our variance partitioning analysis showed that the lowest hierarchical level of organisation, intra-ramet variation, explained the majority of total variation in leaf length and leaf width, followed by intra-clone variation. Additionally, variation in plant height was primarily explained by the intra-clone level. This is congruent with what has been observed in previous studies, where intra-ramet (or intra-individual variation) also explained more variation in leaf traits, fruits and seeds than the variation among plants (Herrera *et al.*, 2015; Herrera, 2017). These striking patterns in the two lowest hierarchical levels could be due to leaf traits changes through time while the plant develops, whereas at higher hierarchical levels the trait differences are already summarised across ramets and therefore average out the intra-ramet variation (*i.e.*, leaves on different ramets are on average more similar to each other than leaves within a ramet). Taken together, these results support the notion that intra-individual variation is responsible for the larger share of the total intra-specific variation, potentially driving population performance and plastic responses to environmental changes. The topic of intra-individual variation is thus worthy of future investigation, and our results justify further research on a deeper mechanistic explanation for intra-individual variation and how these might enhance population variation and persistence.

Genetic differentiation in intra-individual variation

In our common garden experiment, any differences in intra-clone and intra-ramet CV among regions, populations, or genets of origin suggest the existence of genetically based variation in intra-individual variation. We revealed a general pattern of the highest hierarchical levels of region and population having the best goodness of fit, thereby being the primary factors explaining genetic variation in CV at the lower hierarchical levels of intra-genet and intra-ramet. This pattern could well reflect the strong versatility of clonal plants, allowing individuals to adjust their life-history in terms of vegetative reproduction, the placement of ramets and the degree of integration between ramets according to the local environmental conditions (Fischer and van Kleunen, 2001). An individual from a fine-grained heterogeneous environment and microclimate is more likely to invest in increased variation in order to deal with environmental changes and predictability on a small spatial scale, compared to an individual from a coarse-grained heterogeneous or even homogenous environment (Winn, 1996b; Stark *et al.*, 2017). The observed genetic variation in the CV of plant height, leaf length and width, primarily explained by region and population, may very well have been driven by the different environments among regions (ALB, HAI, and SCH) and among all sampled populations (see next section).

Effects of microclimate of origin and treatments on CV

Mean annual soil temperature from the populations of origin was found to have a significant effect on variation in height at all hierarchical levels of organisation, as well as on variation in leaf length at intra-population level. The negative association between CV in height and mean annual soil temperature indicates that individuals originating from colder sites show increased variation, in support of a stress-induced variability hypothesis, stating that abiotic stress increases intra-specific variation (Kuppler *et al.*, 2020). Colder plots from the Biodiversity Exploratories are primarily within coniferous forests, resulting in more or less constant shaded conditions (Willems *et al.*, 2021). Therefore, a possible explanation for the observed increased height variation in individuals originating from colder plots could be, that these individuals have evolved an optimized way to capture light under light-limited conditions by increasing their variation, potentially by number of leaves and the positioning of them (Valladares and Brites, 2004). This relationship thus suggest that intra-individual variation may respond adaptively to various environmental conditions (Herrera *et al.*, 2015) may and ultimately lead to adaptation to long-term microclimatic conditions (May *et al.*, 2017).

At the intra-clone level, intra-individual variation in leaf length was found to increase under two-weeks earlier shading, indicating a rapid phenotypic change within clonal plants in response to environmental drivers. Under shaded conditions, common phenotypic plastic responses are an increased mean leaf size and specific leaf area (Pigliucci and Kolodynska, 2002; Sultan, 2003). After shading was applied, leaf length values may have adjusted in emerging whorls on the developing ramets, causing the intra-individual variation to increase under the early shading treatment. However, at the intra-ramet level the interaction between the shading and drought treatment had a significant effect on the intra-individual variation in leaf width: compared to control shading without drought, early shading with drought, and early shading without drought treatment combinations, the intra-individual variation was considerably lower in individuals exposed to the control shading with drought treatment combination. Possibly, the increase in intra-individual variation observed in the early shading with drought treatment could be a result of plant responses in various directions in response to drastic environmental conditions (Winn, 1996b; Sun *et al.*, 2021), potentially benefitting the individual performance under the drought conditions, perhaps through facilitating drought tolerance. Another strategy to deal with drought is to avoid unnecessary evapotranspiration by minimizing leaf area (Marron *et al.*, 2003; Liu and Stützel, 2004; Wellstein *et al.*, 2017). Newly developing leaves have the opportunity to respond to current environmental conditions, given that there is sufficient phenotypic plasticity for rapid adjustments. However, the low intra-ramet variation in leaf width observed under the drought treatment implies that the individuals did not respond to the drought conditions by further minimizing their leaf area, leading to the low intra-individual variation.

With regard to intra-individual responses, it is important to consider the morphology of the study species. *Galium odoratum* has its leaves positioned in multiple whorls on a single ramet. These whorls and leaves generally increase in size with increasing distance from the ground surface, with the exception of the top whorl which is typically still under development. Therefore, some variation would always be present, even under a hypothetically constant environment, simply due to the structure and architecture of the leaves and whorls (Fischer and van Kleunen, 2001). Nevertheless, this inherent intra-individual variation could very well be a result of evolution of *G. odoratum*, for instance to maximize light capture (Chazdon and Kaufmann, 1993; Reich *et al.*, 2003).

Relationship between trait means and coefficient of variation

Significant relationships between trait means and CV would suggest that these two components are interdependent and that one could drive the other. However, if means and CV are independent, intra-individual variation should be considered as an individual property in itself (Herrera, 2017), able to respond and evolve unconstrained with respect to mean trait values. We observed both linear and quadratic relationships between trait means and the CV on intra-genet, intra-clone and intra-ramet level, but also in some cases an absence of any relationship. The quadratic functions suggest a more complex relationship, where substantially low and high intra-individual variation values reduce mean plant responses while intermediate intra-individual variation values could enhance a plant's performance, in line with stabilizing selective regimes found in previous studies (March-Salas et al. 2021). In this sense, the optimal strategy for some plants could be to invest in intermediate intra-individual variation.

At the intra-clone level, we found that the relationship between mean leaf length and leaf length CV depends on the drought treatment: Under the control conditions, there were no relationships between mean leaf length and its variation, whereas under drought conditions there existed a slight concave relationship that shows a decreasing mean leaf length with increasing intra-individual variation. Thus, under drought stress, plants with decreasing leaf length become less uniform in their leaf traits. This difference between treatment responses indicates that drought conditions affect the relationship between trait means and their variation. In this sense, it could be that plants promote intra-individual changes to optimise final plant trait values, or potentially facilitate eco-physiological traits through water use efficiency and decreased evapotranspiration under stressful conditions (Marron *et al.*, 2003; Liu and Stützel, 2004; Wellstein *et al.*, 2017). Smaller plants may therefore be particularly prone to increase their intra-individual variation in order to adjust to the stressful drought conditions due to their smaller leaf sizes.

Conclusion

In sum, using a common-garden experiment we demonstrated that intra-individual variation introduces an important component of variation in populations. Intra-ramet variation explained the vast majority of total population variation, suggesting a relevant role of phenotypic variation within individuals for adjustments to the local environmental conditions and ultimately for population performance. Furthermore, our results indicate that intra-individual variation in vegetative traits may be partially genetically based, indicating that it could be under the influences of neutral or selective evolutionary processes. Relationships between mean annual soil temperature at the sites of origin and intra-individual variation in vegetative traits suggest that the environment shaped genetically based among-population variation in intra-individual variation. We also showed that intra-individual variation responds plastically to drought and shading treatments, which may allow plants to maintain or improve overall performance under stressful or varying environmental conditions. However, we lack strong support for mechanistic explanations for most patterns in intra-individual variation, potentially mediated by eco-physiological and epigenetic modifications. We therefore conclude that, overall, the observed patterns in intra-individual variation suggest its important role for genetic adaptation to microclimatic variation among populations and for plastic adjustments to rapidly changing microclimatic conditions within populations, respectively.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

CM, MMS, PDF and JFS designed the study. CM conducted the field sampling and the common garden experiment. CM, MMS, and JFS analysed the data with input from PDF and JK. CM wrote the first draft of the manuscript with all co-authors contributing to revisions.

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Table 1. Comparison of models for genetic variation in CV of height, leaf length and width in *Galium odoratum* at different hierarchical levels. Hierarchical levels were added one at a time as nested random factor, and goodness of fit was compared using Akaike Information Criterion (AIC). Significantly lower AIC values (ΔAIC ≥ 2) that explain the best goodness of fit are indicated in bold.

Akaike Information Criterion (AIC) values for genetic basis of trait CV			
	Region	**Region/Population**	**Region/Population/Genet**
INTRA-GENET (CV_{IG}, N = 71)			
Height	– 123.6	**– 126.4**	
Leaf length	– 137.8	– 136.7	
Leaf width	**– 165.3**	– 163.3	
INTRA-CLONE (CV_{IC}, N = 135)			
Height	– 183.6	– 183.7	– 184.2
Leaf length	– 204.1	– 205.8	– 203.8
Leaf width	– 251.8	– 250.9	– 248.9
INTRA-RAMET (CV_{IR}, N = 517)			
Leaf length	– 545.6	– 549.8	– 549.5
Leaf width	**– 609.2**	– 607.2	– 605.9

Table 2. Results of mixed-effects models of CV in height, leaf length and leaf width in *Galium odoratum* at different hierarchical levels explained by environmental variables and experimental treatments. Stepwise backwards selection was applied to obtain the minimum adequate models. Parameters used in the minimum adequate model are indicated with bold. Marginally significant effects (0.1 > P > 0.05) are shown in italics. If stepwise backwards selection did not lead to removing model parameters, estimates from the full model are shown. Parameter estimates (PE) for continuous variables are indicated with ↑ for positive and ↓ for negative. Chi-square (X^2) test values, degrees of freedom (df) and P-values (P) are given.

INTRA-POPULATION (CV_{IP}, N = 22)												
	Height				**Leaf length**				**Leaf width**			
Predictors	PE	X^2	df	P	PE	X^2	df	P	PE	X^2	df	P
Soil temperature	↓	**5.237**	**1**	**0.022 ***	↓	**3.870**	**1**	**0.049 ***	↓	*2.844*	*1*	*0.092*
SMI	↑	0.546	1	0.460	↓	0.036	1	0.850	↑	0.054	1	0.817
INTRA-GENET (CV_{IG}, N = 71)												
	Height				**Leaf length**				**Leaf width**			
Predictors	PE	X^2	df	P	PE	X^2	df	P	PE	X^2	df	P
Soil temperature	↓	**7.682**	**1**	**0.006 ****	↓	1.012	1	0.314	↓	0.130	1	0.718
SMI	↓	0.045	1	0.831	↑	0.482	1	0.488	↑	1.616	1	0.204
INTRA-CLONE (CV_{IC}, N = 135)												
	Height				**Leaf length**				**Leaf width**			
Predictors	PE	X^2	df	P	PE	X^2	df	P	PE	X^2	df	P
Soil temperature	↓	**8.686**	**1**	**0.003 ****	↓	0.658	1	0.417	↓	0.050	1	0.823
SMI	↓	0.077	1	0.782	↑	0.002	1	0.965	↑	0.477	1	0.490
Shade (S)		2.250	1	0.134		**4.299**	**1**	**0.038 ***		*3.127*	*1*	*0.077*
Drought (D)		0.046	1	0.831		0.662	1	0.416		1.407	1	0.236
S × D		1.813	2	0.404		0.004	1	0.948		0.510	1	0.475
INTRA-RAMET (CV_{IR}, N = 517)												
					Leaf length				**Leaf width**			
Predictors					PE	X^2	df	P	PE	X^2	df	P
Soil temperature					↓	0.799	1	0.371	↓	0.869	1	0.351
SMI					↓	0.308	1	0.579	↓	0.031	1	0.861
Shade (S)						2.592	1	0.107		**2.348**	**1**	**0.125**
Drought (D)						1.254	1	0.263		**1.508**	**1**	**0.220**
S × D						*3.219*	*1*	*0.073*		**6.229**	**1**	**0.012 ***

Table 3. The full models of all the predictors for trait means. Stepwise backwards selection was applied to obtain the minimum adequate models. Parameters used in the final model are indicated with bold. Marginal effects (0.1 > P > 0.05) are shown in italics. If the stepwise backwards selection did not lead to removed parameters in the model, estimates from the full model are shown. X^2 values, degrees of freedom (df) and P-values (P) are given. No significant results were found for intra-population (CV_{IP}), and thus this level is not shown.

INTRA-GENET (CV_{IG}, N = 71)

	Height			**Leaf length**			**Leaf width**		
Predictors	X^2	df	P	X^2	df	P	X^2	df	P
Trait CV	*3.453*	*1*	*0.063*	0.604	1	0.437	**7.395**	**1**	**0.007 ****
Trait CV^2	**4.178**	**1**	**0.041 ***	**6.386**	**1**	**0.012 ***	0.023	1	0.880

INTRA-CLONE (CV_{IC}, N = 135)

	Height			**Leaf length**			**Leaf width**		
Predictors	X^2	df	P	X^2	df	P	X^2	df	P
Trait CV	2.482	1	0.115	1.173	1	0.279	**20.149**	**1**	**<0.001 *****
Trait CV^2	**3.951**	**1**	**0.047 ***	**10.965**	**1**	**<0.001 *****	0.006	1	0.937
Shade (S)	0.430	1	0.512	0.790	1	0.374	0.359	1	0.549
Drought (D)	0.002	1	0.962	**1.656**	**1**	**0.198**	1.137	1	0.286
Trait CV × S	0.258	1	0.612	0.010	1	0.929	0.098	1	0.754
Trait CV × D	0.363	1	0.547	0.121	1	0.728	0.080	1	0.777
Trait CV^2 × S	0.386	1	0.534	0.551	1	0.458	0.174	2	0.917
Trait CV^2 × D	0.081	1	0.776	**4.705**	**1**	**0.030 ***	0.224	2	0.894
Trait CV × S × D	0.338	1	0.561	0.068	1	0.794	0.515	2	0.773
Trait CV^2 × S × D	1.937	3	0.586	2.611	2	0.271	1.053	4	0.902

INTRA-RAMET (CV_{IR}, N = 517)

	Leaf length			**Leaf width**		
Predictors	X^2	df	P	X^2	df	P
Trait CV	0.087	1	0.768	**112.22**	**1**	**< 0.001 *****
Trait CV^2	**67.162**	**1**	**< 0.001 *****	0.760	1	0.384
Shade (S)	0.434	1	0.510	0.646	1	0.422
Drought (D)	*3.071*	*1*	*0.080*	1.995	1	0.158
Trait CV × S	0.002	1	0.965	0.001	1	0.970
Trait CV × D	0.521	1	0.771	0.014	1	0.907
Trait CV^2 × S	0.215	1	0.643	0.331	1	0.565

Trait CV^2 × D	0.004	1	0.950	1.524	2	0.469
Trait CV × S × D	0.036	2	0.982	0.050	2	0.975
Trait CV^2 × S × D	0.533	2	0.766	0.050	1	0.823

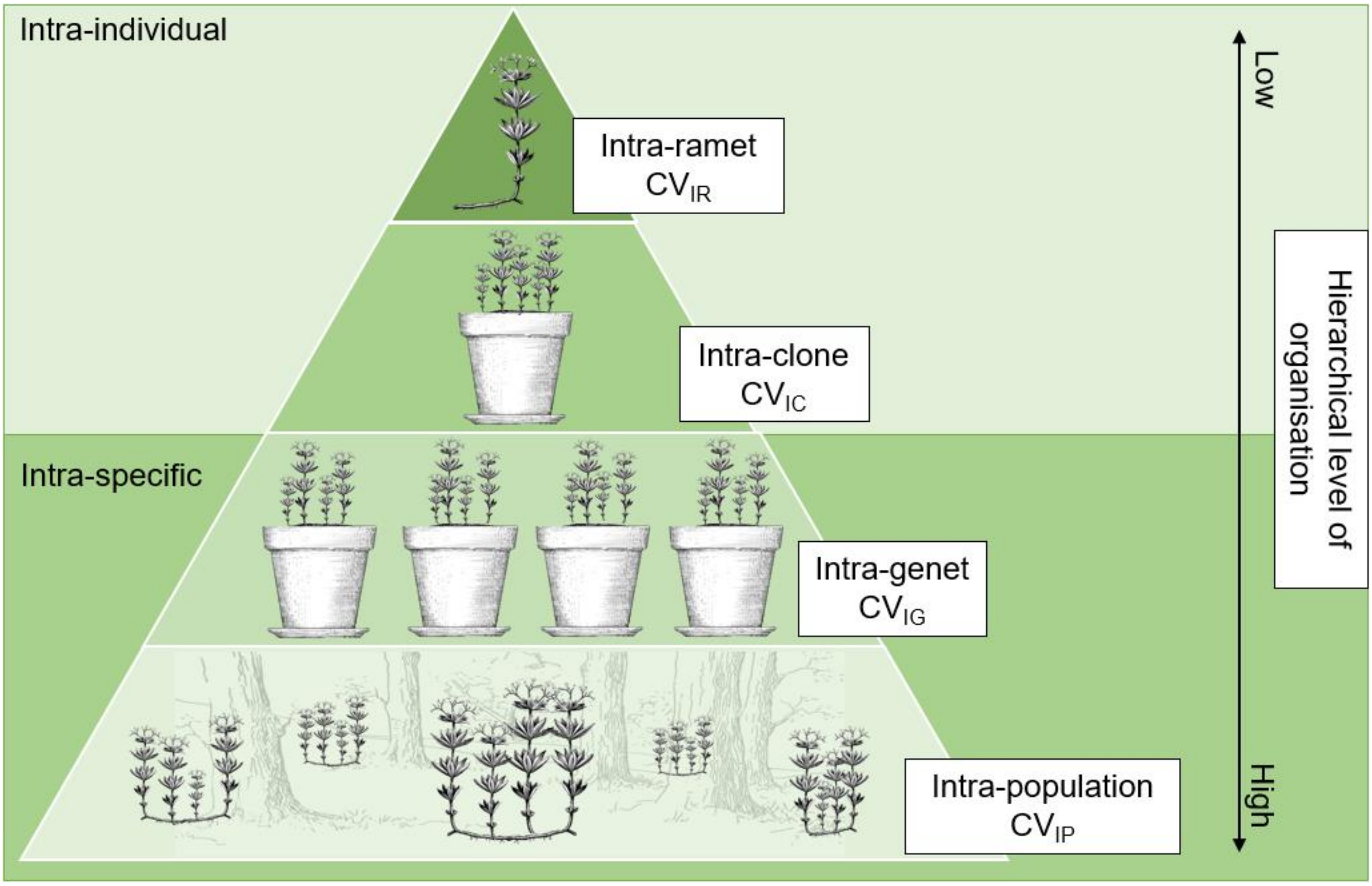

Fig. 1. Schematic figure of the hierarchical levels of organisation of *Galium odoratum*. In each population, five genets were sampled allowing to calculate intra-population coefficient of variation (CV). Each genet was separated by cutting its stolons into four separate ramets, that were each planted in their own individual pot, which allows to calculate intra-genet CV. Each pot was under one of the four treatments (control shade + no drought, early shade + no drought, control shade + drought, early shade + drought). Over time, the individually planted ramets in each pot reproduced vegetatively and became a clonal individual made up of several ramets, allowing to calculate intra-clone and intra-ramet CV, respectively. The CV abbreviation is shown at each hierarchical level.

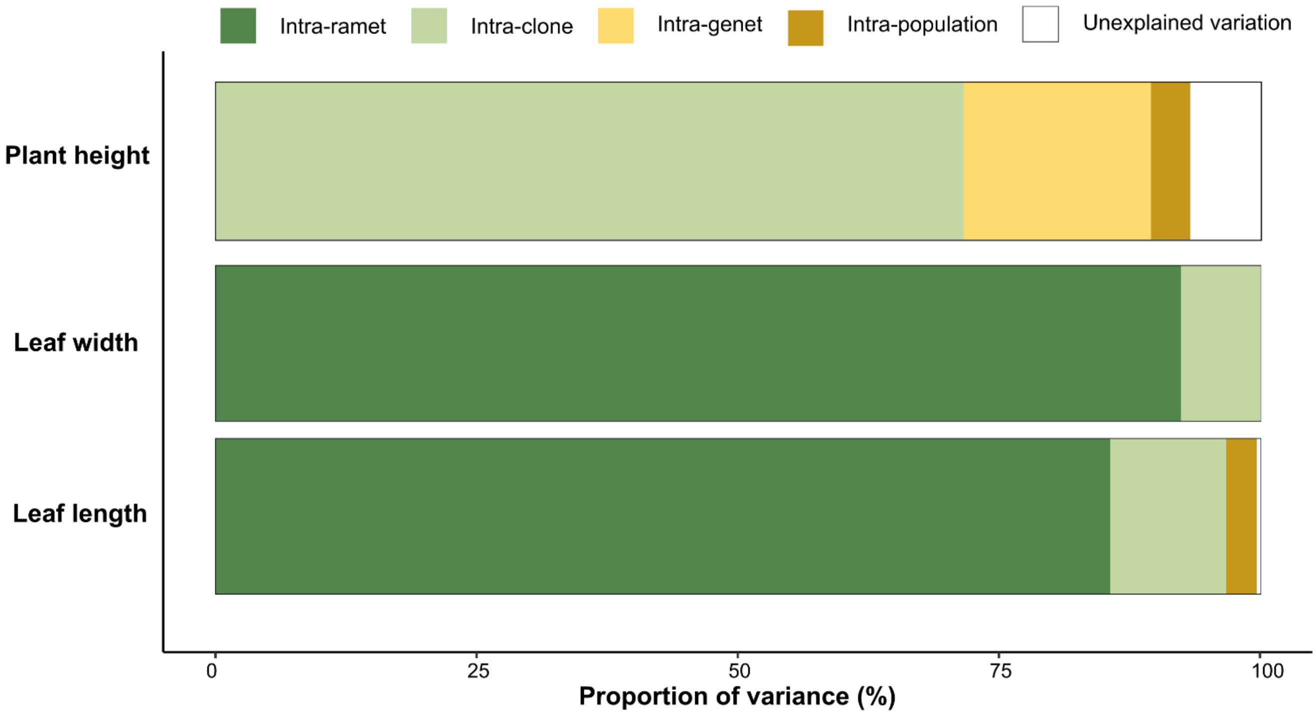

Fig. 2. Proportion of variation in plant height, leaf length and leaf width of *Galium odoratum* explained by variance partitioning among different levels of organisation: intra-population, intra-genet, intra-clone, intra-ramet or unexplained.

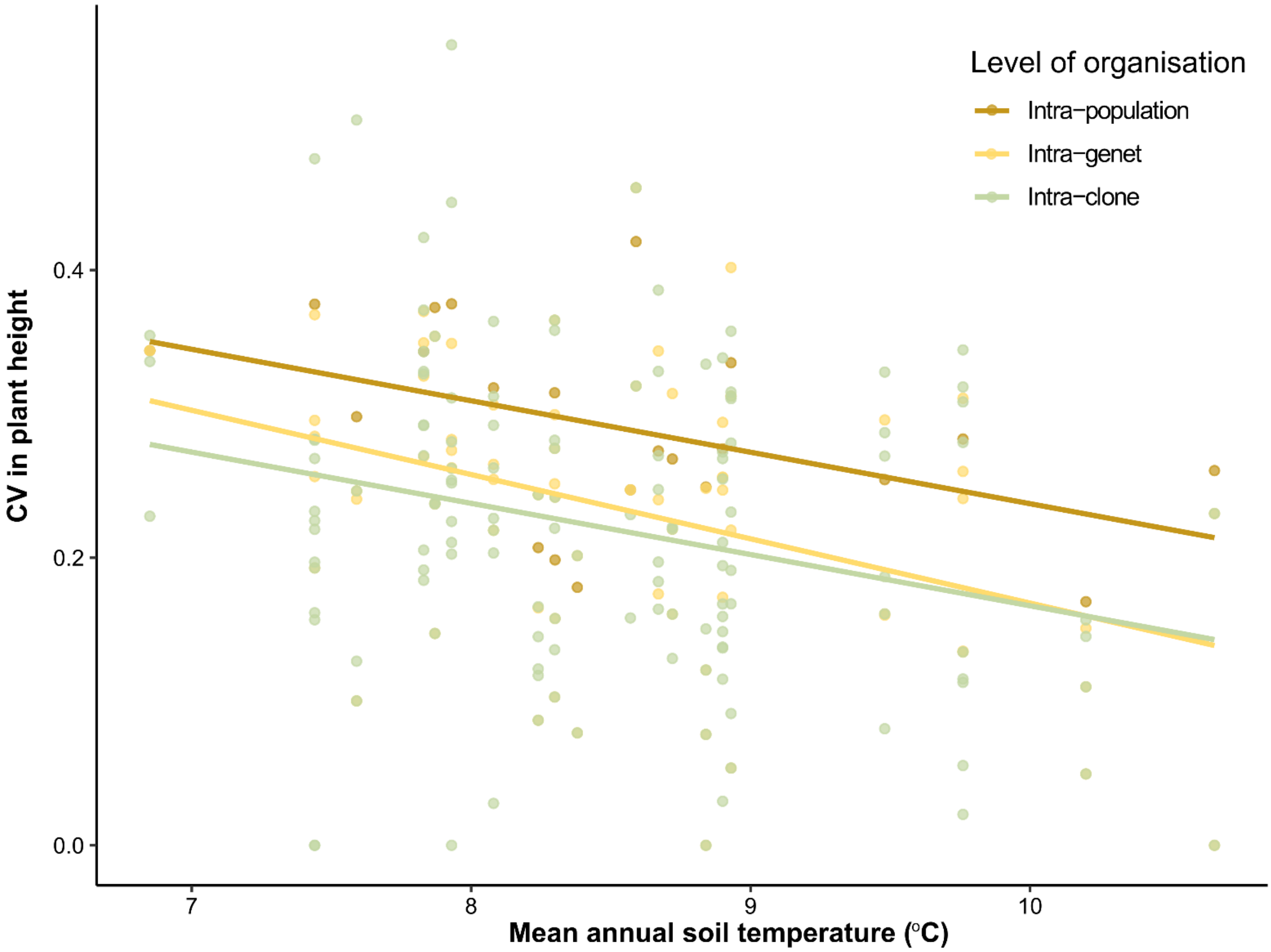

Fig. 3 Relationship between mean annual soil temperature at the site of origin with the intra-individual variation in height in *Galium odoratum*. Regression lines through data (dots) from different hierarchical levels of organisation are shown.

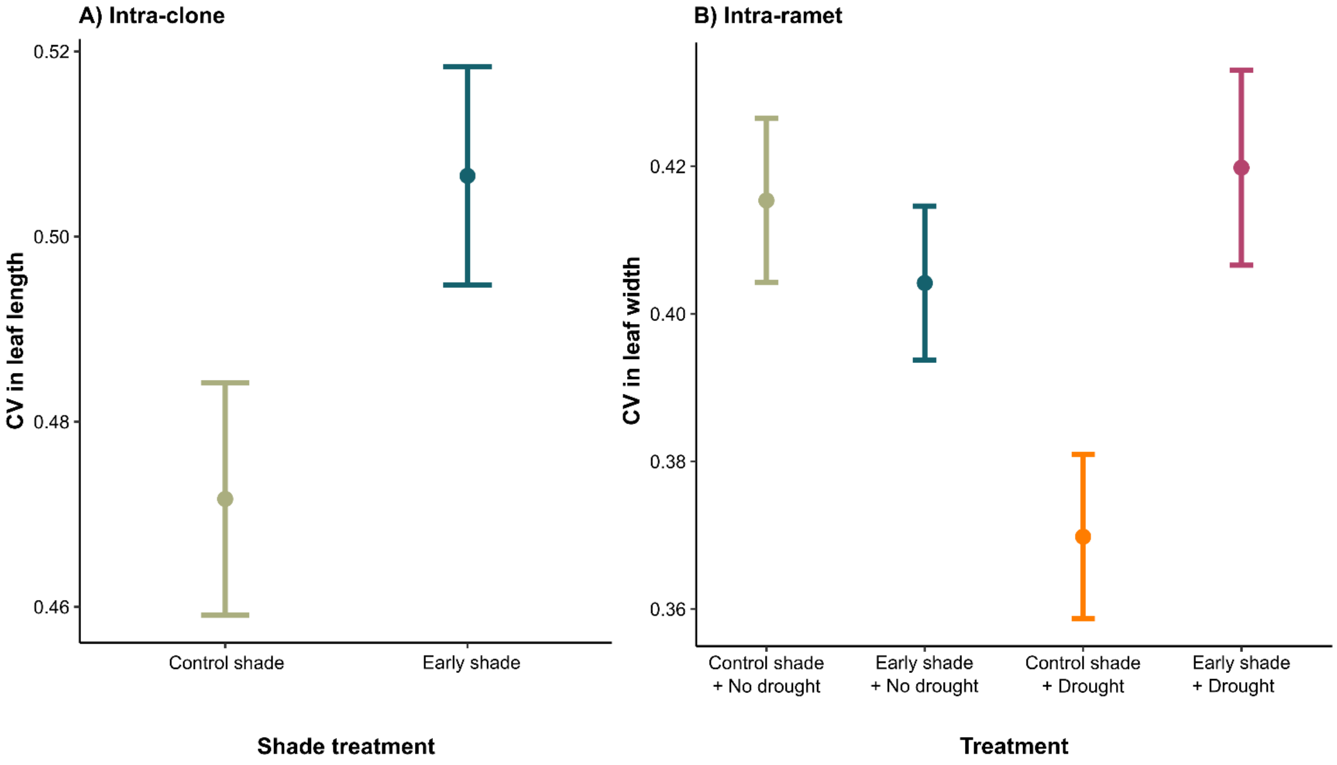

Fig. 4. Shading and drought treatment effects on the coefficient of variation (CV) at two intra-individual levels in *G. odoratum*. A) Effect of control and early shade treatment on CV in leaf length at the intra-clone level. B) Effect of the four different treatment combinations on CV in leaf width at intra-ramet level.

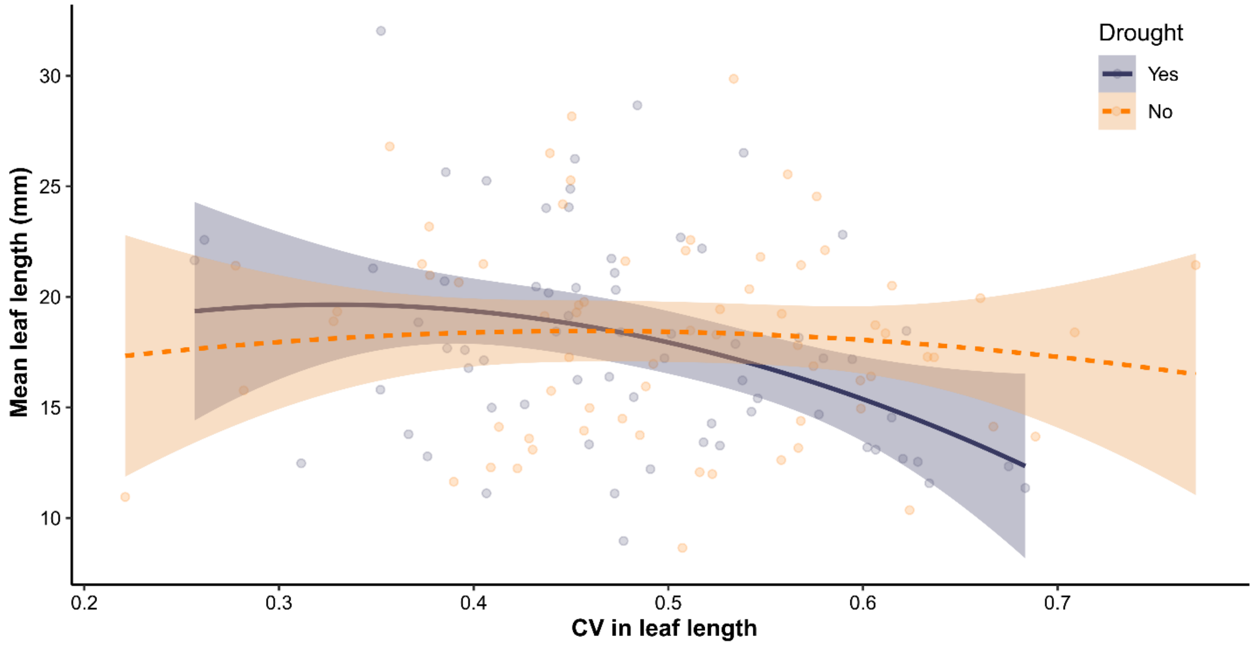

Fig. 5. Significant two-way interaction between the drought treatment and quadratic intra-clone CV in leaf length on the mean leaf length in *Galium odoratum*. The dashed and orange curve represents the control treatment while the blue curve represents the drought treatment. 95% confidence intervals are shown around the curves.