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RESEARCH ARTICLE

Ecological significance of intraplant variation: Epigenetic mosaicism in *Lavandula latifolia* plants predicts extant and transgenerational variability of fecundity-related traits

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Abstract

1. Intraindividual epigenetic mosaicism is probably widespread among long-lived plants, yet its ecological significance as a potential source of variation in fitness-related traits in plant populations remains virtually unexplored. This paper examines the hypothesis that extant epigenetic variation within plants can have both current and transgenerational fecundity correlates which could eventually translate into fitness variations among different parts of the same individual and their respective offspring.
2. Five modules, each consisting of a terminal branchlet bearing one inflorescence and its subtending leaves, were collected from each of 15 wild-growing *Lavandula latifolia* (Lamiaceae) plants. They were characterized epigenotypically by the methylation state of methylation-sensitive amplified fragment length polymorphism (MS-AFLP) markers, and phenotypically by fecundity-related traits (inflorescence length, and size, number and mass of seeds produced). Seeds from the different modules were sown in the greenhouse and resulting 'subprogenies' characterized phenotypically (germination probability and time to emergence, seedling size, susceptibility to fungal disease).
3. All plants sampled were internally heterogeneous with regard to the methylation state of 1%–13% of MS-AFLP markers. Predictable relationships were found between epigenotypic and phenotypic variation across the extant modules of individual *L. latifolia* shrubs. Phenotypes of subprogenies from different modules of the same plant grown under homogeneous conditions in the greenhouse were predictably related to the epigenotype of the maternal module which produced the seeds.
4. *Synthesis*. The variable epigenotypes of different modules in the same plant not only predicted extant phenotypic variation among the modules themselves, but also phenotypic differences among the subprogenies produced by different modules. These relationships linking intraplant epigenotypic mosaicism with both extant and transgenerational heterogeneity in fitness-related traits

support the 'epigenetic mosaicism hypothesis' for plant variation, and also suggest hitherto unexplored ecological consequences of epigenotypically enhanced variation in the context of plant populations and communities.

KEYWORDS
DNA methylation, epigenetic mosaicism hypothesis, fecundity-related traits, intraplant variation, *Lavandula latifolia* (wild lavender), methylation-sensitive amplified fragment length polymorphism (MS-AFLP), transgenerational effects

1 | INTRODUCTION

The notion that individual plants are nonunitary entities can be traced back at least to the work of Erasmus Darwin (1800), who emphasized that '…every bud of a tree is an individual vegetable being; and that a tree therefore is a family or swarm of individual plants'. In more recent times, the ecological, evolutionary and physiological implications of the internal multiplicity inherent to modular plant growth have been examined in considerable detail from different perspectives and with reference to a variety of subindividual units (Herrera, 2009). These latter include, for instance, physiologically autonomous sectors (Orians & Jones, 2001; Watson, 1986; Watson & Casper, 1984), branches and ortostichies (Orians et al., 2004; Sprugel et al., 1991; Zwieniecki et al., 2003), elemental architectural modules (Hallé, 1986), differentially proliferating cell lineages (Klekowski, 1988; Otto & Hastings, 1998; White, 1979) and genomically differentiated mosaics (Pineda-Krch & Lehtilä, 2004b). The fusion of these kaleidoscopic perspectives on plant subindividuality with the idea that subindividual selection (i.e. among the different units that form the individual) and inheritance of somatic mutations can have evolutionary consequences (Buss, 1983a, 1983b; Otto & Hastings, 1998; Pineda-Krch & Fagerström, 1999; Pineda-Krch & Lehtilä, 2004a; Steele, 1979), provided conceptual grounds for a 'genetic mosaicism hypothesis' (GMH) of the ecology and evolution of plant variation (Whitham & Slobodchikoff, 1981; Whitham et al., 1984; Gill et al., 1995; Pineda-Krch & Lehtilä, 2004b; reviews in Herrera, 2009; Gerber, 2018).

Simplifying from Gill et al. (1995), the GMH rests on the simultaneous fulfilment of four central premises: (a) spontaneous mutations occur among the proliferating meristems; (b) the meristematic and modular basis of plant development assures that many of these mutations are preserved and expanded hierarchically among modules as the plant grows; (c) the differential growth and survival of ramets, branches and shoots should alter the genotypic configuration of the plant as it grows; and (d) intraplant trait heterogeneity arising from genotypic heterogeneity will affect individual fitness through effects on progeny traits, plant responses to the environment and/or responses of animal consumers (Herrera et al., 2021). Studies on wild plants have so far produced relatively few examples of spontaneous genetic mosaics within individuals and, whenever such mosaics have been identified, the estimated somatic mutation rates were extremely low and/or there was no evidence of extant or transgenerational phenotypic correlates of the genetic mosaicism (Cloutier et al., 2003; Gerber, 2018; Orr et al., 2020; Padovan et al., 2013; Ranade et al., 2015; Schmid-Siegert et al., 2017; Wang et al., 2019; but see Hanlon et al., 2019; Holeski et al., 2009). The proposal that genomically different parts of the same plant could contribute differentially to the next generation (i.e. differ in fitness) by virtue of their genomic distinctness is central to the GMH, but lack of empirical verification has probably hindered its acceptance in the long run despite strong theoretical foundations (Gerber, 2018; Herrera, 2009; Otto & Hastings, 1998; Pannell & Eppley, 2004; Pineda-Krch & Fagerström, 1999; Pineda-Krch & Lehtilä, 2004a).

Somatic mutations altering DNA sequences are not the only molecular mechanism with the capacity to produce stable genomic and phenotypic heterogeneity within individual plants. Potentially heritable epigenetic changes, such as those involving DNA cytosine methylation, also have the capacity to induce stable genomic heterogeneity and phenotypic variation within individual plants through their effects on gene expression, transposon activity, and plant growth and development (Cokus et al., 2008; Finnegan et al., 2000; Lister et al., 2008). This is supported by reports of homologous organs in different parts of the same genetic individual differing in extent and/or patterns of DNA methylation (Bian et al., 2013; Bitonti et al., 1996; Gao et al., 2010), and associations between subindividual epigenetic variation and intraplant phenotypic heterogeneity (Alonso et al., 2018; Herrera et al., 2019; Herrera & Bazaga, 2013; Marfil et al., 2009). Furthermore, persistent epigenetic mosaicism can arise within individuals of long-lived plants as a consequence of steady internal epigenetic diversification over lifetime (Herrera et al., 2021; Yao et al., 2021). These lines of evidence motivated Herrera et al.'s (2021) proposal of an 'epigenetic mosaicism hypothesis' (EMH) of plant variation consisting of the same elements (a)–(d) above as the original GMH but in which the terms 'mutation' and 'genotype' were replaced with 'epimutation' and 'epigenotype', respectively (see also Alonso et al., 2018; Herrera et al., 2019; for additional motivation). Support for the genealogical basis of epigenotypic mosaicism, and its dynamic nature over individuals' lifetimes (elements a–c), was recently provided by Herrera et al. (2021) for wild lavender (*Lavandula latifolia* Med., Lamiaceae). The objective of this paper is to further test on this species that extant intraplant epigenotypic variation has current and transgenerational phenotypic correlates which could eventually have ecological consequences by inducing fitness variations across different parts of the same individual and their respective offspring. Throughout this paper, 'transgenerational' will thus refer to the effects of maternal epigenetic

mosaicism on phenotypic heterogeneity of offspring from the same maternal parent.

The following two questions will be specifically addressed in this study: (1) Does a predictable relationship exist between epigenotypic and phenotypic variation across different architecturally defined modules of the same *L. latifolia* shrub? and (2) Are the phenotypes of progenies produced by different modules of the same plant predictably related to the epigenotype of the module which produced the seeds, or in other words, Do epigenetically distinct plant parts produce phenotypically distinct progenies? The traits of maternal plants and progenies chosen for study were all directly or indirectly related to fecundity so that plausible inferences about fitness variations could be drawn from our results. To add strength to our conclusions, each of the preceding two questions will be addressed by considering epigenotypes and phenotypes of maternal plants and progenies from both multivariate (all traits considered simultaneously) and univariate (traits considered individually) perspectives. Our results show that modules of the same plant with different epigenotypes not only differ in extant traits, but they also predictably produce offspring which differ in fitness-related traits.

2 | MATERIALS AND METHODS

2.1 | Study species

Wild lavender (*Lavandula latifolia* Med.) is an evergreen shrub inhabiting open shrublands and woodlands in the Iberian Peninsula and southern France (Upson & Andrews, 2004). Branching is dichasial and generally conforms to Leeuwenberg's development model (a sympodial succession of equivalent sympodial units, each of which is orthotropic and has determinate growth; Hallé, 1986; Hallé et al., 1978). Crowns of adult plants are made up of morphological units consisting of distinct leaf clusters borne by short stems, many of which produce one terminal inflorescence in early summer (see Alonso et al., 2018: Figure 1; and Wetzel, 2021: Figure 2; for drawings and photographs). Each of these clusters of even-aged leaves borne by young (≤3 year), short terminal branchlets will be hereafter termed a 'module' following Hallé's (1986) definition ('the leafy axis in which the entire sequence of aerial differentiation is carried out, from the initiation of the meristem that builds up the axis to the sexual differentiation of its apex'). Further details on natural history, reproductive biology and demography of *L. latifolia* can be found in Herrera (1991), Herrera and Jovani (2010), Herrera and Bazaga (2016) and references therein.

2.2 | Field and greenhouse methods

Field sampling was conducted in September 2014 at a large *L. latifolia* population near Arroyo Aguaderillos in the Guadahornillos watershed (Sierras de Cazorla, Segura y las Villas Natural Park, Jaén province, southeastern Spain), under permit of the Consejería de Medio Ambiente, Junta de Andalucía. Fifteen widely spaced shrubs roughly similar in size were selected for study. These plants were the same studied by Alonso et al. (2018), and a superset of those studied by Herrera et al. (2021). Five modules, each consisting of a single inflorescence plus its subtending basal leaves were collected from each shrub. Sampled modules were distributed as evenly as possible across the shrub's crown in each plant, subject to the constraint that all leaves in the sampled module should be healthy and free of any visible damage. Leaves were placed in paper envelopes and dried at ambient temperature in containers with silica gel for subsequent DNA extraction. Inflorescences were placed individually inside fine mesh bags, and kept indoors at room temperature until all seeds were shed naturally. Sound seeds produced by each inflorescence were counted and weighted collectively. For the purpose of the analyses presented here, each maternal module was characterized by inflorescence length (measured from the tip to the base of the uppermost leaf), mean seed mass, and the log transforms of total number and total mass of seeds produced. Seed size and seed production are mainly determined by maternal genetic and/or maternal environmental effects in the vast majority of species studied so far (e.g. Lipow & Wyatt, 1999; Roach & Wulff, 1987; Thiede, 1998; Zas & Sampedro, 2015), and in *L. latifolia* seed production by each inflorescence depends closely on the number of flowers produced (Herrera, 1991). Seed size and seed production were thus treated here as maternal phenotypic traits.

Seeds were kept at ambient temperature in paper envelopes until sown in the greenhouse 2 months later. Depending on availability, up to 26 sound seeds per maternal module (mean ± SE = 21.6 ± 0.6 seeds per module; range = 4–26) were randomly selected and shallowly buried in 48-cell plastic germination trays filled with a mixture of universal substrate (COMPO SANA®) and perlite (3:1). Subsets of 4–5 seeds from the same maternal module were planted as spaced as possible in each cell of the tray. Plants and modules were distributed within and among trays, and these were randomly rearranged regularly to account for possible heterogeneity of conditions within the greenhouse. A total of 1623 seeds were sown, all plants and modules combined. During the germination period, trays were watered daily and maintained at a 16:8 h light/dark photoperiod under ambient temperature (20–25°C). Seed germination was recorded daily during 8 weeks, when germination had practically ceased and around 60% of all seeds sown had already germinated. Seeds with protruding radicles ≥2 mm were considered as successfully germinated. Around 900 seedlings were obtained in total (mean ± SE = 12.7 ± 0.6 seedlings per maternal module, range = 2–23, $N = 71$). Between 48 and 64 days after the first seedling emerged, a total of 601 seedlings which were roughly similar in size and age and had at least three pairs of true leaves completely expanded were selected and its aerial part harvested by cutting main stems at ground level. They were stored individually in labelled envelopes and dried immediately at ambient temperature in containers with abundant silica gel. Each harvested seedling was weighed to the nearest 0.01 mg in an analytical balance (XS105 Mettler-Toledo). The presence/absence of visible signs of fungal disease in some of the leaves of each seedling was recorded

at harvesting. Although we did not anticipate that some seedlings should be infested by fungi in the course of the experiment, we took advantage of this and used fungal susceptibility as another phenotypic feature of seedlings, given the evident ecological significance of possible variation in this trait in relation to seedling survival.

Within the greenhouse progeny of each maternal plant, those from the same maternal module will be named 'subprogeny'. Each subprogeny was characterized phenotypically by seed germination rate (proportion of sown seeds which had germinated when the experiment was terminated), mean time to germination (days elapsed from seed sowing until cotyledons were visible at the substrate's surface), mean seedling dry mass at the end of the experiment and proportion of seedlings whose leaves had signs of fungal disease. This latter variable was included as a proxy for intrinsic susceptibility of seedlings to fungal disease.

2.3 | Laboratory methods

Dried leaf material from maternal modules was homogenized to a fine powder using a Retsch MM 200 mill and total genomic DNA was extracted from approximately 35 mg of ground leaf material using the Bioline ISOLATE II Plant DNA Kit and the manufacturer protocol. The DNA extract of each module ($N = 75$) was used for obtaining its epigenetic fingerprint as detailed below.

Epigenetic fingerprints of maternal modules were investigated using a variant of the amplified fragment length polymorphism (AFLP) technique which allowed to identify instances of intraplant polymorphism in the methylation state of methylation-susceptible anonymous 5′-CCGG sequences. Since we were interested in detecting instances of epigenotype heterogeneity among modules from the same plant, our AFLP method used exclusively primer combinations based on the methylation-sensitive HpaII enzyme. HpaII cleaves 5′-CCGG sequences but is inactive when either or both cytosines are fully methylated, and cleaving may be impaired or blocked when one or both of the cytosines are hemi-methylated (McClelland et al., 1994; Roberts et al., 2007). In the absence of DNA sequence variation among samples, as expected for leaves from the same plant, any observed intraplant polymorphism in these methylation-sensitive AFLP markers (MS-AFLP hereafter) will reflect subindividual heterogeneity in the methylation state of the associated 5′-CCGG site (see Verhoeven et al., 2010; Herrera & Bazaga, 2013; for earlier applications of this method in plant epigenetic research). Analyses were performed following the AFLP protocols described in Herrera and Bazaga (2008). Leaf samples were fingerprinted using eight primer combinations, each with two (HpaII) or three (MseI) selective nucleotides, which were chosen on the basis of repeatability and ease of scoring (Table S1). Amplified products were analysed on an ABI PRISM 3130xl DNA sequencer, and fingerprint profiles were scored manually by visualizing electrophoregrams with GeneMapper 5.0 software. As other genetic markers, AFLPs are subject to genotyping errors which can distort results (Bonin et al., 2004; Pompanon et al., 2005). Scoring error rates were determined for each individual marker obtained ($N = 688$) by running replicated analyses for 20 samples (26.7% of total), and estimated as the ratio of the number of discordant scores in the two analyses to the total number of replicated samples. To minimize the likelihood of spurious intraplant polymorphisms arising from scoring errors rather than actual epigenetic variation within plants, only the $N = 232$ markers whose scoring error rates were equal to zero were retained for analysis (Table S1).

2.4 | Data analysis

Epigenotypic information for maternal modules was coded as a module × MS-AFLP marker binary matrix whose elements were the methylation state of each polymorphic marker ($N = 70$ markers; Table S1) in the given maternal module (1 = unmethylated; 0 = methylated). Four DNA samples whose MS-AFLP scores in multivariate space departed anomalously from the rest, possibly because of imperfect DNA amplification, were excluded from all analyses. Phenotypic information for maternal modules (inflorescence length, mean seed mass, log transforms of total number and total mass of seeds) and greenhouse subprogenies (germination rate, mean time to germination, mean seedling dry mass, frequency of fungal disease) were likewise arranged as two module × trait matrices. The three matrices thus obtained furnished the raw data for all analyses (Herrera et al., 2022). No seedlings remained alive by the end of the experiment for four subprogenies from three maternal plants. To correct convergence problems when fitting some mixed-effects models (see below), missing data for seedling mass and fungal frequency for these modules were imputed using the simple procedure of filling in the means of recorded values for the trait concerned (Little & Rubin, 1987). The effect of this imputation on results, if any, is expected to be negligible given the small proportion of modules involved (5% of total). All statistical analyses reported in this paper were carried out using the R environment (R Core Team, 2020).

2.4.1 | Multivariate approach

Pairwise dissimilarities matrices between maternal module phenotypes and epigenotypes, and between phenotypes of greenhouse subprogenies, were obtained by applying Gower or Jaccard indices to phenotypic and epigenotypic data, respectively. Principal coordinate analyses were run on each of these dissimilarity matrices, and sets of coordinates were obtained for each module or subprogeny in epigenotypic and phenotypic spaces of reduced dimensionality. Each module or subprogeny was then characterized epigenotypically and phenotypically by its distance to the corresponding plant centroid. These values provided standardized estimates of the degree of multivariate epigenotypic or phenotypic deviation of each maternal module or subprogeny within the particular domain of the maternal plant it belonged to. We will refer to these distances as 'intraplant distinctness' hereafter. They are well suited for performing the tests of extant and transgenerational

epigenotypic–phenotypic relationships pursued in this paper. Computations were performed with function betadisper of the VEGAN package (Oksanen et al., 2019). Partitions of epigenotypic and phenotypic variances into within-and among-plant components were obtained by applying the adonis function in VEGAN to the pairwise dissimilarities matrices.

Questions on the intraplant relationships between the multivariate epigenotypes of maternal module, on one side, and the multivariate phenotypes of modules (extant relationships) and subprogenies (transgenerational relationships), on the other, were analytically addressed by fitting two linear mixed-effects models using the lmer function of the LME4 package (Bates et al., 2015). In each model, intraplant distinctness in epigenotypic space was included as the single fixed effect, continuous predictor, and plant identity was treated as a random categorical effect. The latter ensured that inferences on the fixed effect referred to variation within individual plants (i.e. among modules) and applied to the 'broad inference space' (sensu McLean et al., 1991) comprising the local *L. latifolia* population (see further elaboration in Section 4.2). All distinctness values were scaled to mean zero and standard deviation unit so that fixed-effect parameter estimates represented standardized effects. Confidence intervals for model parameter and variance estimates were obtained using function confint.merMod. The function ggpredict from the GGEFFECTS package (Lüdecke, 2018) was used to compute the predicted marginal effects of epigenotypic intraplant distinctness on intraplant phenotypic distinctness for modules and subprogenies.

Both extant (among modules) and transgenerational (among subprogenies) phenotypic variation were found associated with extant epigenotypic variation among modules; thus, the transgenerational association could arise from direct (inheritance) or indirect (through effects of maternal phenotypes) causal effects. The relative magnitudes of these two causal pathways were explored by regressing phenotypic distinctness of subprogenies against the epigenotypic and phenotypic distinctnesses of extant maternal modules (all plants combined), and then computing the 'relative importance' (sensu Grömping, 2015) of each predictor with the calc.relimp function in the RELAIMPO package (Grömping, 2006) and normalized to sum 100%. Figures obtained were the proportional R^2 contribution averaged over orderings among regressors.

2.4.2 | Univariate approach

To evaluate in an univariate context whether epigenotypic differences between modules of individual *L. latifolia* plants predicted differences in phenotypic characteristics between the modules themselves and between their subprogenies, we looked for MS-AFLP markers significantly associated with every phenotypic trait considered. For each phenotypic trait (four traits each for modules and subprogenies), separate linear mixed-effects models were fit for each polymorphic MS-AFLP marker ($N = 70$) using the lmer function. In each model, the phenotypic trait was the dependent variable, marker methylation state (1 = unmethylated; 0 = methylated) the single fixed effect, and plant the random effect. *p*-values for the effect of marker methylation state on a given trait were used to identify significant marker–trait associations. Given the large number of models fit for every trait, Storey and Tibshirani's (2003) *q*-value method was applied to estimate false discovery rates. Using the QVALUE package (Storey et al., 2020), we calculated for every trait the set of *q*-values for all marker–trait models fitted, and found the largest *q*-value leading to an expectation of less than one falsely significant model [i.e. *q*-value × (number of models accepted as significant) < 1]. Predicted marginal effects of MS-AFLP markers with statistically significant effects on phenotypic traits were computed with function ggpredict.

In addition to identifying single MS-AFLP markers significantly associated with each phenotypic trait, we were also interested in quantifying their combined explanatory value as predictors of phenotypic differences among modules and subprogenies of individual plants. Separate linear mixed-effects models were fit for each trait, which had trait value as the response variable and included the methylation state of all significantly associated MS-AFLP markers as fixed effects (predictors). As done in models fitted to identify significant markers, plant was also included as a random effect. The marginal R^2 for each model, which represents the variance explained collectively by fixed factors alone (Nakagawa & Schielzeth, 2013), was used to evaluate quantitatively the combined value of significant epigenetic markers as predictors of intraplant variation in phenotypic traits of modules or subprogenies. Function r2_nakagawa from package PERFORMANCE (Lüdecke et al., 2021) was used for computations.

3 | RESULTS

All *L. latifolia* plants sampled were internally heterogeneous with regard to the methylation state of one or more of the MS-AFLP markers considered ($N = 232$). Between 0.9% and 13.4% of markers (mean ± SE = 4.9 ± 1.1%; $N = 15$ plants) were polymorphic in methylation state across the modules sampled in the same plant, that is, occurred either in methylated or in unmethylated states in different modules. Intraplant epigenotypic variation accounted for 5.9% of total epigenotypic variance in the sample. There was substantial heterogeneity among the multivariate phenotypes of extant modules and greenhouse subprogenies from the same plant, as revealed by the high intraplant variance components (51.2% and 58.7% of total phenotypic variance for modules and subprogenies, respectively).

3.1 | Multivariate analyses

Intraplant epigenotypic distinctness of individual modules predicted phenotypic distinctness of both the modules themselves and their greenhouse subprogenies, as shown by the statistical significance of fixed effects in the two linear mixed-effects models fitted to the data (Table 1). Positive fixed-effect parameters denote direct linear relationships between epigenotypic distinctness and the phenotypic distinctness of both modules and subprogenies, as shown by

TABLE 1 Results of linear mixed models testing the effect of intraplant epigenotypic distinctness of modules (fixed effect) as predictor of the phenotypic distinctness of modules and subprogenies (response variables). 'Distinctness' stands for the standardized distance of each module or subprogeny to its plant's centroid in epigenotypic or phenotypic space, and it measures the epigenetic or phenotypic disparity of modules or subprogenies relative to the plant average

	Fixed effect: Epigenotypic distinctness of maternal module				Random effect: Plant	
Response variable	Parameter estimate	95% confidence interval	Chi-square	*p*-value	Variance	95% confidence interval
Phenotypic distinctness of plant module	0.335	0.128–0.542	10.07	0.0015	0.286	0.257–0.860
Phenotypic distinctness of subprogeny	0.219	0.036–0.404	5.50	0.019	0.547	0.468–1.117

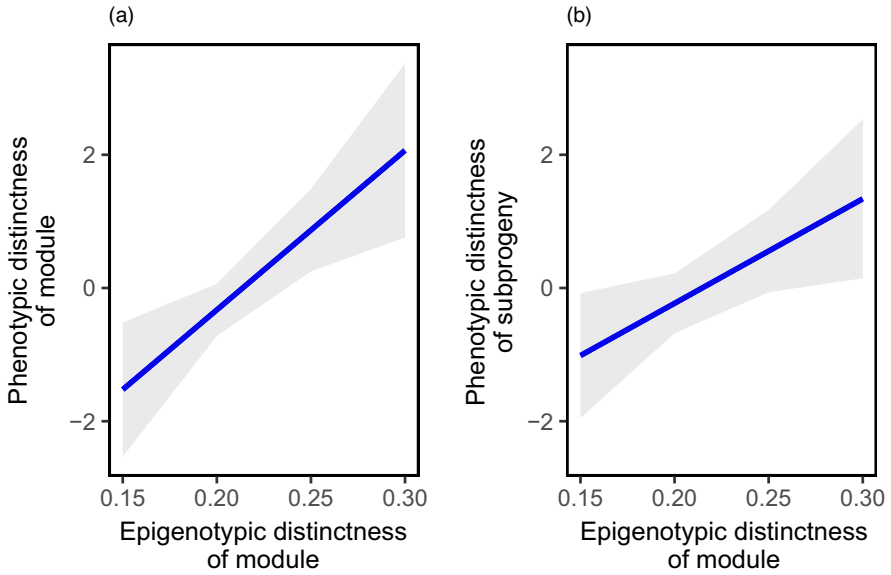

FIGURE 1 Mean predicted marginal effect of intraplant epigenotypic distinctness of a module on the phenotypic distinctness of the module itself (a) and on the phenotypic distinctness of the module's subprogeny obtained in the greenhouse (b). Confidence intervals (95%) of prediction shown as shaded areas. 'Intraplant distinctness' stands for the standardized distance of each module or subprogeny to the plant's centroid in the given epigenotypic or phenotypic space, which provides a measurement of epigenetic or phenotypic disparity relative to the plant mean value. All axes have comparable standardized scales, in standard deviation units with respect to the mean. See Table 1 for analytical results, statistical significance levels and additional details.

the plots of marginal effects in Figure 1. Within *L. latifolia* plants, therefore, the more epigenotypically distinct a particular module, the more phenotypically distinct the module itself and its greenhouse subprogeny.

Relative importance analysis of the regression of phenotypic distinctness of subprogenies against epigenotypic and phenotypic distinctnesses of extant maternal modules revealed that the predictive importance of maternal modules' epigenotypes (72.4%) was considerably greater than that of maternal modules' phenotypes (27.6%).

3.2 | Univariate analyses

Variation among modules of the same plant in the methylation state of some MS-AFLP markers did significantly predict variation in phenotypic traits among the modules themselves and among their greenhouse subprogenies. Variation in module phenotypic traits was associated with intraplant variation in the methylation state of 12 different markers (Table S2). Each module trait was significantly associated with 2–9 different markers, which together predicted between 16% and 51% of the trait's intraplant variation (Table 2). Trait variation among subprogenies of the same plant was significantly associated with the methylation state in the maternal module of 11 different markers (Table S2). Each subprogeny trait was associated with 3–4 markers, which collectively predicted 26%–34% of among-subprogeny variation in each trait (Table 2).

Most statistically significant MS-AFLP marker–trait associations across modules or across subprogenies of the same plant involved a decline in trait value from the methylated to the unmethylated state of the marker (Figure 2). Markers predicting intraplant variation in traits of modules and subprogenies were largely non-overlapping, as only two markers out of 21 were simultaneously associated with the two groups of traits (Table S2).

TABLE 2 Number of methylation-sensitive amplified fragment length polymorphism (MS-AFLP) markers significantly associated with intraplant, among-module variation in phenotypic traits of the modules themselves and among subprogenies in the greenhouse, and their combined predictive value of intraplant phenotypic variation. See Table S2, for marker identification and further details

		MS-AFLP markers associated with extant and transgenerational intraplant variation	
Sampling units	**Trait**	***N***	**Combined predictive value (marginal R^2)**
Modules	Inflorescence length	5	0.402
	Mean seed mass	2	0.157
	Log total seed number	9	0.514
	Log total seed mass	9	0.466
Subprogenies	Seed germination rate	3	0.269
	Time to germination	3	0.259
	Mean seedling mass	4	0.261
	Fungal disease frequency	3	0.337

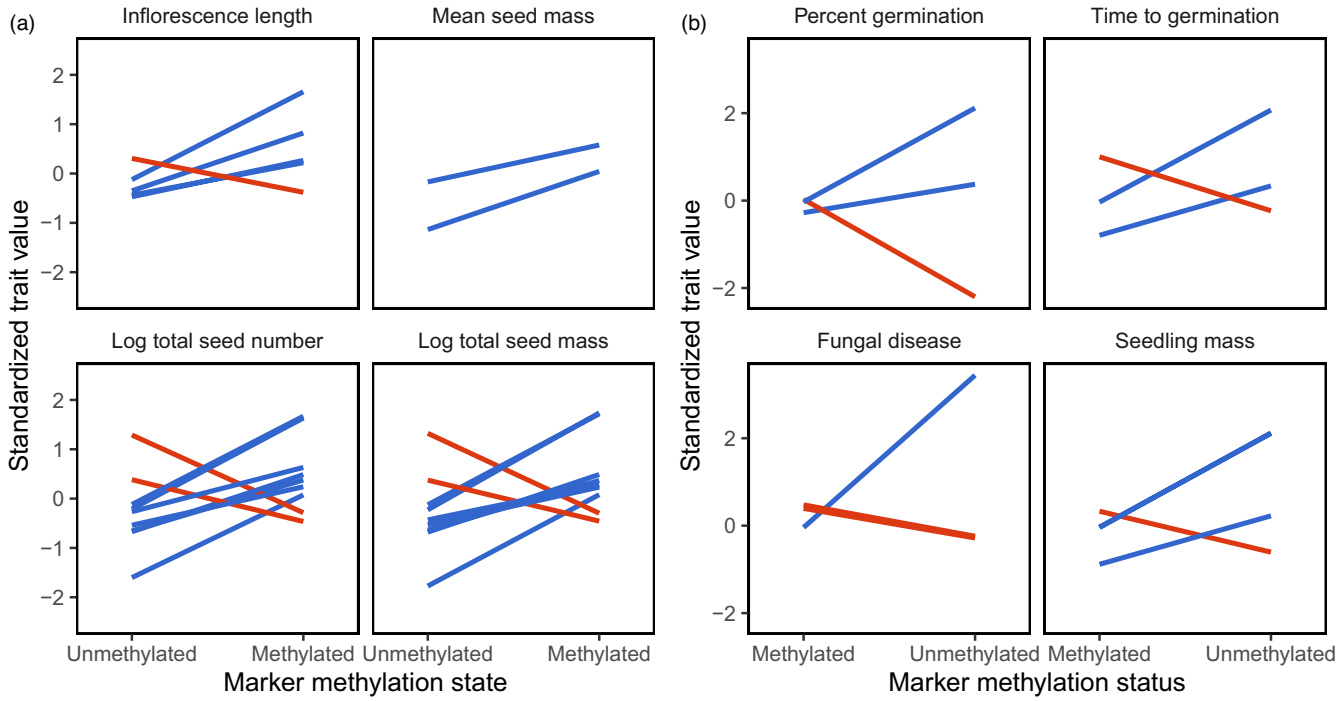

FIGURE 2 Mean predicted effects of intraplant variation in the methylation state of methylation-sensitive amplified fragment length polymorphism markers significantly associated with the phenotypic traits of modules (a) and subprogenies (b) considered in this study. Lines in each plot correspond to the markers significantly associated with intraplant phenotypic variation. A negative relationship (red lines) predicts a decline in trait value with a change from unmethylated to methylated state of the marker, while the reverse is true for a positive relationship (blue lines). Vertical axes are scaled in standard deviation units with respect to the mean. See Table S2 for individual markers involved, slope estimates and statistical significance levels.

4 | DISCUSSION

Results of both multivariate and univariate analytical approaches support a role of intraplant epigenetic mosaicism as a source of individual- and population-level variation in ecologically relevant plant traits, as envisaged by the EMH. First, predictable relationships were found between epigenotypic and phenotypic variation across the modules of individual *L. latifolia* shrubs. And second, phenotypes of subprogenies from different modules of the same plant grown under homogeneous conditions in the greenhouse were predictably related to the epigenotype of the maternal module which produced the seeds. These two aspects of results will be discussed in turn below.

4.1 | Extant phenotypic and epigenotypic mosaicism

Interest on the ecological significance of subindividual variation in plants has historically focused on the relatively infrequent examples of discrete variation involving discontinuous variants of leaves

(heteroblasty, heterophylly), fruits (heterocarpy) or seeds (heterospermy, heteromorphism) (Imbert, 2002; Mandák, 1997; Matilla et al., 2005; Wells & Pigliucci, 2000; Zotz et al., 2011). In contrast, continuous intraplant variation in quantitative traits remains comparatively unexplored despite generally representing a major source of population-wide variance in functionally important traits (Herrera et al., 2015; Ishii & Harder, 2012; Sobral et al., 2013; Zywiec et al., 2012). Results found here for *L. latifolia* conform to this pattern (see also Alonso et al., 2018), as variation among modules of the same individual in the set of fecundity-related traits considered was found to account for 51% of total phenotypic variance in the whole set of plants sampled.

Associations between epigenotypes and phenotypes across individuals, populations or species have been frequently documented in recent years for model and non-model plants, either in the wild or under experimental conditions (e.g. Amoah et al., 2012; Chano et al., 2021; Foust et al., 2016; Medrano et al., 2014; Paun et al., 2010; Róis et al., 2013; Wang et al., 2020; Wilschut et al., 2016). In contrast, similar epigenotype–phenotype associations across different homologous parts of the same plant individual remain basically unreported, and the few documented instances refer to discrete phenotypic variation in leaves or flowers (Bitonti et al., 1996; Herrera & Bazaga, 2013; Marfil et al., 2009; but see Alonso et al., 2018; Bian et al., 2013). Results of the present study, showing that epigenotypic and phenotypic variation were significantly correlated across modules of the same *L. latifolia* shrub, provide one of the few examples to date of intraplant association between epigenotypes and continuous traits. We found that disparity of modules relative to the plant's mean phenotype was directly related to their disparity relative to the plant's mean epigenotype, and that intraplant variation in the methylation state of a subset of MS-AFLP markers predicted variation in all phenotypic traits considered. All traits considered exhibited a significant association across modules of the same plant with at least two MS-AFLP markers.

Interpretation of these results is subject to the caveat that marker–trait associations provide only indicative evidence and should not be taken as proofs of causality (Platt et al., 2010). Keeping this in mind, circumstantial evidence is compatible with the interpretation that marker–trait associations found here can stem from the markers involved falling within some genomic region directly involved in the control of the associated traits. Cytosine methylation states in anonymous 5′-CCGG motifs probed with MS-AFLP markers are stably associated with phenotypic effects in other species, including some of the traits considered here such as seed size and inflorescence length (Akimoto et al., 2007; Amoah et al., 2012; Johannes et al., 2009; Long et al., 2011; Marfil et al., 2009; Xiao et al., 2006; Xu et al., 2016). Further circumstantial support for a causal interpretation of the relationship between epigenetic and phenotypic variation within *L. latifolia* plants is provided by experimental results of Herrera et al. (2019) with the perennial herb *Helleborus foetidus*. In that study, experimental manipulation of genomic DNA methylation in different ramets of the same individual induced intraplant variation in inflorescence and fecundity traits, and trait values declined in ramets with experimentally hypomethylated genomes. The latter agrees with the prevailing relationships found here across modules of *L. latifolia* plants between trait values and methylation state of individual MS-AFLP markers. Irrespective of the actual mechanistic basis underlying them, however, extant intraplant associations between epigenotypes and phenotypes revealed by this study provide empirical support for the link between phenotypic and epigenotypic mosaics proposed by the EMH.

4.2 | Transgenerational correlates of extant epigenotypic mosaicism

An important result of this study was that the variable epigenotypes of different modules of the same plant not only predicted extant phenotypic distinctness of these modules, but also phenotypic differences among subprogenies from different modules. The transgenerational effects of maternal features (both genetic and environmentally induced) on progeny traits have been thoroughly documented for many plants including *L. latifolia* (Biere, 1991a, 1991b; Herrera, 2000b; Mazer, 1987; Mazer & Gorchov, 1996; Roach & Wulff, 1987). To the best of our knowledge, however, the possibility that architecturally defined sectors of the same maternal individual can sexually produce progenies with distinct phenotypes has never been explicitly envisaged or tested in studies of parental effects in non-clonal wild plants (Herman & Sultan, 2011; Mazer & Gorchov, 1996; but see Holeski et al., 2009, for an example with asexually produced progenies). The transgenerational associations between epigenotypes and subprogenies found here, albeit statistically significant, were quantitatively weaker than the extant associations between epigenotypes and phenotypes of modules, as shown by the smaller fixed-effect parameter estimates (Table 1) and marginal R^2 (Table 2) of the corresponding mixed models. This weaker transgenerational epigenotype–phenotype association should be expected. Although *L. latifolia* flowers are self-compatible, seeds from cross-pollination are more likely to produce successful seedlings (Herrera, 2000b). Seeds used in the greenhouse experiment were from naturally pollinated flowers; hence, subprogeny phenotypes most likely reflected unaccounted paternal effects to an unknown extent.

Transgenerational associations between module epigenotypes and subprogeny phenotypes most likely were the outcome of direct and indirect causal pathways. Direct pathways probably involved the inheritance of influential markers. The methylation state of MS-AFLP markers in maternal parents has extensive transgenerational transmission in *L. latifolia* (Herrera et al., 2018); thus, some of the module–subprogeny associations found here could reflect the inheritance of methylation state in markers which are causatively linked to progeny traits, as found in other species. For example, phenotypic variation of oak seedlings is related to their methylation level (Browne et al., 2020); in the shrub *Pistacia lentiscus*, progeny performance (time to emergence, seedling size) is related to inherited methylation patterns (Albaladejo et al., 2019); and transgenerational defence induction often depends on

epigenetic inheritance (Holeski et al., 2012; Sobral et al., 2021). Indirect effects, on the other hand, would arise whenever the associations found between maternal modules' epigenotypes and the phenotypes of their subprogenies were mediated by the influence of the modules' phenotypes on their offspring. In *L. latifolia*, seed size influences germination rate and seedling size, and inflorescence length is related to pollinator composition, which, in turn, influences seed quality and seedling performance including emergence rate (Herrera, 2000a; C. M. Herrera, unpublished data). We attempted to statistically dissect the relative importance of such direct and indirect effects on phenotypic heterogeneity of subprogenies by application of 'relative importance' analysis (Grömping, 2006, 2015). Results showed that direct effects had higher quantitative importance than indirect ones in the prediction of phenotypic differences between subprogenies (72% vs. 28%, respectively). Although these figures must be interpreted cautiously, they suggest that inheritance of the heterogeneous epigenetic features of the maternal modules was the main factor accounting for phenotypic heterogeneity among subprogenies of the same maternal parent.

All seed and seedling traits considered here have well-known effects on the number and quality of offspring contributed to the next generation (e.g. Kalisz, 1989; Mazer, 1987; Stanton, 1984, 1985; see Herrera, 2000a, 2000b, for *L. latifolia*). Consequently, given the transgenerational associations between epigenotypes and phenotypes, modules of the same *L. latifolia* plant that differ in epigenotype would also most likely differ in fitness under field conditions, and the greater the epigenotypic distinctness of modules, the greater the expected variance in fitness of their subprogenies. Our results are particularly robust because associations between fitness-related phenotypic traits and epigenotypes were tested by means of mixed-effects models where plants were treated as random effects. A property of mixed-effects models is their potential for making inferences that apply to different populations of effects, or 'inference spaces' (Littell et al., 2006). In the context of the present study, the local *L. latifolia* population represents the 'broad inference space' (sensu McLean et al., 1991) and our conclusions refer specifically to that space, not just the 15 plants sampled. This means that model parameter estimates for fixed effects (Table 1, Table S2) refer to the local *L. latifolia* population as a whole, and do not depend on the particular sample of individual plants studied ($N = 15$) insofar as they are representative of the population (Bolker, 2015; Littell et al., 2006).

5 | CONCLUDING REMARKS

Understanding the ecological causes and consequences of epigenetic variation was included by Sutherland et al. (2013) among fundamental ecological questions, and they also stressed how little was known on these aspects at the time. Despite the considerable progress experienced by ecological epigenetics since then (Richards et al., 2017), the ecological significance of intraplant epigenetic variation still remains essentially unexplored. In addition to providing compelling support to key elements of the EMH, the relationships found here linking intraplant epigenotypic mosaicism with both extant and transgenerational heterogeneity in fitness-related traits suggest some intertwined ecological and evolutionary questions which have been not explored so far. The diversifying influence of epigenotypic mosaicism on progeny traits important for population recruitment (e.g. seed size and germination time, seedling size and susceptibility to disease) can act enhancing offspring establishment in heterogeneous and/or environmentally unpredictable habitats through diversifying bet-hedging mechanisms (Simons, 2009, 2011). In the case of abundant plants with dense populations like *L. latifolia*, the main expected consequence from this effect will be a greater resilience of local populations in the face of environmental changes. The quick shift in epigenotypic profile of the *L. latifolia* population studied here following experimental disturbance (Herrera & Bazaga, 2016) is consistent with this expectation. In the case of rare plants with sparse populations, epigenetically enhanced heterogeneity of individual progenies could eventually contribute to species survival via rescue effects (Carja & Plotkin, 2019). Insofar as conspecific individuals in wild plant populations differ in extent of intraplant epigenotypic variation, the preceding effects would lead to the magnitude of epigenetic mosaicism becoming itself a target of natural selection. Variable selective pressures could eventually lead to variable levels of epigenetic mosaicism across habitat types that differ in environmental predictability (Herrera, 2009).

Individual plants' ontogenies add a temporal dimension to the preceding ecological and evolutionary effects. Extant epigenetic mosaics exhibited by adult plants at a given point in their lifetimes, such as those studied here, are the instantaneous manifestation of a dynamic lifelong process of internal epigenetic diversification which takes place steadily, apparently at a constant epimutation rate (Herrera et al., 2021; Yao et al., 2021). Intraplant epigenotypic diversity, and possibly also phenotypic diversity, are thus expected to increase with plant age. Enhanced resilience or rescue effects derived from epigenetic mosaicism are thus predicted to vary with the age structure of populations, being most ecologically and evolutionarily significant in those dominated by older, epigenotypically most diverse individuals. These expectations have direct implications for plant population management and conservation which deserve further research.

AUTHORS' CONTRIBUTIONS

C.M.H. designed the research, did the field sampling, conducted the data analyses and led the writing; C.A. and M.M. designed and carried out the greenhouse experiment; P.B. performed the MS-AFLP analyses; all authors reviewed and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Epigenotypic and phenotypic data of maternal modules, and phenotypic data of greenhouse subprogenies used for this study are deposited at the public institutional repository of Consejo Superior de Investigaciones Científicas (Digital.CSIC), https://doi.org/10.20350/digitalCSIC/14692 (Herrera et al., 2022).

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