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Phenotypic dissimilarity index: Correcting for intra- and interindividual variability when quantifying phenotypic variation

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Abstract

In trait-based ecology, phenotypic variation (PVar) is often quantified with measures expressing average differences between populations standardized in the range 0–1. However, these measures disregard the within-population trait variability. In addition, some of them cannot be partitioned between populations. These aspects can either alter their interpretation or limit their applicability. To overcome these problems, we propose a new measure, the phenotypic dissimilarity (PhD) index, to quantify PVar between populations in scenarios of varying within-population interindividual trait variability. PhD can also quantify within-population PVar while accounting for intraindividual trait variability. Using simulated and real data, we show that using the PhD index becomes important when the within-population trait variability is not negligible, as in all ecological studies. By accounting for within-population trait variability, the PhD index does not overestimate PVar across an environmental gradient compared to other estimators. Traits inherently vary within species. Accounting for such variability is essential to understanding species' phenotypic responses to environmental cues. The proposed PhD index will provide ecologists with a tool for quantifying PVar within species and compare it between species at different levels of biological organization. We provide an R function to calculate the PhD index.

KEYWORDS
functional trait, interindividual trait variability, intraindividual trait variability, intraspecific trait variability, phenotypic plasticity, phenotypic plasticity index, phenotypic variation

INTRODUCTION

Functional traits, or trait (sensu Sobral, 2021) variability within species, are a key determinant of species' ability to cope with, and eventually adapt to, environmental factor variations. Accounting for this variability is considered essential to explaining the success of a species under contrasting environmental conditions (Albert et al., 2011). However, although intraspecific trait variability (ITV) can account for a significant amount of trait variation (for plants 19%–31% of the total plant trait variability within community at the global scale) (Siefert et al., 2015), ITV, or phenotypic variation (PVar), has been generally overlooked in trait-based ecology,

mostly for practical reasons (e.g., sampling effort/cost), coupled with a disproportional interest in interspecific studies (McGill et al., 2006). In recent years, many studies have spotlighted the adaptive role of ITV in organismal responses to environmental changes at different scales (e.g., Siefert et al., 2015). As a result, there has been increasing interest in developing methodological approaches to quantifying ITV in functional trait studies (e.g., de Bello et al., 2011; Niu et al., 2020).

One of the major challenges in developing these methods is the difficulty to include all the ITV underlying structure. ITV structure includes three levels of PVar among individuals of a species: (i) differences between populations of a species, (ii) interindividual variability (Bolnick et al., 2003), defined as the trait variability among individuals within a population/subpopulation, and (iii) within-individual variability (e.g., Herrera, 2017). Developing methods accounting for ITV structure is a pressing task for trait-based ecology.

Various indexes of phenotypic variation (Valladares et al., 2006, for a review) are generally used to quantify ITV. We refer to PVar indexes, instead of phenotypic plasticity indexes (PIs), as in Valladares et al. (2006), to clarify that PVar indexes indeed quantify ITV, but they cannot explicitly discriminate between the sources of ITV (genetic variability vs. phenotypic plasticity). A key advantage of using PVar indexes when quantifying ITV is that they are relatively simple to calculate. In addition, they are bounded between 0 and 1, which simplifies comparisons among species and facilitates straightforward ecological and evolutionary interpretations (Valladares et al., 2006). Among the many available PVar indexes, the most used remain the coefficient of variation (CV), the PI (Valladares et al., 2000), and the relative distance plasticity index (RDPI; Valladares et al., 2006). However, these measures disregard within-population and within-individual trait variability (Figure 1a–c). In addition, some of them cannot be partitioned between populations (Figure 1b). These aspects can either alter their interpretation or limit their applicability.

Here, by building on the RDPI proposed by Valladares et al. (2006), we aimed to formulate a PVar, called the phenotypic dissimilarity (PhD) index (Figure 1d), that is able to quantify ITV between populations, or groups of individuals growing in different environmental conditions, by accounting for the PVar within each population/group. PhD can also be used to quantify ITV across individuals within a single population while accounting for intraindividual variability. As such, the proposed PhD index is a good candidate to effectively estimate PVar between populations/groups by simultaneously accounting for different ITV components depending on the comparison being made. Using a worked example, we demonstrate that accounting for within-population variability is essential when calculating PVar between populations. We also provide an R function to calculate the PhD index.

METHODS

Index formulation

Studies focused on the PVar within a species are usually based on the evaluation of how a target trait changes across a set of varying environmental conditions, either experimentally or in the field. For a species that occupies K positions along an environmental gradient or levels of a treatment (henceforth referred to as environmental states), let N_k be the number of individuals in environmental state k $(k = 1, 2, \ldots, K)$ and τ_{ik} be the value of trait τ for individual i $(i = 1, 2, \ldots, N_k)$ in environmental state k.

According to Valladares et al. (2006), in the simplest case of only two environmental states, k and m, we can summarize the PVar of trait τ as the expected trait dissimilarity between two individuals drawn at random, one from each environmental state:

$$\mathrm{RDPI} = \sum_i^{N_k} \sum_j^{N_m} \frac{1}{N_k} \frac{1}{N_m} \times d_{ij}. \quad (1)$$

where $1/N_k$ is the probability of drawing individual i from state k, $1/N_m$ is the probability of drawing individual j from state m, and d_{ij} is any symmetric dissimilarity measure between the trait values of individuals i and j such that $d_{ij} = d_{ji}$ and $d_{ii} = 0$. For a single trait τ, Valladares et al. (2006) proposed to calculate d_{ij} as $d_{ij} = \frac{|\tau_{ik} - \tau_{jk}|}{(\tau_{ik} + \tau_{jk})}$. This measure is basically the univariate version of the Bray and Curtis (1957) dissimilarity, a multivariate dissimilarity index that has been extensively used by ecologists. With this index, since d_{ij} is bounded between zero and one, RDPI is also bounded in the same range.

Note that for the sake of generality, in Equation (1) we assume that the number of individuals in environmental states k and m does not necessarily have to be the same. Note also that by replacing the univariate measure d_{ij} with one of the many available multivariate dissimilarity measures in the ecologist's toolbox (e.g., Pavoine et al., 2009), we can easily generalize the calculation of PVar to multiple traits of various statistical types, for example, nominal, fuzzy, and ordinal (Legendre & Legendre, 2012; Pavoine et al., 2009).

In the context of biodiversity theory, the same index was independently proposed by Rao (1982) to measure the functional or phylogenetic dissimilarity between two species' assemblages. However, Rao (1982) noted that Equation (1) (i.e., RDPI) could not be immediately used as a measure of PVar between environmental states k and m. This is because

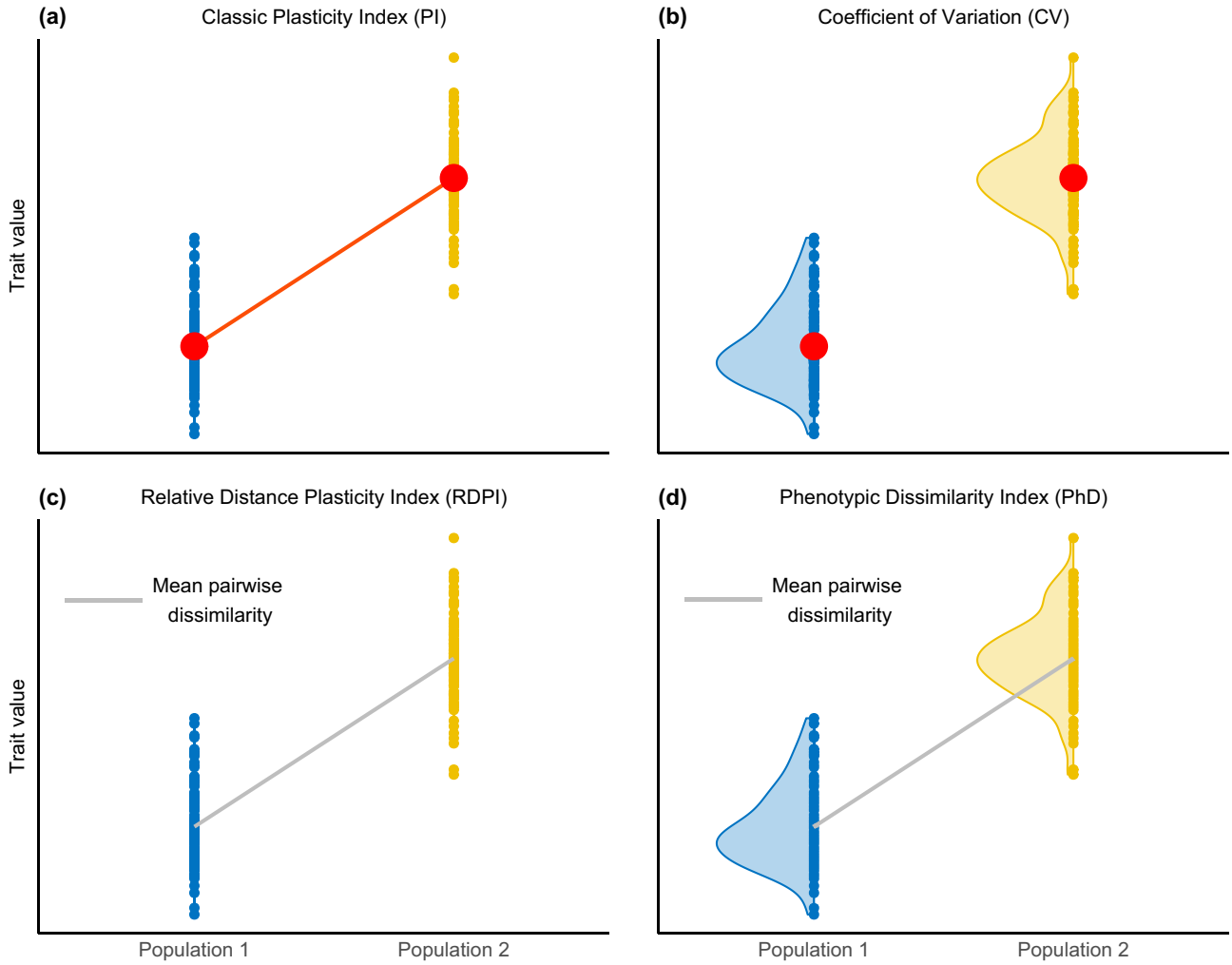

FIGURE 1 Representation of calculation of major estimators of PVar available in the literature compared to phenotypic dissimilarity (PhD) index proposed in this paper. (a) Classic plasticity index (PI, Valladares et al., 2000)—only trait means within populations are used to calculate PI (red dots) between populations. Mean differences between populations can, however, be biased if some individuals within a population contribute to the population mean largely compared to other individuals. (b) Coefficient of variation (CV)—mean and within-population trait variability (shaded areas) of a trait are used for CV calculation within each considered population, but CV cannot be partitioned between populations. (c) Relative distance plasticity index (RDPI)—calculates the mean phenotypic pairwise dissimilarity among all the individuals from different populations (gray line) without taking into account within-population trait variability. RPDI is an individual-based phenotypic variation (PVar) that could in principle account for both between- and within-population trait variability component of intraspecific trait variability (ITV). However, by not accounting for intrapopulation variability, RDPI values depend on the trait variability within each group under comparison. Moreover, by not applying this correction, resolved with the PhD index, RDPI incurs the same pitfall as PI. (d) PhD index—calculates the mean phenotypic pairwise dissimilarity among all individuals from different populations (gray line) by taking into account within-population trait variability (shaded areas).

the value of RDPI depends on the trait variability within both environmental states k and m, which can be defined as

$$D_k = \sum_{i,j}^{N_k} \frac{1}{N_k}\frac{1}{N_k} \times d_{ij} = \frac{1}{N_k^2}\sum_{i,j}^{N_k} d_{ij} \quad \text{and} \quad D_m = \sum_{i,j}^{N_m} \frac{1}{N_m}\frac{1}{N_m} \times d_{ij} = \frac{1}{N_m^2}\sum_{i,j}^{N_m} d_{ij}. \tag{2}$$

D_k and D_m thus summarize the expected trait dissimilarity between two individuals i and j drawn at random from environmental state k and m, respectively.

The consequence of the dependence of RDPI on D_k and D_m is that RDPI violates the basic condition that for two identical environmental states with the same number of individuals in each state and identical trait distribution, that is, if for each individual i in environmental state k there is an individual j in m such that $\tau_{ik} = \tau_{jm}$, the measure takes the value zero. In other words, if we compare a given environmental state with itself, the resulting value of RDPI can be larger than zero, thereby violating the intuitive assumption that a measure of trait dissimilarity for two identical environmental

states and trait distributions cannot be larger than zero (Pavoine & Ricotta, 2014).

To overcome this problem, Rao (1982) demonstrated that if the dissimilarity matrix **D** with elements d_{ij} is squared Euclidean, we have RDPI $\geq \frac{1}{2}(D_k + D_m)$. A dissimilarity matrix **D** with elements d_{ij} is said to be squared Euclidean if the associated dissimilarity matrix **Δ** with elements $\sqrt{d_{ij}}$ is Euclidean so that **Δ** can be associated with clouds of points in Euclidean space without distortions (Gower & Legendre, 1986). Accordingly, for a squared Euclidean dissimilarity coefficient d_{ij} bounded in the range [0, 1], Pavoine and Ricotta (2014) proposed the following normalized version of the RDPI:

$$\mathrm{PhD}_{km} = \frac{\mathrm{RDPI} - \frac{1}{2}(D_k + D_m)}{1 - \frac{1}{2}(D_k + D_m)}. \quad (3)$$

where PhD expresses the expected trait dissimilarity across environmental states in the range [0, 1].

In Equation (3), PhD_{km} is obtained in the usual way by linearly rescaling RDPI between its minimum and maximum value, $\mathrm{PhD}_{km} = \frac{(\mathrm{RDPI} - \min_{\mathrm{RDPI}})}{(\max_{\mathrm{RDPI}} - \min_{\mathrm{RDPI}})}$. This scaling method ensures that the same magnitude of PhD_{km} quantifies the same degree of PVar independently of the trait variability within each environmental state (Pavoine & Ricotta, 2014). Note here that the trait dissimilarity between pairs of individuals originally proposed by Valladares et al. (2006), $d_{ij} = \frac{|\tau_{ik} - \tau_{jm}|}{(\tau_{ik} + \tau_{jm})}$, is squared Euclidean (Legendre & Legendre, 2012). Therefore, it can be used to calculate PhD_{km} in a meaningful way. Note also that if d_{ij} is squared Euclidean, the resulting matrix of trait dissimilarities PhD_{km} among K environmental states $(k, m = 1, 2, ..., K)$ is Euclidean (Pavoine & Ricotta, 2014). Therefore, the PhD_{km} dissimilarities can be analyzed without distortions with standard distance-based ordination methods, such as principal coordinate analysis (PCoA). Ricotta et al. (2015) further showed that PVar could be related to the distances among the trait centroids of the K environmental states in the PCoA ordination space of the d_{ij} coefficients. Finally, a simple and intuitive way to generalize PhD_{km} to more than two environmental states consists in calculating the mean value of PhD_{km} for all possible $\frac{K(K-1)}{2}$ pairs of environmental states (e.g., Legendre & De Cáceres, 2013):

$$\mathrm{PhD}_K = \frac{\sum_{k>m}^{K} \mathrm{PhD}_{km}}{\frac{K(K-1)}{2}}. \quad (4)$$

PhD_K thus represents a generalization of Equation (3) to any number of environmental states. The R function to calculate the PhD index is available in Puglielli et al. (2022a).

Testing the effect of interindividual variability on PhD and RDPI estimates

To test the effect of the RDPI correction provided by the PhD index, we analyzed the linear relationship (slope and R^2) PhD–RDPI in two simulated scenarios (Figure 2) (Puglielli et al., 2022b). In each scenario, we simulated four populations, all of them always characterized by the exact same mean trait value of 20, but changing trait variance. In Scenario 1, we let the trait standard deviation within each population increase in 10 steps from 0.02 to 10 (for the full vector see Appendix S1: Table S1) while retaining an equal value of standard deviation for all populations at each step. We expected the PhD index and the RDPI to behave similarly—that is, both slope and R^2 tend to one. In this scenario, the PhD index and the RDPI should in fact scale proportionally since the RDPI is corrected for the same quantity in each pairwise comparison between populations. In Scenario 2, we let the trait standard deviation differ between populations both within and across the 10 simulated steps (vectors in Appendix S1: Table S1). Under this scenario, we expected a complete lack of relationship between the PhD index and RDPI because the differences in the trait variance between populations should render, by formulation, PhD index estimates independent of those of the RDPI (see *Index formulation* section). Full methodological details for the worked example are provided in Appendix S1: Section S1, where we also present two additional worked examples. In one example, we analyzed the relationship between PhD and CV, PI, and RDPI (Appendix S1: Section S2, Table S2, and Figure S1). In the other, we compared how PhD index and RDPI estimate within-populations PVar in a context of contrasting intraindividual variability (Appendix S1: Section S3 and Figure S2; Puglielli et al., 2022c).

RESULTS

The results of the worked example showed that when within-population trait variability increases or is heterogeneous across populations (Figure 3c,d; Appendix S1: Table S1), the PhD–RDPI relationship vanishes. The results from Scenario 1 showed that there was no relationship between PhD and RDPI unless the trait variance was close to zero (Figure 3a,b). In particular, we observed a significant PhD–RDPI relationship at the lowest trait variance case of Scenario 1, followed by a drop in terms of both slope (from 0.55 to ≈0) and R^2 (from 0.81 to ≈0)

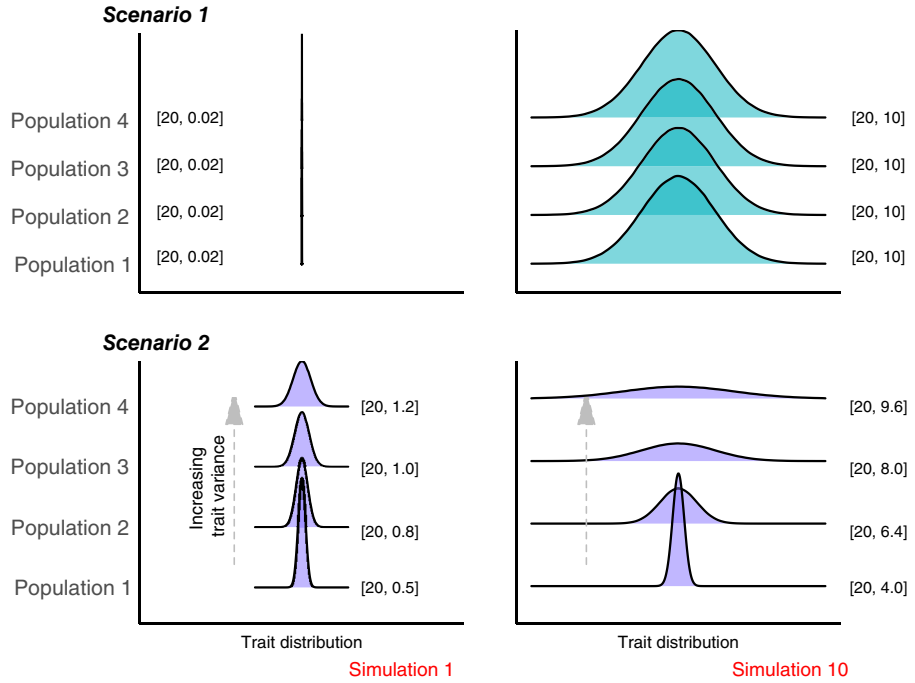

FIGURE 2 Simulated scenarios used in Example 1. The panels represent the extremes of the 10 cases that define each scenario. In each scenario, all the populations have the same mean, but trait standard deviation changes. In Scenario 1, the trait standard deviation is the same within each population at each simulation step, but it continuously increases from Simulation 1 to Simulation 10. In Scenario 2, the trait standard deviation differs between populations (vertical gray dashed arrow) at each simulation step, and it increases across cases as for Scenario 1. The numbers reported in parentheses are the trait mean and standard deviation used to simulate each population for the reported cases. The full list of standard deviation vectors used to simulate all the cases defining Scenario 1 and 2 are reported in Appendix S1: Table S1.

across the remaining nine simulated cases (Figure 3a,b). In Scenario 2, when the trait variance also differed between populations, the slope and R^2 of the PhD–RDPI relationship ranged between 0.01–0.19 and 0.001–0.50, respectively, without any clear pattern across simulations (Figure 3c,d).

DISCUSSION

Our results from the simulated scenarios highlight that the RDPI and the PhD index lose their coordination as soon as trait variance increases above zero. Thus, measures of mean differences between populations based on individual phenotypic dissimilarity not accounting for within-population trait variability, such as the RDPI, are sensitive to those extreme distances that largely contribute to the mean dissimilarity between populations. Such extreme distances indeed depend on the trait variance within populations. We argue that ignoring within-population trait variability might not return reliable PVar measures between populations. The same applies when quantifying interindividual variability within populations by accounting for intraindividual variability (Appendix S1: Section S3 and Figure S2). The proposed PhD index provides a solution to this problem because it quantifies PVar, or ITV, across populations of a species by accounting for the effect of interindividual trait variability within populations. We have shown that the PhD index is an important asset to estimate PVar in contexts where the trait variance cannot be considered negligible. Within-population trait variability can be considered negligible when it tends to zero, but this is impossible in ecological studies.

Another important consideration can be drawn from the results of an additional example (Appendix S1: Section S2). We show that, across an environmental gradient, and on average, the most common PVar indexes overestimate PVar compared to the PhD index (intercept test in Appendix S1: Figure S1). Thus, it appears that the overestimation of PVar can be a major effect of not accounting for within-population and within-individual trait variability when quantifying the mean dissimilarity between and within populations, respectively. This in turn depends on the elements of the intraspecific variability structure accounted for in the PVar index calculation (Appendix S1: Section S2). We call for more studies investigating how accounting for within-population trait variability affects PVar estimates across environmental gradients. This aspect is of particular interest because PVar estimates are often used to identify traits that are

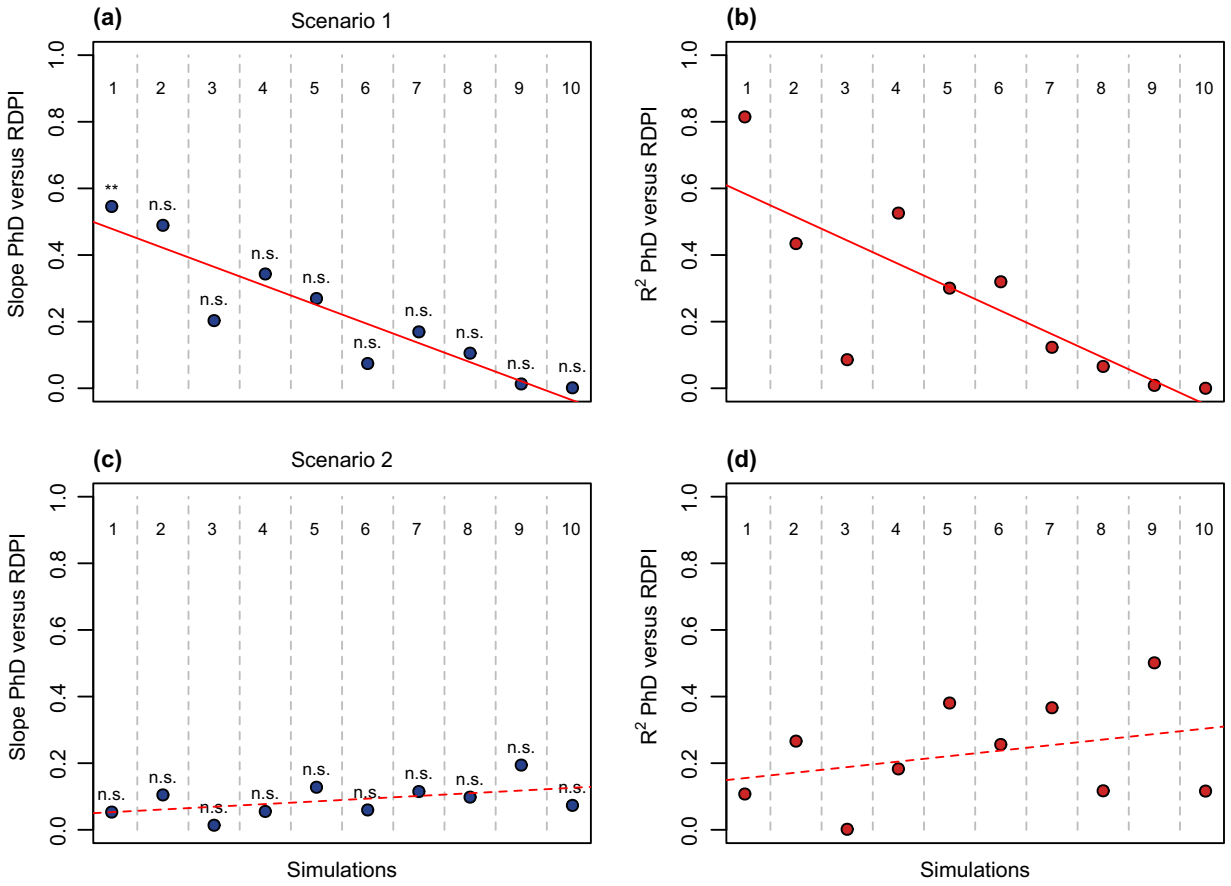

FIGURE 3 Changes in slope and coefficient of determination (R^2) for the relationship between phenotypic dissimilarity (PhD) index and relative distance plasticity index (RDPI) across simulated cases defining (a, b) Scenario 1 and (c, d) Scenario 2. More information on the simulated scenarios is reported in Figure 2 and in the *Methods* section. Solid and dashed lines represent significant and nonsignificant changes of the considered parameters across simulations just for a graphical purpose. ** and n.s. indicate, respectively, a significant or a nonsignificant PhD–RDPI relationship at $p \leq 0.05$.

most responsive to environmental gradients, and these traits are usually interpreted as drivers of species responses to environmental changes, including global climate change (Puglielli et al., 2017).

The PhD index we propose corrects the RDPI for the within-population inter- and intraindividual trait variability when the target is quantifying PVar between or within populations, respectively. When does accounting for within-population interindividual or intraindividual trait variability matter? Interindividual variability is a central topic in ecology and evolution because this variability represents the raw material for natural selection (e.g., Van Noordwijk, 1988). Each individual in a population can in fact use a subset of the available resources in a "population's niche" (Bolnick et al., 2003), and this can shape individuals' resource use strategies accordingly. Individual specialization within populations increases the variance of traits linked to the use of alternative resources, and such trait variance can ultimately differ between populations arranged along an environmental gradient. Therefore, we argue that the proposed PhD index is an essential tool for ecologists working with traits, as in the field within-population trait variability is always likely relevant, and it can alter quantifying PVar between populations.

By returning measures of intraspecific variability bounded between 0 and 1, and corrected for within-population trait variability, the PhD index can be used to compare species by their degree of PVar across the same environmental gradient. The PhD index also provides an alternative to classic analyses that can be used to test for between-population differences in phenotypes by controlling for the within-population trait variability/dissimilarity. These include analyses such as ANOVA or PERMANOVA, whose applicability is, however, constrained by the response variable residuals' distribution/dispersion, and their results, expressed in variance units, are not always immediately interpretable.

In sum, we showed that the PhD index represents a generalization of the RDPI, which is just a limit of the PhD index. Moreover, the PhD index is a PVar estimator that reaches the greatest level of generality compared to other estimators in terms of sources of trait variance accounted for in its calculation (Figure 1), and decomposability at different levels of biological organization.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The novel R function to calculate the PhD index (with an example) is available in Figshare at https://doi.org/10.6084/m9.figshare.17121134.v2 (Puglielli et al., 2022a). The simulated scenarios of Example 1 are available in Figshare at https://doi.org/10.6084/m9.figshare.19977500.v1 (Puglielli et al., 2022b). The underlying data of Example 3 are available in Figshare at https://doi.org/10.6084/m9.figshare.19977518.v1 (Puglielli et al., 2022c). Specific leaf area and soil water content data used in this analysis are available in Dryad at https://doi.org/10.5061/dryad.53550 (Carmona et al., 2014).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Appendix S1

Section S1

Detailed description of the simulated scenarios in Example 1

We analyzed the relationship PhD-RDPI indexes in two simulated scenarios (**Fig. 2** in the main text). Each scenario included 4 populations (i.e. 6 contrasts), all of them characterized by the exact same mean trait value but changing trait variance.

Scenario 1 - Given the same mean trait value per each population (trait value = 20), we allowed the trait standard deviation within each population to increase in ten steps from 0.02 to 10 (the full standard deviation vector is shown in **Table S1**). In other words, at each step, all the populations had the same trait mean and standard deviation, and only the standard deviation magnitude changed across steps (Fig. 2). At each step, we evaluated the linear relationship – i.e., slope and coefficient of determination (R^2) – between the PhD and the RDPI. We expected the PhD index and the RDPI to behave similarly - i.e., both slope and R^2 tend to one. This is because the trait variance within each population is always the same at each simulation step, and only the variance magnitude change. Thus, at each step, the PhD and the RDPI should vary proportionally considering that the RDPI is always corrected for the same quantity in each pairwise comparison between populations.

Scenario 2 - Given the same mean trait value per each population (trait value = 20), we allowed the trait standard deviation to vary along two dimensions (Fig. 2) : (i) trait standard deviation differed between populations at each of the ten simulated steps; (ii) trait standard deviation continuously increased across the ten steps (variance vectors are shown in **Table S1**). As for Scenario 1, at each step, we evaluated the linear relationship – i.e., slope and coefficient of

determination (R^2) – between the PhD and the RDPI. Under this scenario, we expected a complete lack of relationship PhD-RDPI, as the differences in the trait variance within populations should render, by formulation, PhD index estimates independent of that of the RDPI (see Index formulation section).

Table S1. Standard deviation values used to simulate trait distribution across the ten simulated cases for the four populations (*Pop.1* to *Pop.4*) included in the two simulated scenarios defining Example 1. The mean value of the trait was set to 20 for all the populations at each step.

		Simulation									
		#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**
Scenario 1	*Pop.1-4*	0.02	1.13	2.24	3.35	4.46	5.57	6.67	7.78	8.89	10.00
Scenario 2	*Pop.1*	0.04	0.16	0.36	0.64	1.00	1.44	1.96	2.56	3.24	4.00
	Pop.2	0.06	0.26	0.58	1.02	1.60	2.30	3.14	4.10	5.18	6.40
	Pop.3	0.08	0.32	0.72	1.28	2.00	2.88	3.92	5.12	6.48	8.00
	Pop.4	0.10	0.38	0.86	1.54	2.40	3.46	4.70	6.14	7.78	9.60

Section S2

Comparing the PhD index with other phenotypic variation (*PVar*) estimators

For this example, we used an already published dataset of some Mediterranean grassland species growing along a topographical and soil water content gradient (**Table S2**) (Carmona et al. 2015). From this dataset, we used specific leaf area (SLA) measurements. Following Valladares et al. (2006), we selected the following *PVar*: (i) coefficient of variation (CV); (ii) classic plasticity index (PI); and iii) Relative Distance Plasticity Index (RDPI) (see **Table 1** and **Fig. 1a-c** in the main text for details on PI, CV and RDPI calculations). For this example, we selected only species that were present in at least four plots along the considered gradient (**Table S2**). Seventeen species were included in the final analysis, and we used these species set to calculate species-specific mean PhD index and means of the other four *PVar* across plots - i.e. species mean phenotypic shift across a soil water content gradient. Thus, the final dataset included species-specific mean values of PhD, CV, PI and RDPI of SLA calculated across the gradient (**Table S3**). We explored the relationship between PhD index and the other four *PVar* estimators using linear regression analysis. Finally, to further characterize the differences between the indexes in terms of similarity and magnitude of yielded results, we tested if the slope and the intercept of each linear relationship between the PhD index and the other *PVar* indexes differed from the 1:1 relationship. This test was carried out using the slope.test and elev.test functions included in the 'smatr' R package (Warton et al. 2006, 2012) at $p \leq 0.05$.

Results of Example 2 (**Fig. S1a-c**) showed that the PhD index is positively related with the three considered *PVar* indexes, with R^2 values ranging between 0.26 and 0.47 (p always ≤ 0.05). All the relationships had intercepts significantly greater than zero (**Fig. S1b-d**). Thus, across an

environmental gradient, and on average, the most common *PVar* indexes overestimate *PVar* compared to the PhD index (intercept test, **Fig. S1a-c**). In the case of CV and PI, this was true at any value of PhD (slopes of the relationship CV-PhD and PI-PhD did not differ from unity, **Fig. S1a-b**). Only the slope of the linear relationship RDPI-PhD (0.60; $CI_{95\%}$ = 0.25 – 0.96) statistically differed from unity (**Fig. S1b-d**), suggesting that the two indexes converge towards the 1:1 line at very high values of PhD index, which are in turn linked to a rather small/negligible within population trait variance, in line with the considerations in the main text. The relationships PhD-RDPI and PhD-CV had a greater R^2 than the relationship PhD-PI (**Fig. S1a,c**), possibly owing to the fact that only CV and RDPI account for at least one of the elements defining the PhD index itself. In the Discussion section of the main text, we argue that the overestimation of *PVar* can be the main effect of not accounting for within-population trait variability when quantifying the mean dissimilarity between populations. This statement is supported by the intercept of the relationship CV-PhD being close to zero (**Fig. S1a**), meaning that accounting for the trait variance, necessary for CV calculation, partly buffers the differences in mean *PVar* estimates returned by CV and PhD. However, CV does not include individual-based dissimilarities for its calculation and this is still sufficient to inflate average *PVar* estimates.

Table S2. Subset of the Mediterranean grassland species from Carmona et al. (2015) included in Example 2. Species presence along the soil water gradient (1 = highest soil water content; 40 = lowest soil water content) is shown.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Anthemis arvensis									■			■			■	■		■		■		■		■	■					■	■			■	■	■	■		■	■
Anthyllis cornicina						■	■	■					■	■		■																								
Biserrula pelecinus		■				■	■	■	■	■	■	■	■	■	■	■		■		■	■	■	■	■	■	■					■	■	■	■		■				■
Bromus hordeaceus									■				■		■		■							■		■	■	■	■				■		■		■				
Bromus rubens																								■		■	■	■	■	■	■	■	■	■						
Crepis capillaris												■								■						■									■						
Dactylis glomerata	■		■									■	■					■			■						■			■			■						
Echium plantagineum					■	■		■						■	■	■	■	■	■	■	■	■			■		■	■	■			■				■	■	■	■
Eryngium campestre																			■				■	■		■												■		
Hordeum murinum																					■			■	■	■	■	■				■							■	■
Medicago sativa																								■	■			■			■						■			
Plantago lagopus	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■				■			■	■	■	■	■	■	■	■		■	■
Tolpis barbata	■		■		■				■			■				■																							
Trifolium cherleri	■	■	■	■	■	■	■				■	■	■	■	■			■		■		■	■								■		■	■	■	■			■
Trifolium glomeratum													■		■							■	■			■													
Trisetum paniceum																	■	■	■	■	■	■																		
Vulpia ciliata																	■	■	■	■															■					

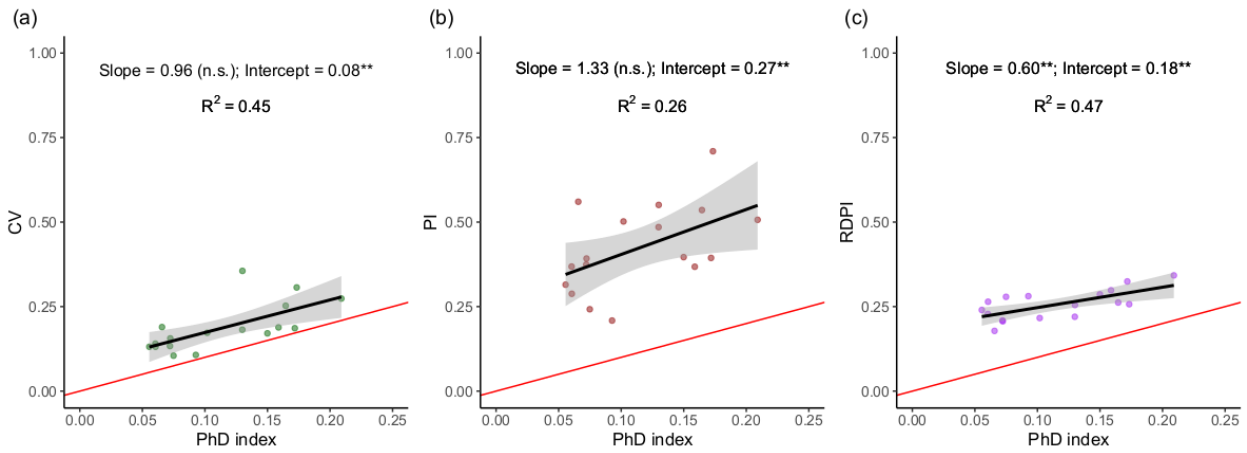

Fig. S1. Relationship among Phenotypic Dissimilarity Index (PhD) and common estimators of phenotypic plasticity across 17 grassland species from the Carmona et al. (2015) dataset (see **Table S2-3**). **(a)** coefficient of variation (CV); **(b)** classic Plasticity Index (PI, Valladares et al. 2000); **(c)** Relative Distance Plasticity Index (RDPI, Valladares et al., 2006). All the relationships were significant at $p \leq 0.05$. Red line represents the 1:1 relationship. ** indicates when the slope or the intercept of the observed relationship statistically differ from the slope or the intercept of the 1:1 relationship. The R^2 of each relationship is also shown.

Section S3

Testing the effect of intraindividual variability on PhD and RDPI estimates

In the main text, we compared the behavior of the proposed Phenotypic Dissimilarity Index (PhD) to that of the RDPI index (Valladares et al., 2006) in summarizing phenotypic differences between populations under multiple scenarios of changing trait variance (**Example 1**). In particular, as the PhD index corrects the RDPI for within population trait variability when quantifying phenotypic variation between populations, the two indexes converge only when the trait standard deviation within populations is zero, impossible in ecological studies. As such, when the trait standard deviation is greater than zero, the relationship between the two indexes rapidly vanishes. In view of the correction provided by the PhD index, in the main text, we also propose that the PhD index can be similarly used to quantify interindividual variability within a site while controlling for within-individual trait variance. Here we present a worked example to display the potential applicability of the PhD index to such a study case.

To this aim, we used an unpublished dataset including intraindividual measurements of leaf length from two population of *Betula nana* (10 individuals per population) naturally growing in Estonia and in the Svalbard Islands under contrasting environmental conditions. For the Estonian population, the number of measured leaves at the individual level ranged between 20 and 281. For the Svalbard population, the number of measured leaves at the individual level ranged between 46 and 302. *Betula nana* individuals from Estonian population were sampled in July 2016 from Männikjärve bog in Jõgeva county (58° 87’ N, 26° 25’ E). Individuals from the Svalbard population were sampled during July 2015, on a shrubland near Colesbukta (78° 10’ N, 14° 96’ E). Individuals from both populations were sampled from the middle of the populations,

from as uniform conditions within the community as possible. The sampled individuals were removed from the ground level, placed into a plant frame, and stretched between newspapers. In the lab, all the individuals were dried for 24 hours at 50°C. After drying, all the leaves were removed from the plants and measured individually, and leaf length (mm) was measured as the distance from where the petiole is attached to the tip of the leaf.

Svalbard individuals showed greater intraindividual variability in leaf length compared to the individuals from the Estonian population (**Fig. S2a,b**). In particular, the mean coefficient of variation at the individual level was 0.30 and 0.20 for Svalbard and Estonian population, respectively. However, the median leaf length value at the individual level varied 1.6 and 1.2 fold for the Estonian and the Svalbard population, respectively. In other words, the intraindividual variability was greater for Svalbard population compared to the Estonian one, while interindividual variability was greater for the Estonian population compared to the Svalbard one (**Fig. S2a,b**).

Accordingly, when separately calculating mean PhD and RDPI indexes for leaf length across individuals for the Estonian and the Svalbard population, the results show that both RDPI and the PhD index are greater for the Estonian than for the Svalbard population. However, the median difference in RDPI values between sites is of only 0.03 units (**Fig. S2a,b**), indicating a relatively similar degree of interindividual variability within each site, considering that the index can assume values between 0 and 1. This seems in contrast with the previous observation, as the individuals seems to be, on average, more phenotypically distant within the Estonian than within the Svalbard population. Such difference in the interindividual variability within population is in

turn better captured by the PhD index (0.03 and 0.005 for the Estonian and the Svalbard population, respectively) (**Fig. S2a,b**). In sum, the PhD index, by accounting for intraindividual variability when quantifying interindividual variability within populations, better expresses the average differences in phenotypic variability among individuals within a population.

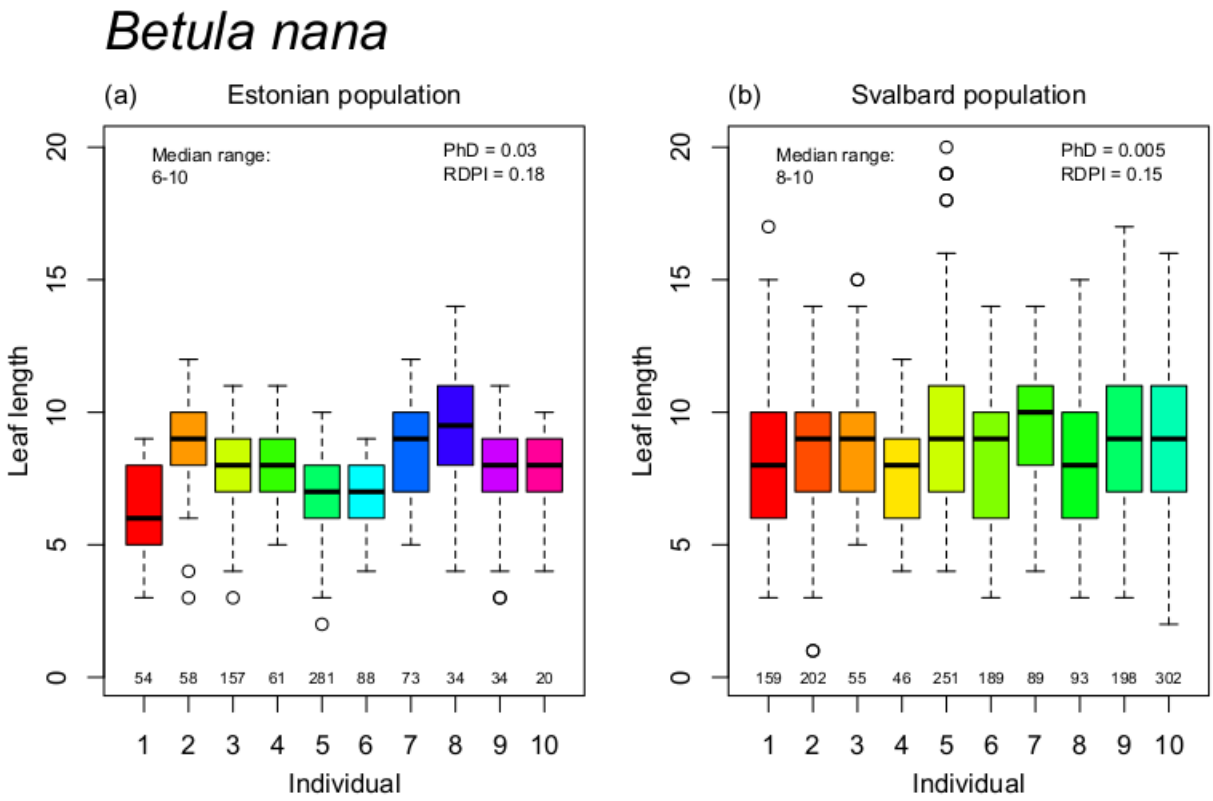

Fig. S2. Testing the effect of intraindividual variability on quantification of interindividual phentotypic variation for leaf length in two populations of *Betula nana* naturally growing in Estonia and in the Svalbard Islands. **(a)** Boxplot summarizing the intra- and interindividual variability in leaf length within each individual sampled in the Estonian population. **(b)** Boxplot summarizing the intra- and interindividual variability in leaf length within each individual sampled in the Svalbard population. The leaf length median range within population and the sample size per each individual are also shown. Interindividual phenotypic variation quantified using the Phenotypic Dissimilarity Index (PhD) and the Relative Distance Plasticity Index (RDPI, Valladares et al., 2006) is shown in each panel.

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