horticulturae

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Heterogeneity in Seed Samples from Vineyards and Natural Habitats Along the Eurasian *Vitis vinifera* Range: Implications for Domestication and Hybridization

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Abstract: By exploring seed samples from vineyards and natural habitats across the Eurasian range of *Vitis vinifera*, our analysis revealed substantial morphological variation within populations. Through the analysis of domestication index values, probabilities, and entropy, we assessed seed diversity. Samples with high domestication probability values—predominantly from vineyards—exhibited low heterogeneity and entropy, with similar patterns observed in natural habitats, suggesting the presence of feral vines. In parallel, seeds with low domestication index values, found mainly in natural habitats, also displayed low entropy and are likely associated with *Vitis sylvestris* or other wild Vitaceae species. Intermediate domestication values pointed to hybrid swarms, highlighting the crucial role of hybridization in the development of modern grapevine cultivars. The study identified mixed populations across the Iberian Peninsula, Italy, and the South Caucasus, which act as significant gene reservoirs. A domestication gradient is evident, with higher domestication rates in the South Caucasus compared to Western Europe and East Asia. The results demonstrate the significance of these mixed populations as repositories of genetic diversity, underscoring their conservation value, particularly considering the negative impact of habitat alterations, especially in riparian forests due to major public works.

Keywords: feral; grapevine; *Vitis sylvestris*; introgression; heterogeneity estimate; Shannon index; hybridization; wild relatives; biodiversity

Academic Editors: Qian Zha and Meiling Tang

Received: 30 November 2024
Revised: 5 January 2025
Accepted: 8 January 2025
Published: 15 January 2025

Citation: Rivera, D.; Valera, J.; Maghradze, D.; Kikvadze, M.; Nebish, A.; Ocete, R.; Ocete, C.Á.; Arnold, C.; Laguna, E.; Alcaraz, F.; et al. Heterogeneity in Seed Samples from Vineyards and Natural Habitats Along the Eurasian *Vitis vinifera* Range: Implications for Domestication and Hybridization. *Horticulturae* **2025**, *11*, 92. https://doi.org/10.3390/horticulturae11010092

1. Introduction

Heterogeneity represents a fundamental organizing principle of biological systems, manifesting across multiple scales from the molecular to the ecosystem level. This inherent variability encompasses the morphological, functional, and interactive dimensions of organisms and their populations. In cultivated species such as *Vitis vinifera* L., population heterogeneity is particularly evident in the phenotypic and genetic variation among individuals within defined cultivar groups.

The application of entropy—a concept derived from information theory and thermodynamics—provides a robust quantitative framework for analyzing such biological diversity. In population biology, entropy metrics effectively quantify the distribution patterns of phenotypic traits, genetic markers, or physiological responses within populations [1]. Higher entropy values correspond to increased population heterogeneity, reflecting greater informational complexity and reduced predictability in population structure [2].

In viticultural systems, entropy values demonstrate significant variation between genetically diverse populations and clonally propagated monocultures. Higher entropy metrics in heterogeneous vineyards reflect increased informational complexity across multiple parameters, including genotypic diversity, pathogen-resistance profiles, and yield characteristics. This quantitative framework for assessing population heterogeneity has substantial implications for viticulture, informing germplasm selection, disease-resistance breeding programs, and the development of sustainable cultivation strategies. Moreover, understanding the relationship between population entropy and phenotypic plasticity provides valuable insights for enhancing vineyard resilience in the context of contemporary viticultural challenges.

Grapevine domestication is a complex process that has likely occurred over thousands of years, starting in the Near East and South Caucasus at least since the Neolithic period, about 11,000 years ago [3]. The wild grapevine species *Vitis sylvestris* C.C. Gmelin is widely regarded as the primary ancestor of the cultivated grapevine *Vitis vinifera* L., although the species rank for *Vitis sylvestris* is not universally accepted. The domestication process has resulted in the selective modification of key phenotypic traits, including sexual system evolution from dioecy to monoecy, increased berry and foliar dimensions, enhanced sugar accumulation, and altered seed morphology characterized by larger seeds with a reduced proportional size relative to the berry dimensions [4]. *Vitis sylvestris* is a dioecious species, meaning that male and female flowers are found on separate plants. In contrast, *Vitis vinifera* is predominantly hermaphroditic, with both male and female reproductive organs present in the same flower. The transition in reproductive strategy facilitated the domestication process through enabling a consistent fruit set and yield stability [4].

The domestication of *Vitis vinifera* from its wild progenitor *V. sylvestris* represents a pivotal transformation in agricultural development. This process, characterized by the selection of desirable traits, has had profound cultural and economic implications. The emergence of viticulture and oenology as specialized fields underscores the grapevine's importance in various ancient civilizations. The dissemination of grapevine cultivation facilitated intercultural exchange, encompassing technological innovations and diverse cultural practices across regions of the Old World [4]. This agricultural advancement has not only shaped ancient societies but continues to influence modern viticultural practices and the global wine industry.

Genetic studies have provided substantial insights into the complexities of grapevine domestication, with molecular evidence suggesting, though not conclusively, the possibility of multiple domestication events involving diverse *V. sylvestris* populations or related taxa. This hypothesis is corroborated by the high genetic diversity observed in modern cultivated grapevines, reflecting a rich evolutionary history. The genetic diversity preserved in *V.*

sylvestris populations, despite traits such as small bunches, poor-quality fruit, flowers that fail to ripen, or the lack of fruit production in male individuals, represents a valuable resource for contemporary grapevine breeding programs. This diversity harbors valuable traits, including disease resistance and tolerance to environmental stress, which could be introgressed into cultivated grapevines to enhance their resilience in a changing climate [4].

The range of wild grapevine (*V. sylvestris*) spans from the Hindu Kush mountain range to Western Europe and the Mediterranean region, covering latitudes from 30–31° N (Ourika River, Morocco) to 49–50° N (Rhine River, Ludwigshafen am Rhein, in Germany) [5,6]. Wild grapevines are primarily dioecious, meaning they have separate male and female individuals [7]. Female flowers exhibit morphological hermaphroditism, but their pollen is non-functional. On the other hand, male flowers carry functional pollen and a non-functional gynoecium, although there can be exceptions where male flowers exhibit hermaphroditic characteristics, referred to as androids by Levadoux [8].

In *Vitis* species, pollen grain morphology is closely associated with the plant's reproductive strategy. Male and hermaphroditic vines produce tricolporate pollen grains, characterized by three germinal furrows (colpi) and pores. These structures facilitate the release of male gametes. In contrast, female vines produce acolporate pollen grains, which lack these apertures, rendering them functionally sterile [9,10].

Hermaphroditism in grapevine populations emerged through mutations affecting male reproductive development. This evolutionary modification of sexual morphology enabled self-fertilization capacity, a characteristic that significantly enhanced cultivar stability during domestication [11]. Cultivated grapevine varieties (*Vitis vinifera*) exhibit high levels of heterozygosity and genetic diversity [11]. This genetic variability is a consequence of the species' evolutionary history, multiple domestication events, ongoing hybridization with wild relatives, and human-mediated selection processes.

The differentiation between wild grapevines (*Vitis sylvestris*) and their domesticated counterparts (*Vitis vinifera*) has been a subject of extensive research in ampelography and viticulture. While several morphological characteristics are commonly cited as distinguishing features, recent studies have highlighted the complexity and variability of these traits.

Wild grapevines are generally characterized by a greater environmental hardiness and distinct reproductive features. They typically produce small, black berries with a rounded shape and acidic flavor, arranged in loose, small to medium-sized clusters. The plants are dioecious, exhibiting sexual dimorphism in leaf morphology. Additionally, wild grapevines are often reported to exhibit an open U-shaped or very open petiolar sinus, though variations in this trait may occur under different stress conditions. Their seeds are generally described as small, rounded, and characterized by a short beak [12–14].

Turkovic [15] proposed that the open petiolar sinus, reminiscent of American *Vitis* rootstock genotypes or cultivars like *V. rupestris* Scheele "du Lot" or *V. riparia* Michx. "Gloire de Montpéllier" could serve as a key distinguishing feature. However, this characteristic is not exclusive to wild grapevines and may occur in some cultivars.

The purported enhanced disease resistance in wild grapevines, with the notable exception of susceptibility to the erineum strain of *Colomerus vitis*, has been historically cited as a distinguishing characteristic between wild and cultivated *Vitis* species. Recent investigations have challenged this generalization, revealing that heightened resistance is not a consistent trait across all wild grapevine populations. Instead, empirical evidence indicates that significantly elevated resistance levels are confined to a limited subset of wild accessions. This finding underscores the complexity of disease-resistance mechanisms in *Vitis* species and highlights the need for nuanced approaches in comparative studies of wild and cultivated grapevines [16].

Distinguishing between *V. vinifera*, female *V. sylvestris*, and their hybrids presents significant challenges. The primary differentiating factors include leaf size and trunk thickness, which are generally larger in *V. vinifera*. Sexual dimorphism in leaf morphology is observed in *V. sylvestris* populations, with geographical variations [17]. Subtle differences in rhytidome (bark) structure may also be present. Historical observations (19th century) by Rathay [18] in the upper Danube valley noted sexual dimorphism in wild grapevines, with females exhibiting nearly entire leaves and males possessing highly divided (three-lobed) leaves [19].

Levadoux [8] demonstrated the relativity of these distinguishing traits, observing that wild grapevines can exhibit characteristics typically associated with cultivated varieties, while some primitive cultivars may resemble wild grapevines. Certain traits, such as black berry color, small size, rounded shape, high acidity, and loose clustering, tend to dominate in sexually propagated cultivars. While cultivar characteristics are maintained through vegetative propagation, they can undergo significant modifications during sexual reproduction. For instance, seeds of cultivars like Pinot Noir or Petit Verdot may acquire traits resembling those of *V. sylvestris* within a few generations of sexual reproduction.

This complexity underscores the need for integrative approaches, combining morphological, genetic, and ecological data, to accurately distinguish between wild and domesticated grapevines. The intricate relationship between wild and cultivated grapevines continues to challenge researchers, highlighting the importance of comprehensive studies in grapevine biology and evolution.

We emphasize the significance of investigating seed heterogeneity in both vineyards and natural habitats to evaluate the phenotypic and genetic diversity within grapevines, and as an indicator of introgression events. The examination of grape seeds serves as a valuable tool for assessing the domestication levels of grapevines, both in contemporary populations and archaeological contexts [11,16–35].

This study builds on previous research to distinguish between indigenous wild grapevines, hybrids, and feral varieties. The term "wild" is commonly associated with organisms growing without human intervention in natural habitats. However, "wild" plants in the ecological sense constitute a very heterogeneous complex in terms of their origin and morphology [35], as Levadoux [8] clearly stated in the case of the grapevine (Figure 1). In this context, "wild" is used to denote any type of grapevine in natural settings, encompassing a broad range of origins and morphologies. The term "wild" or "autochthonous wild" refers to naturally occurring grapevines classified at the species level as *Vitis sylvestris*. In contrast, grapevines exhibiting clear domestication traits are classified as *Vitis vinifera*, where they are referred to as feral or sub-spontaneous when found in natural habitats and as cultivars when cultivated in vineyards. Hybrids between indigenous and cultivated wild individuals are termed hybrids, without a specific name. Asian and American species, along with their hybrids, are designated by their accepted scientific names as listed in POWO [36] or GRIN [37] except for *Vitis sylvestris*.

Our research questions and hypotheses are rooted in the work of Levadoux [8] that defines a wild grapevine, *lambrusque* in French, *lambrusca* in Italian, and *labrusca* in Spanish, as any grapevine seemingly growing in a wild state.

Various types of wild grapevines within the complex *V. vinifera*–*V. sylvestris* are identified based on their habitat and origin, and the major types are illustrated in Figure 1:

- Post-cultural wild or post-cultural lambruscae thrive in abandoned vineyards, closely resembling the ampelographic characteristics and seed parameters of the same cultivar in cultivated vineyards.
- Sub-spontaneous feral lambruscae grow in uncultivated soil from seeds originating in domesticated grapevine vineyards. While sharing most characteristics with the parent

grapevine cultivar, they exhibit greater differences from wild grapevines in the same natural habitat.

- Spontaneous wild *lambruscae* represents a natural autochthonous element of Western Eurasian flora. This category has multiple origins:
    - (a) Colonial wild grapevines that arise from wild sub-spontaneous plants that have found favorable conditions for returning to the wild. They are naturalized but of cultivated origin.
    - (b) Autochthonous wild (spontaneous native) grapevines descended from ancestors that were likely never cultivated; these grapevines are predominantly dioecious, wild, native, and autochthonous.
    - (c) Wild mestizo grapevines resulting from the hybridization of native wild plants with post-cultural or sub-spontaneous lambruscae. They are naturalized but non-native due to exogenous parentage.

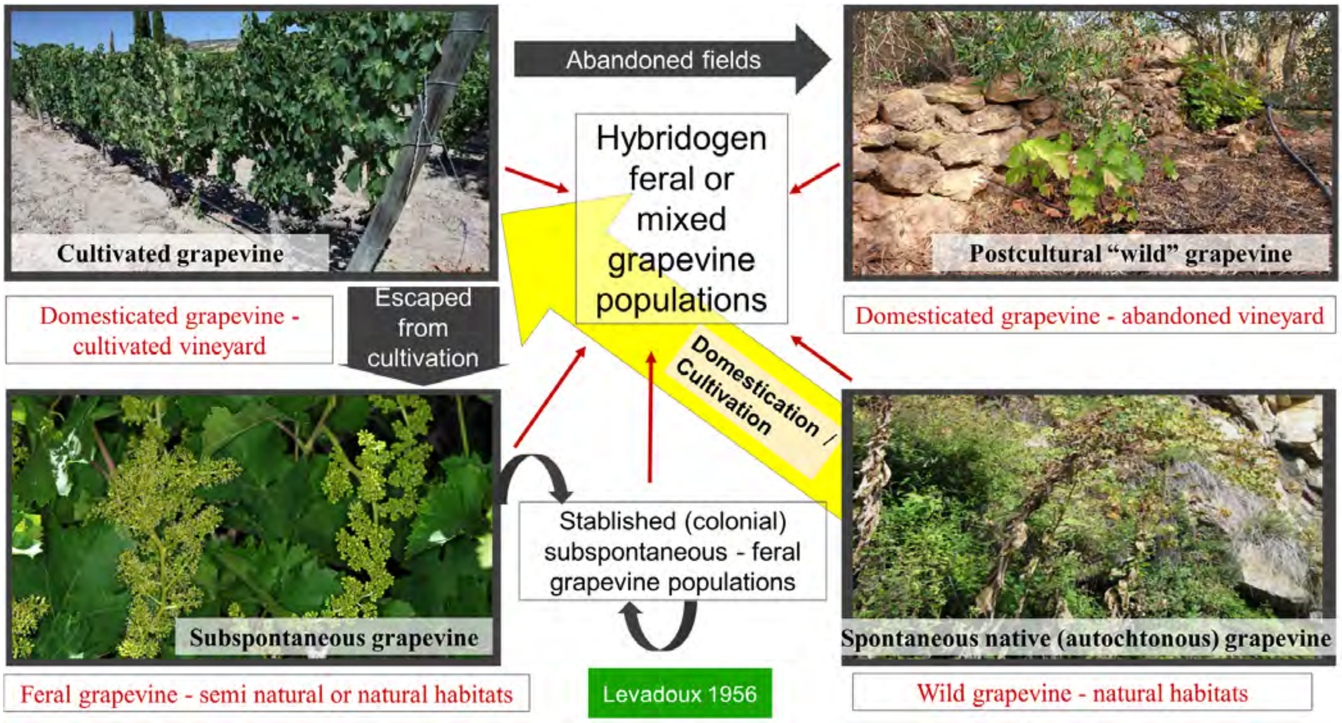

Figure 1. Relationships between cultivated and wild grapevine populations and different "wild" types. Image from D. Rivera based on Levadoux [8]. The yellow arrow represents a synthesis of widely accepted presumptions, scientific scenarios, and interpretations of findings that link *Vitis sylvestris* to the origins of *Vitis vinifera*. However, the potential contribution of other ancestors cannot be disregarded.

This implies that at least four different types of "wild" vines may occur and often overlap in natural habitats: wild sub-spontaneous lambruscae, wild colonial, wild mestizo, and wild autochthonous native. Their identification is a challenge that needs to be addressed.

Furthermore, various interspecific hybrids involving American *Vitis* species (*V. berlandieri* Planch. × *V. riparia*, *V. rupestris* × *V. berlandieri*, *V. solonis* (Planch.) Millardet × *V. riparia*) were developed in the 19th century to create phylloxera-resistant rootstocks for grapevine cultivation. These American species and their hybrids have escaped cultivation, entering natural habitats along European rivers, where they produce hybrids with *V. sylvestris* and even *V. vinifera*, as observed in Hungary. The local presence of American species and their hybrids has displaced preexisting *V. sylvestris* populations in certain areas of Hungary and France [38,39]. In Italy, numerous feral populations of American *Vitis* species have led to the discovery of interspecific hybrids, such as *V.* ×*baccoi* Ardenghi, Galasso, & Banfi (=*V. riparia* × *V. vinifera*), *V.* ×*goliath* Ardenghi, Galasso, & Banfi (=*V. riparia* × *V. rupestris* × *V. vinifera*), *V.* ×*instabilis* Ardenghi, Galasso, Banfi, & Lastrucci (=*V. riparia* × *V. rupestris*), *V.* ×*koberi* Ardenghi, Galasso, Banfi, & Lastrucci (=*V. berlandieri* × *V. riparia*), and *V.* ×*ruggerii* Ardenghi, Galasso, Banfi, & Lastrucci (=*V. berlandieri* × *V. rupestris*), growing in natural habitats. This complexity further complicates the study of wild grapevine populations [40]. Similarly, in Spain, hybrids have been named after natural populations, such as *V.* ×*gallica* F.M. Vázquez (=*V. berlandieri* × *V. vinifera*) and *V.* ×*hispanica* F.M. Vázquez & D. García (=*V. rupestris* × *V. vinifera*) [41]. In the Valencian hinterland, feral American grapevine rootstocks are known as *parrizos*, *parrizas*, or *vidueños* in Spanish.

The study aims to explore and differentiate the ampelographic characteristics and seed allometric relationships among the subtypes in the gradient from purely domesticated cultivars to native autochthonous wild grapevine (cultivars, postcultural vines in abandoned vineyards, wild sub-spontaneous *lambruscae*, wild colonial, wild mestizo, and wild autochthonous native), and in parallel determine their heterogeneity or entropy.

Our primary goal is to analyze and compare wild and domesticated Eurasian grapevine seed samples in terms of their heterogeneity, entropy, and the amount of information required to describe them. This analysis aims to identify links between heterogeneity and potential introgression or hybridization between wild and domesticated grapevines.

The fundamental objective is thus to develop a methodology for detecting introgressed or hybrid *Vitis* populations (wild mestizo) and to distinguish them from wild autochthonous native grapevines, through the study of seed morphology. Another goal is to identify feral populations of *Vitis vinifera* (wild sub-spontaneous lambruscae and wild colonial) in natural habitats based on the different degrees of domestication observed in their seeds.

To achieve this, we plan to utilize domestication index values and domestication probabilities, and the estimate of the heterogeneity within the seed ensemble of each single sample as indicators of introgressions and hybrid swarms, providing a quantitative estimate of variability within individuals and populations. Lastly, we seek to determine geographical patterns at different levels (countries and regions) and the connections between wild and domesticated grapevine diversity, shedding light on domestication events in these regions.

2. Materials and Methods

2.1. Geographic Locations of the Vineyards and Natural Habitats Sampled

A total of 4341 grape seeds from 816 samples were analyzed. But only 491 samples consisted of between 3 and 26 seeds, averaging eight seeds per sample. Of the 816 samples and 4341 seeds, 107 and 147, respectively, were fossils, 195 and 398 archaeobotanical, and 514 and 3796 modern. Modern samples included, in addition to *Vitis vinifera* and *V. sylvestris*, 5 samples of the genus *Ampelopsis*, 38 of American *Vitis* species, not only collected in America, but also escaped in Europe, and 8 Asian *Vitis* species, collected in India, China, and Japan.

From a geographical viewpoint, most samples were collected in Europe and the South Caucasus. In France, 20 samples were collected, primarily sourced from vineyards (19), with only 1 from a natural habitat. Georgia contributed 36 samples, with a balanced representation from both natural habitats (16) and vineyards (20). Armenia stands out with 73 samples, where 29 came from natural habitats and 44 from vineyards. Italy provided 50 samples, predominantly from natural habitats (38), while 12 were from vineyards. Spain exhibited the highest sample count at 183, comprising 80 from natural habitats and 103 from vineyards. Turkey and the USA, with 24 and 26 samples, respectively, showcased an inverse pattern. In the case of Turkey, 19 out of 24 samples came from vineyards, while in the USA, only 1 sample came from a vineyard, and 25 came from natural habitats. Further details can be found in Supplementary Tables S1 and S2.

2.2. Process of Seed Collection and Criteria for Inclusion in the Study

We aimed to achieve a comprehensive representation of variability within *Vitis*, encompassing both cultivated and wild populations, as well as archaeological and fossil materials. In the assessment of heterogeneity, we endeavored to sample wild grapevine populations extensively in Spain and Italy, supplemented by substantial samplings in Armenia and Georgia. In the case of cultivars, they were directly sampled from production vineyards or collections within various germplasm banks, notably in the Rioja's collection at Mendavia (Navarra, Spain), San Michele all'Adige (Trentino-Alto Adige, Italy), Agullent (Valencia, Spain), and Geilweilerhof—Institut für Rebenzüchtung (Siebeldingen, Germany).

Ripe grapes were manually collected in vineyards, natural habitats, and repositories by members or collaborators of this research team. Care was taken to collect each sample from one single individual, and when possible, from a couple of bunches of grapes. In natural populations, this was very difficult as many bunches had a very low number of grapes, so more were used.

The seeds were manually extracted from the grapes and then left to dry at room temperature and stored in glass test tubes, 100 × 16 mm, fitted with a screw cap, labeled inside and outside.

2.3. Methods Used for Calculating the Domestication Index and Probabilities of Domestication

Each of the 4341 seeds was individually described using 14 characters. Among these, 11 were quantitative, including the total seed length, maximum breadth and thickness, breadth of the stalk at the junction and seed base, length of the beak in the dorsal and ventral views, thickness of the beak at the seed base, total length of the chalaza scutellum, maximum breadth of the chalaza scutellum, and distance from the chalaza apex to the seed apex [27,35,42]. Additionally, there are three qualitative characteristics: contour type, with options like ovoid, quadrangular, triangular, rounded, and pentagonal; the arrangement of the fossettes, with choices such as parallel, furcate, convergent, and divergent; and the presence/absence of radial furrows. But, for the present work, only the 3970 seeds of the samples with three or more seeds were considered.

The quantitative and qualitative traits were assessed by analyzing digital images of seeds. These seeds were photographed in the dorsal, ventral, and lateral views. Measurements were conducted utilizing the open-source Fiji software 2.9.0, released on 14 September 2022 [43]. Additionally, scaled images of fossilized and archaeological seeds, sourced from specialized literature, were incorporated for reference measurements. All recorded traits were documented in an Excel spreadsheet, and allometric relationships were automatically computed through dedicated algorithms.

The first step was to calculate six classical domestication indices for each individual seed: Stummer's [12], Facsar and Perret's [20–23,26], and Mangafa and Kotsakis' 1 to 4 indices [25]. The next step was to combine these indices into a single index, which we call the domestication index (DI), and its complementary wildness (WI) index [35,42].

We used logistic regression models [35,44,45] and in parallel the random forest technique [35,46–49] to evaluate, using morphometric data and comparison collections, the probability of individual seeds coming from a domesticated grapevine, or a wild native autochthonous one, or presenting intermediate characteristics that prevent a clear allocation to one of the two categories. In the present study, "randomForest" in R version 4.4.1 (14 June 2024) and R Studio [50,51] assigns a probability value of being "domesticated" to each single seed (PDIrF, probability of domestication estimated with randomForest), which ranges from 0 to 1. And Logit proceeds similarly in R [35] (PDILO, the probability of domestication estimated with Logit). These together with the DI index served to identify the "domestication syndrome" in grapevine seeds [35].

Details of the different calculation methods are given in the references cited and accompany this paper, with Supplementary References [52–54], as Supplementary Materials File S1: Methods.

2.4. Methods Used for Calculating the Heterogeneity and Entropy Levels

The heterogeneity within a sample tends to increase when there are a greater number of distinct categories and a more even distribution of abundance or representation across those categories. To quantify the degree of sample heterogeneity, various statistical estimators can be employed, based on the behavior of the domestication values observed for the individual seeds within each sample. Indicators reflecting the dispersion or spread of values within each sample, such as the standard deviation or the range between the minimum and maximum values, can effectively represent this sample-level heterogeneity. Such heterogeneity may potentially be associated with the hybrid or admixed origin of the sample under investigation.

The introgression index (HI), denoted by the standard deviation of domestication index values (σ (DI)), serves as an indicator of heterogeneity within the sample.

The proportion of wild seeds (PW) within each sample refers to the fraction of seeds below the domestication threshold ($DI \leq 0.2$), denoted as "wild," ranging from 0 to 1. The term "wild" here signifies phenotypic characteristics, not merely habitat, and its interpretation considers values across the entire sample and other contextual data, aiding in distinguishing native wild plants, hybrids or mestizo, ferals (sub-spontaneous or colonial), or complex populations with different types cohabiting.

The wild sum/2 ($WS/2$) for each sample is calculated using Equation (1).

$$\frac{WS}{2} = \frac{mean\ (1 - DI) + PW}{2} \tag{1}$$

This index integrates factors such as the wildness index (1 − domestication index) and proportion of wild seeds, contributing to a comprehensive assessment of the sample. The wild sum index is calculated by dividing the sum by 2 to ensure that the range of values remains between 0 and 1. This approach maintains comparability with other indexes, where 0 represents fully domesticated and 1 represents fully wild. The mean value of $1 - DI$ provides an indication of the wildness degree of the sample (with values > 0.8 meaning wild autochthonous native), and when combined with PW, the proportion of wild seeds in the sample, it allows for a preliminary evaluation of the heterogeneity within the sample. A purely wild sample will yield a value of 1 due to $DI = 0$ and $PW = 1$, while conversely, a purely domesticated sample will yield 0. Intermediate values suggest introgression.

The maximum and minimum value of the domestication index (max (DI) and min (DI), respectively) within the sample allow us to calculate the range $\rho(DI)$ (max (DI) − min (DI)), which also serves as a distinctive parameter, facilitating the differentiation of major types.

2.5. Methods Used for Calculating the Information and Entropy Levels

Theoretically, addressing the heterogeneity of a seed sample can involve calculating information using the Shannon index. While this approach has found success in the case of the *Phoenix* palms' genus [2], its direct application to seed observations may lack informativeness. However, when calculated based on individual values of DI, $PDILO$ (the probability of domestication estimated with Logit), and $PDIrF$ (the probability of domestication estimated with random forest), the Shannon index proves to be extremely useful.

To compute the Shannon index for grapevine seed sample heterogeneity (*Ht*), we start with the premise that *DI* (domestication index) may have seven distinct values (0, 0.17, 0.33, 0.5, 0.66, 0.83, 1), while *PDILO* (the probability of domestication estimated with Logit) and *PDIrF* (the probability of domestication estimated with random forest), ranging continuously from 0 to 1, are discretized into ten intervals of 0.1 each. Probabilities are computed based on the frequency distribution of possible values within each sample. The index is then calculated using Equation (2).

$$H_t = -\sum_{i=1}^{7} p(DI_i) \times log_2\ (p(DI_i) - \sum_{i=1}^{10} p(PDILO_i) \times log_2\ (p(PDILO_i) - \sum_{i=1}^{10} p(PDIrF_i) \times log_2\ (p(PDIrF_i) \quad (2)$$

The magnitude of the total heterogeneity index (H_t) serves as a quantitative indicator of phenotypic diversity within seed assemblages. Higher H_t values denote greater morphological heterogeneity within a given seed sample, whereas lower H_t values indicate reduced phenotypic variation. When $H_t = 0$, this signifies complete homogeneity, representing a monomorphic sample containing solely one domestication phenotype from the possible morphological spectrum. This metric thus provides insights into the degree of domestication-related diversity present within modern and archaeobotanical assemblages.

We employed the Shannon uniformity (or equitability) index (E_h) to quantify the relative abundance distribution of morphological variants within each seed assemblage. This metric enables the assessment of phenotypic evenness, thereby elucidating the distributional patterns of domestication-related traits and providing insights into the taxonomic origin and cultivation status of the source population. The *Eh* index functions as a reliable discriminatory metric for differentiating among domesticated cultivars, their wild progenitors, and the intermediate hybrid populations that constitute the domestication gradient. This differentiation is achieved through the quantitative assessment of seed morphotype uniformity patterns, which reflect the varying degrees of phenotypic stabilization characteristic of populations at distinct stages along the domestication continuum. The presence of hybrid swarms, representing admixed populations with intermediate phenotypes, can be effectively identified through their distinctive *Eh* values (Equation (3)).

$$E_h = \sum_{i=1}^{3} \frac{H_t}{log_2(N_{types}) \times 3} = \frac{H_t(DI)}{log_2\ (7) \times 3} + \frac{H_t(PDILO)}{log_2\ (10) \times 3} + \frac{H_t(PDIrF)}{log_2\ (10) \times 3} \quad (3)$$

The parameter E_h varies between 0 and 1, where $E_h = 0$ indicates minimal evenness, suggesting pure samples of one single type, while the rest are missing. On the other hand, maximum evenness, with $E_h = 1$, is observed in samples where all potential types are present and in equal proportions. The values of equitability are approximately 1/10 of the Shannon index scores.

3. Results

3.1. Phenotypic Seed Patterns of Variability Within Samples

The samples analyzed exhibit a broad spectrum of variation, ranging from phenotypically purely wild to fully domesticated (Figure 2A,B). Notably, there are several transitional degrees in between, some of which may correspond to ongoing introgression processes and represent the full range from cultivars ($PW = 0$, $1 - DI < 0.2$) (Figure 2A) to postcultural vines in abandoned vineyards, wild sub-spontaneous lambruscae, wild colonial, wild mestizo, and wild autochthonous native plants ($PW > 0.6$, $1 - DI > 0.8$) (Figure 2B).

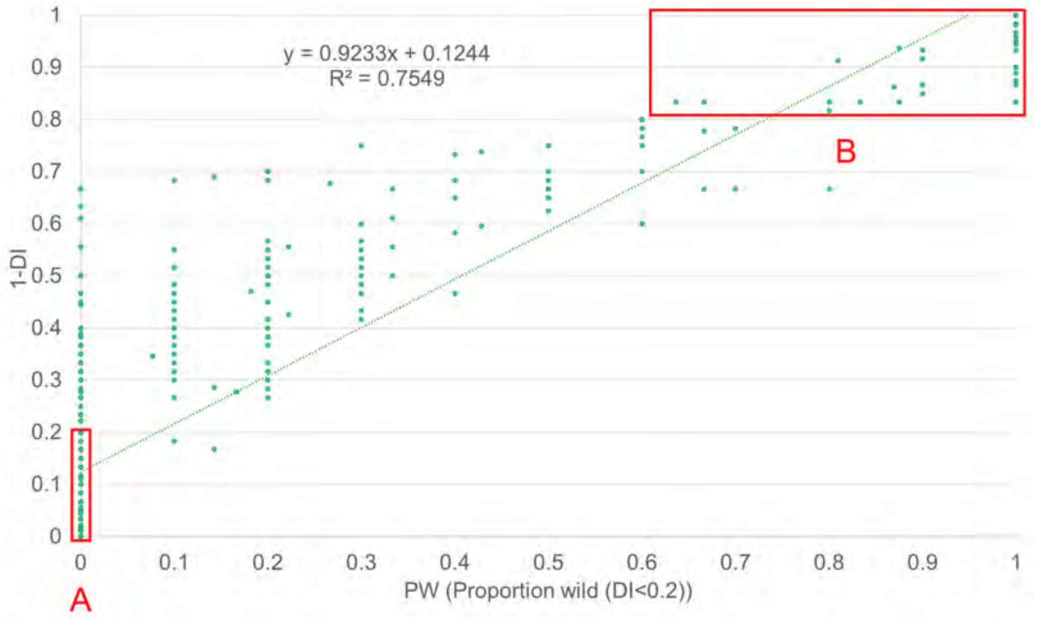

Figure 2. Relationships between the *PW* index (based on the proportion of wild seeds) and average $1 - DI$. Wild index $(1 - DI) = 1$ minus the average of the domestication index values of the seeds in each sample. (**A**) cultivars, purely domesticated; (**B**) purely wild autochthonous native plants. "Proportion wild", *PW*, = proportion of seeds below the wild threshold ($DI < 0.2$). Green dots, data from seed samples; Dotted line of best fit, that models the data's trend.

An inverse relationship is observed between the proportion of seeds in the sample exhibiting the wild phenotype and the mean values of the domestication index. More specifically, this relationship is directly correlated with the square of the mean wildness degree within the sample (Figure 2). However, the significant dispersion of points for a given *PW* value suggests the high heterogeneity of *DI* values across a substantial portion of the samples. This method shows the variability and characterizes the cultivars well but does not allow us to accurately characterize the intermediate types of mestizos or wild colonial or sub-spontaneous lambruscae, and only to partially characterize the native autochthonous wild types.

Morphological variability in seeds is depicted in Figure 3, where the mean values of width and stalk length parameters, in mm, per seed sample are compared for different grapevine types. The graph illustrates that the minimum values of stalk length are characteristic of wild grapevines, regardless of their modern or fossil nature or their continental origin, thus including Eurasian and American wild *Vitis* species. In the case of *Vitis vinifera*, variability is notably high, but stalks generally tend to be longer than in the rest of the *Vitis* species. In intermediate positions, we find samples interpreted as hybrids, which we will explore later as they may be attributed to introgression processes from wild vines into domesticated populations—a phenomenon that is not uncommon.

In addition to the above, Figure 3 allows us to distinguish the seeds of wild vines from the South Caucasus, called *V. caucasica*, due to their greater width and longer stalk length compared to *Vitis sylvestris* from central and western Europe. Differentiation of Asian wild vines, American wild vines, and fossil seeds is not possible from *Vitis sylvestris* in Figure 3, although some of the former have extremely short stalks. "Wild" *Vitis vinifera* includes cultivars that in general show "primitive" traits that would link them to *Vitis sylvestris*.

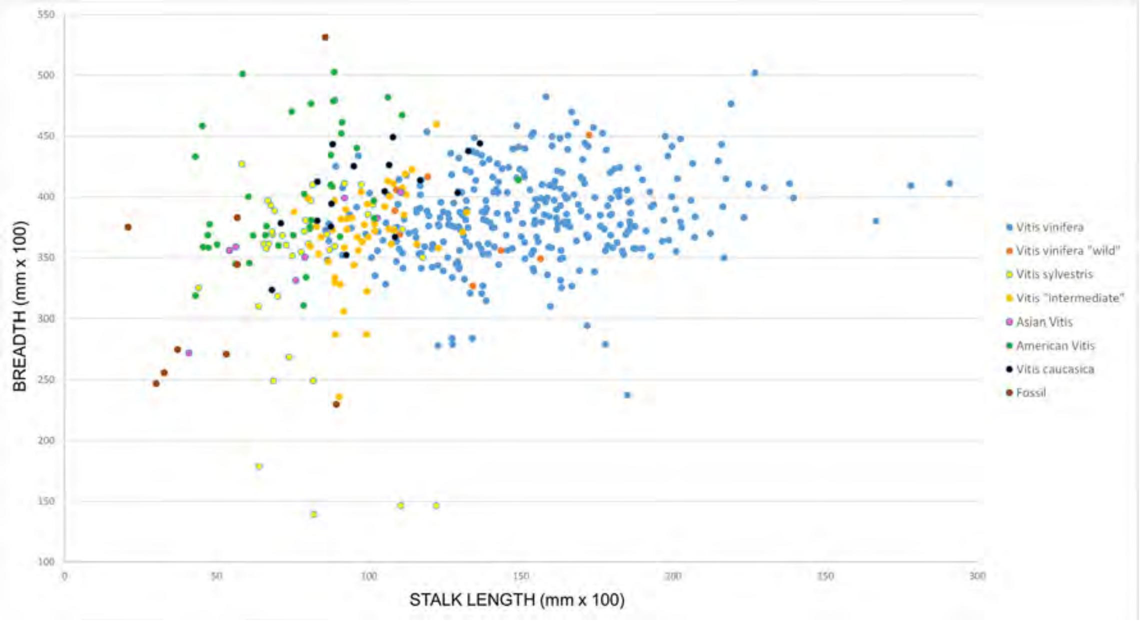

Figure 3. Seed breadth and stalk length as an example of phenotypic differences between wild, intermediate, and domesticated *Vitis*. Note: Asian *Vitis* here includes *Vitis romanetii* Rom. Caill., *V. piasezkii* Maxim. var. *pagnuccii* (Rom. Caill. ex Planch.) Rehder, *V. heyneana* Schult., *V. flexuosa* Thunb., *V. ficifolia* Bunge, and *V. amurensis* Rupr., from China, India, and Afghanistan; and American *Vitis* includes *Vitis vulpina* L., *V. shuttleworthii* House, *V. rupestris* Scheele, *V. rotundifolia* Michx., *V. riparia* Michx., *V. popenoei* J. H. Fennel, *V. peninsularis* M. E. Jones, *V. palmata* Vahl, *V. nesbittiana* Comeaux, *V. mustangensis* Buckley, *V. monticola* Buckley, *V. labrusca* L., *V. cinerea* var. *helleri* (L. H. Bailey) M. O. Moore, *V. cinerea* (Engelm.) Millardet, *V. californica* Benth., *V. bryoniifolia* Bunge, *V. bloodworthiana* Comeaux, *V. blancoi* Munson, *V. biformis* Rose, *V. arizonica* Engelm., *V. aestivalis* var. *linsecomii* (Buckley) L. H. Bailey, *V. aestivalis* Michx. var. *aestivalis*, *V. acerifolia* Raf., *V.* ×*doaniana* Munson ex Viala, and *V. girdiana* Munson, from the USA, Mexico, and natural habitats of Europe (where some are invader species).

3.2. The Place and Role of Intermediate Samples: Markers of Introgression

The detection of intermediate or mixed heterogeneous phenotypes (Figure 3) reveals introgression in varying levels among distinct groups within *Vitis.* Among those initially labeled as "wild" due to their natural habitat, approximately 20% of samples exhibit intermediate phenotypes that suggest introgression, although not only introgression, because wild colonial plants partially reverting to or converging with wild phenotypes could present similar values to those of truly wild mixed varieties and thus would be different to those of wild autochthonous native varieties. This proportion significantly rises to 40–55% for samples categorized as intermediate based on overall seed morphology. Cultivated samples exhibit the lowest introgression, ranging only from 7 to 10%, likely those domesticated phenotypes with wild traits ("wild" *Vitis vinifera* in Figure 3). Interestingly, this supports the hypothesis of Levadoux and other authors regarding the hybrid origin of certain cultivars (Figure 3).

We demonstrate that employing the mean domestication index for the sample μ (DI) and its variation level ρ (DI) can also aid in detecting potential introgression or hybridization cases (Figure 4).

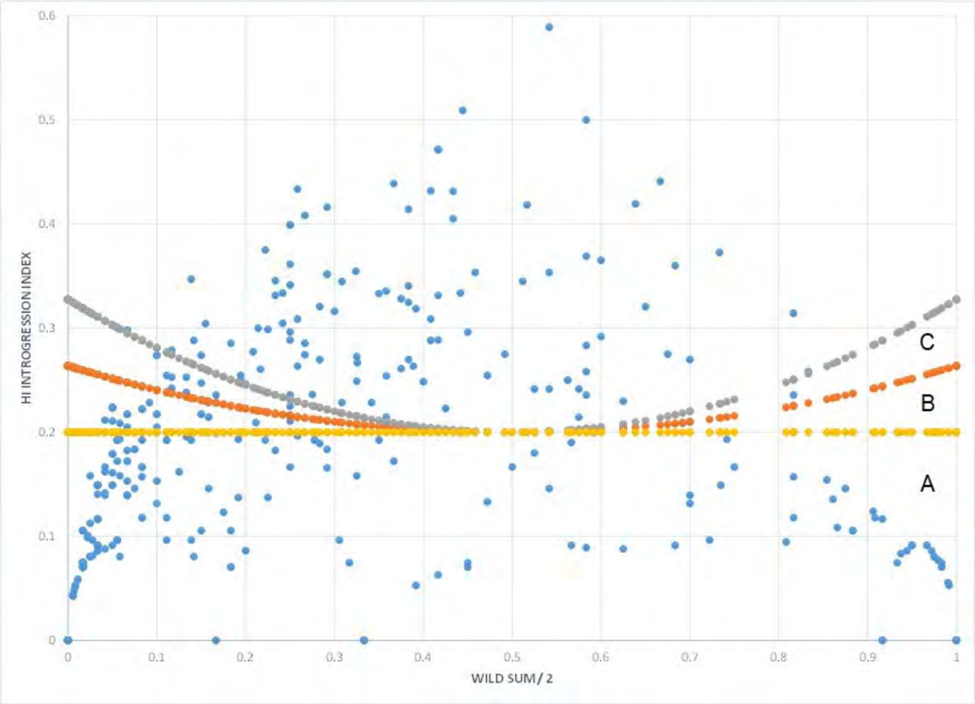

Figure 4. Thresholds for recognizing introgression along the WS/2, wild sum index. The highest values of the introgression index are found in samples that present an intermediate position with respect to the domestication syndrome, expressed in terms of WS/2 (Figure 4). Blue dots represent the 491 samples with 3 or more seeds. (**A**), yellow dots, constant IH = 0.2 threshold, (**B**), orange dots, catenary threshold (Formula (3)), and (**C**), grey dots, hyperbolic cosine threshold (Formula (4)).

Contrary to a constant threshold value (TH(x) = 0.2) (Figure 4A), the introgression index threshold appears to consist of values defined by hyperbolic curves. These curves could take the form of a catenary (Figure 4B) and Equation (4),

$$TH\ (x) = 0.5\cosh\left(x\ /0.5\right) - 0.3, 0 \leq x \leq 1 \tag{4}$$

Or, more likely, a hyperbolic cosine (Figure 4C) and Equation (5),

$$TH\ (x) = \cosh\ \left(x - 0.5\right) - 0.8, 0 \leq x \leq 1 \tag{5}$$

This depends on the sample's domestication level. Notably, higher threshold values are observed towards the lower and upper extremes of the wild sum index (Figure 4).

Most samples identified as hybrid grapevines typically feature small seeds, displaying mean dimensions more akin to those found in wild populations but with higher introgression index (*HI*) values. Interestingly, this occurrence of elevated *HI* values, suggesting introgression or hybridization, is not observed among samples with larger seeds, commonly associated with cultivars.

Furthermore, the introgression index (*HI*) exhibits elevated values in other *Vitis* species, such as *V. girdiana* Munson and *V. nesbittiana* Comeaux from Mexico, or *V. piasezkii* Maxim. var. *pagnuccii* (Rom. Caill. ex Planch.) Rehder and *V. romanetii* Rom. Caill. from China. This could be attributed to the inherent characteristics of each species, implying a notable degree of heterogeneity, or possibly to hybridization events in the history of analyzed accessions. Given that our introgression index is specifically designed to discern the boundaries between *V. sylvestris* and *V. vinifera*, its application to other species is not straightforward. Therefore, it should be considered as an indicator deserving further exploration and testing.

The behavior of the domestication index (*DI*) can facilitate the identification of seeds based on their putative origin. In the context of vineyards, when the mean of *DI* ($\mu(DI)$) is less than or equal to 0.5, the minimum *DI* (min(*DI*)) is less than or equal to 0.5, and the standard deviation of *DI* ($\rho(DI)$) is less than 0.5, these conditions are indicative of phenotypically wild cultivars (Table 1), which may be recognized as potentially primitive. When comparable parameter values of the domestication index (DI, $\mu(DI, min(DI))$ are observed in samples originating from natural habitats across Western Eurasia, these findings may be interpreted as indicative of the presence of the wild grape species *Vitis sylvestris* within those samples. The similarities in *DI* profiles likely reflect the shared phylogenetic affinities and evolutionary histories between the wild *V. sylvestris* populations and the putatively primitive cultivated grapevine accessions exhibiting analogous *DI* characteristics. Similar *DI* parameter values are prevalent in nearly all analyzed ancient plant fossils and approximately half of the Eurasian and American wild *Vitis* species (Table 1 and Figure 5).

Table 1. Joint distribution of seed domestication parameters and major grapevine types.

Parameters			**Types**							
μ (DI)	**min (DI)**	**ρ (DI)**	**Int.**	**Feral**	**Cultivars**	**Wild**	**Others**	**Fossils**	**Primitive**	**S**
Vineyards										
≤0.5	≤0.5	0	-	-	-	-	-	-	2 **	2
		0–0.4	-	-	-	-	-	-	2 **	2
		0.6–1	2 *	-	-	-	-	-	-	2
>0.6	≤0.5	0–0.4	-	-	2	-	-	-	-	2
		0.4–0.6	-	-	12	-	-	-	-	12
		0.6–1	2 *	-	0	-	-	-	-	2
	>0.5	0	-	-	185	-	-	-	-	185
		0–0.4	-	-	79	-	-	-	-	79
Natural habitats										
≤0.5		0	-	-	-	7	12	84		103
		0–0.4	1 ^{o}	-	-	8	17	9	-	35
		0.4–0.6	1 ^{o}	-	-	12	8	-	-	21
		0.6–1	7	-	-	2 a	3	-	-	12
>0.6	≤0.5	0–0.4	-	8	-	-	2	-	-	10
		0.4–0.6	1	12	-	-	1	-	-	14
		0.6–1	20	-	-	-	5	-	-	25
	>0.5	-	-	7	-	-	2	17	-	26
		0–0.4	-	28	-	-	0	0	-	28
			34	55	278	29	50	110	4	

Abbreviations: μ (*DI*), Mean domestication index value; *min* (*DI*), minimum domestication index value; ρ (*DI*), domestication index range; Int., intermediate, introgressed, or hybrid; S, marginal frequencies. Notes: (*) Forcallat, Albariño, Chardonnay Blanc, Chitistvala_Meskhuri, Brancellao, Garnacha Blanca, Merlot, (**) Pardillo, Merseguera, Planta Nova, Rozsaszolo; (^{o}) Guadiana River, Aragon River Dam, Samebis seri (Georgia), (^{a}) Kvetari_04, presents one single seed with $DI = 0.83$ and the rest are clearly wild; similarly, in Huelva, one single seed presents $DI = 0.67$. The colors used in the image are as follows: Pink (used for "Int."), Light gray (used for "Feral with heterogeneous domestication traits"), Dark gray (used for "Feral with homogeneous domestication traits"), Bright blue (used for "Cultivars"), Light blue (used for Fossils), Yellow (used for "Primitive"), Bright green (used for "Wild"), Beige/Orange (used for "Others").

Conversely, in vineyards, the majority of cultivated grapevine accessions exhibit $\mu(DI)$ greater than 0.6, with min(DI) exceeding 0.5 and $\rho(DI)$ ranging from 0 to 0.4. An analogous pattern is observed in samples from natural habitats, which we interpret as indicative of feral grapevine populations (Table 1 and Figure 5). Interestingly, some American wild *Vitis* species and ancient plant fossils also exhibit this cultivar-like *DI* parameter profile (Table 1). This suggests that the domestication syndrome detectable in seeds, while unambiguous, may in some cases replicate preexisting patterns present within wild *Vitis* populations.

Putative hybrid specimens, both from natural habitats and vineyards, are characterized by a high $\rho(DI)$ value, typically ranging from 0.6 to 1.0 (Table 1). The elevated variability of *DI* values within a single sample may imply a process of introgression with domesticated *V. vinifera*, whether of ancient or more recent origin.

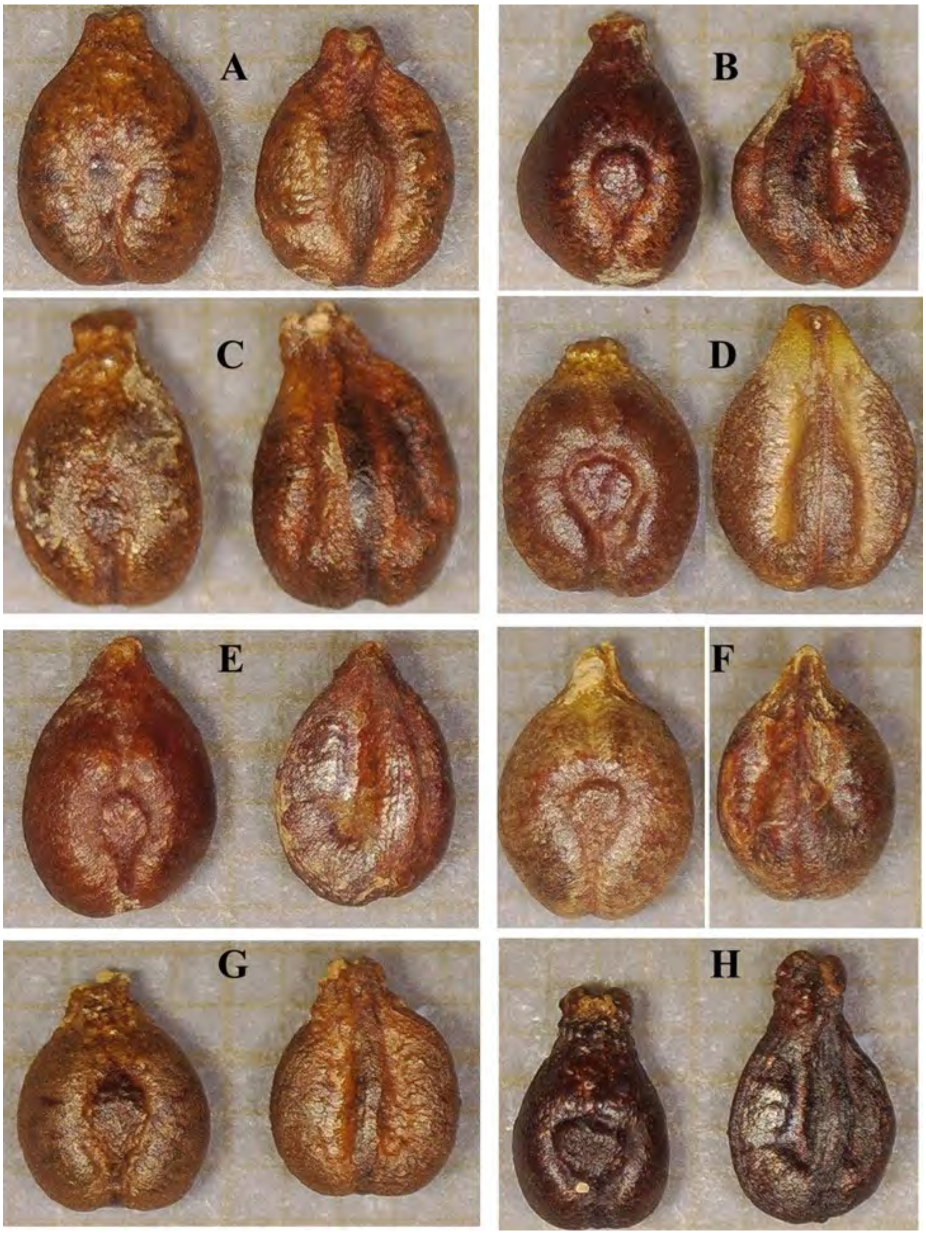

Figure 5. Different seed types recorded from natural habitats in Italy and Spain. Digital microscopy images: background, 1 mm orange graph paper. Samples, their types, and hybridization and domestication index values: (**A**) WILD, 246 Antchatea-Plentzia, *HI* = 0.2, *DI* = 0.27; (**B**) HYBRID, 245 Armintza-Arroyo Amorrada, *HI* = 0.22, *DI* = 0.54; (**C**) INTROGREDED? 244 Balmaseda-Cadegua, *HI* = 0.15, *DI* = 0.65; (**D**) INTROGREDED? 582 Estena River Gorge, *HI* = 0.27, *DI* = 0.7; (**E**) WILD, 249 Urkuleta 1, *HI* = 0.12, *DI* = 0.08; (**F**) FERAL ROOTSTOCK, 260 *V. berlandieri* × *V. riparia* 5BB, *HI* = 0.19, *DI* = 0.13; (**G**) INTROGREDED? 568 Rio Irati (Navarra), *HI* = 0.24, *DI* = 0.43; (**H**) INTROGREDED? 570 Piemonte (Italy), HI = 0.14, DI = 0.33.

Intermediate characteristics are not limited to wild populations colonizing natural habitats; we have identified primitive traits in cultivars such as Albariño, Brancellao, Cainho, Tempranillo (p.p.), Chardonnay Blanc, Forcallat, Garnacha Blanca, Merlot, and Tempranillo Blanco. Samples of varieties like Chardonnay Blanc, Forcallat, and Garnacha Blanca fall within the range of wild-type characteristics. Additionally, varieties such as Rozsaszolo, Planta Nova, Merseguera, or Pardillo display distinctly wild-type seeds and low introgression index (HI) values (0.09–0.15).

3.3. Domestication Probability Variability Within Samples and Entropy Estimates

Obón et al. [35] recently showed that estimating the probability of domestication for each seed using methods such as logistic regression and machine learning, specifically "randomForest", with the use of training sets, allows for a more comprehensive understanding of the overall domestication degree of the samples. When combined with the analysis of heterogeneity, entropy, or information within each sample, we can achieve a more precise insight. In the case of the dataset, the results are summarized in Table 2, where a comparison is drawn between samples obtained from vineyards and those collected from natural habitats (Figure 5).

Table 2. Comparative analysis of heterogeneity and mean domestication probability in samples from vineyards and natural habitats [1].

Vineyards

Het.\PD	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1	*MH*
$0 \leq$ Het. < 1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.6747**	**0.6747**
$1 \leq$ Het. < 2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0683	0.0683
$2 \leq$ Het. < 3	0.0000	0.0000	0.0040	0.0000	0.0000	0.0000	0.0000	0.0000	0.0321	0.0643	0.1004
$3 \leq$ Het. < 4	0.0000	0.0080	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0281	0.0120	0.0482
$4 \leq$ Het. < 5	0.0000	0.0000	0.0000	0.0120	0.0000	0.0000	0.0040	0.0000	0.0241	0.0000	0.0402
$5 \leq$ Het. < 6	0.0000	0.0000	0.0000	0.0040	0.0040	0.0040	0.0000	0.0120	0.0201	0.0000	0.0442
$6 \leq$ Het. < 7	0.0000	0.0000	0.0000	0.0000	0.0000	0.0080	0.0000	0.0000	0.0000	0.0000	0.0080
$7 \leq$ Het. < 8	0.0000	0.0000	0.0000	0.0000	0.0120	0.0040	0.0000	0.0000	0.0000	0.0000	0.0161
MP	0.0000	0.0080	0.0040	0.0161	0.0161	0.0161	0.0040	0.0120	0.1044	**0.8193**	1.0000

Natural Habitats

Het.\PD	0–0.1	0.1–0.2	0.2–0.3	0.3–0.4	0.4–0.5	0.5–0.6	0.6–0.7	0.7–0.8	0.8–0.9	0.9–1	MH
$0 \leq$ Het. < 1	0.1033	0.0083	0.0041	0.0000	0.0041	0.0000	0.0000	0.0000	0.0000	0.0331	0.1529
$1 \leq$ Het. < 2	0.0455	0.0207	0.0000	0.0000	0.0000	0.0041	0.0041	0.0000	0.0000	0.0413	0.1157
$2 \leq$ Het. < 3	0.0165	0.0248	0.0041	0.0083	0.0000	0.0000	0.0000	0.0041	0.0165	0.0331	0.1074
$3 \leq$ Het. < 4	0.0041	0.0207	0.0207	0.0083	0.0000	0.0124	0.0041	0.0083	0.0289	0.0124	0.1198
$4 \leq$ Het. < 5	0.0000	0.0165	0.0372	0.0083	0.0124	0.0041	0.0124	0.0207	0.0496	0.0083	0.1694
$5 \leq$ Het. < 6	0.0000	0.0000	0.0165	0.0331	0.0083	0.0124	0.0124	0.0331	0.0083	0.0000	0.1240
$6 \leq$ Het. < 7	0.0000	0.0000	0.0083	0.0331	0.0537	0.0207	0.0000	0.0165	0.0000	0.0000	0.1322
$7 \leq$ Het. < 8	0.0000	0.0000	0.0000	0.0041	0.0165	0.0413	0.0165	0.0000	0.0000	0.0000	0.0785
MP	0.1694	0.0909	0.0909	0.0950	0.0950	0.0950	0.0496	0.0826	0.1033	0.1281	1.0000

[1] Data are presented as proportions of the total, summing up to 1. Abbreviations: *Het.*, heterogeneity estimate using Shannon index. *PD*: mean domestication probability for the entire sample considering the *DI* (domestication index), *PDILO* (the probability of domestication estimated with Logit), and *PDIrF* (the probability of domestication estimated with randomForest) values. *MH*: marginal sums for each heterogeneity range. *MP*: marginal sums for each probability range. The colors used in the image are as follows: Yellow (used for "probability values of samples from cultivars above 0"), Bright green (used for "probability values of samples from natural habitats above 0").

As expected, vineyards contributed highly homogeneous samples, characterized by an extreme degree of domestication (Table 2). However, occasional instances from vineyards revealed highly heterogeneous varieties with markedly different seeds, which collectively yielded average domestication values. Some varieties even exhibited predominantly wild traits.

Natural spaces harbor significantly higher heterogeneity, encompassing a spectrum of domestication degrees as deduced from the seed phenotype analysis, with intermediate values predominantly associated with elevated heterogeneity (Table 2). Samples exhibiting a maximum domestication probability and low heterogeneity are interpreted as naturalized or semi-spontaneous, while those with minimal domestication probability and low heterogeneity are identified as originating from native wild vines or naturalized American vines, which are indistinguishable using domestication estimates based on seed morphology. Finally, most samples display intermediate domestication values and maximum heterogeneity, indicating the existence of persistent introgression phenomena from cultivated vine populations to wild vines (Figure 6).

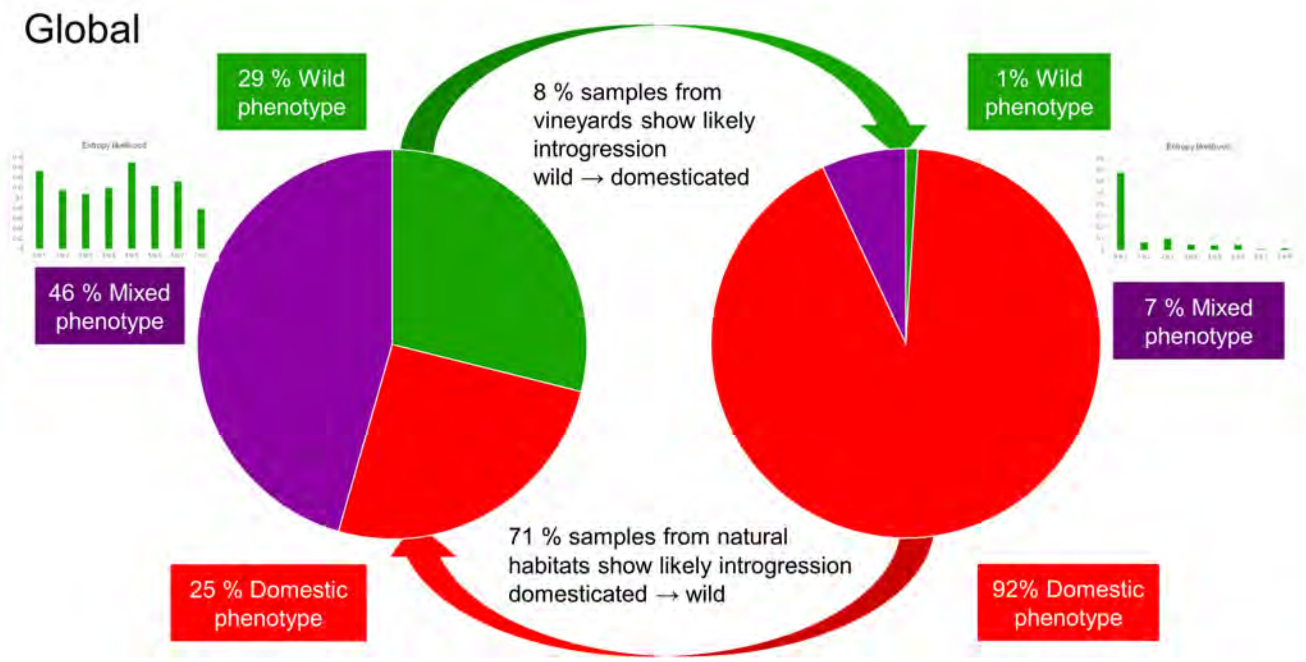

Figure 6. Differences in heterogeneity and the proportion of domesticated, wild, and intermediate types in vineyards and natural habitats, along with an estimation of introgression flows. Color codes: Red, domestic phenotype. Green, wild phenotype. Purple, intermediate or mixed phenotypes.

4. Discussion

4.1. Interpreting the Results in the Context of Domestication and Hybridization

In general, we observe a significant influence of domesticated grapevines on wild populations, which is to be expected given the antiquity of cultivation and domestication processes of Eurasian grapevines, often dating back several thousand years. However, it is crucial to consider the size of both wild and domesticated populations, as this governs the direction of genetic flow. During the initial phases of cultivation, regardless of the local or exotic nature of primitive cultivars, the potential for introgression from wild populations into cultivated ones could have been substantial. Once cultivation is established, the disproportion heavily favors domesticated varieties.

A striking example is found in Castilla–La Mancha, Spain, where more than 150,000 hectares are dedicated to vine cultivation in La Mancha Protected Designation of Origin (PDO) [55], 25,000 in Valdepeñas PDO, 12,500 in Manchuela DO [56], 9000 in

Almansa PDO [57], and 23,000 in Jumilla PDO [58], with an average planting density of about 3000 to 4000 vines per hectare, thus reaching 650,000,000 to 900,000,000 vines. On the other hand, wild vines in the Cuenca Alta del Guadiana, one of the rivers that crosses this re-gion, barely exceed a hundred and, in most stretches, are practically non-existent as also occurs in other rivers of the region such as Tajo, Jucar, Cabriel, or Segura. The dispropor-tion is overwhelming.

In Italy, we have detected a prevalence of domesticated and intermediate vine phenotypes in natural habitats, where it seems that wild vines are reduced to a vestigial pres-ence (Figure 7). In Tuscan populations, D'Onofrio [59] identified redundant genotypic profiles among wild grapevine specimens, indicating clonal propagation in natural conditions. Analysis of specimens from Piedmont, Italy, demonstrated that certain popula-tions initially classified as *V. sylvestris* were identified as naturalized *V. vinifera* (Figure 7). Most identified sylvestris genotypes possessed chlorotype A, while others had chlorotype D, akin to the predominant chlorotype in *V. vinifera* cultivated in Italy. Genotypic analysis of Tuscan populations identified *V. sylvestris* specimens exhibiting hybridization with cul-tivated varieties, demonstrating asymmetric introgression favoring gene flow from *V. vi-nifera* to wild populations [59]. Additionally, certain genotypes seemed to be crosses within *V. sylvestris*, indicating a significant level of sexual exchange between *V. sylvestris* and *V. sylvestris* × *V. vinifera* genotypes.

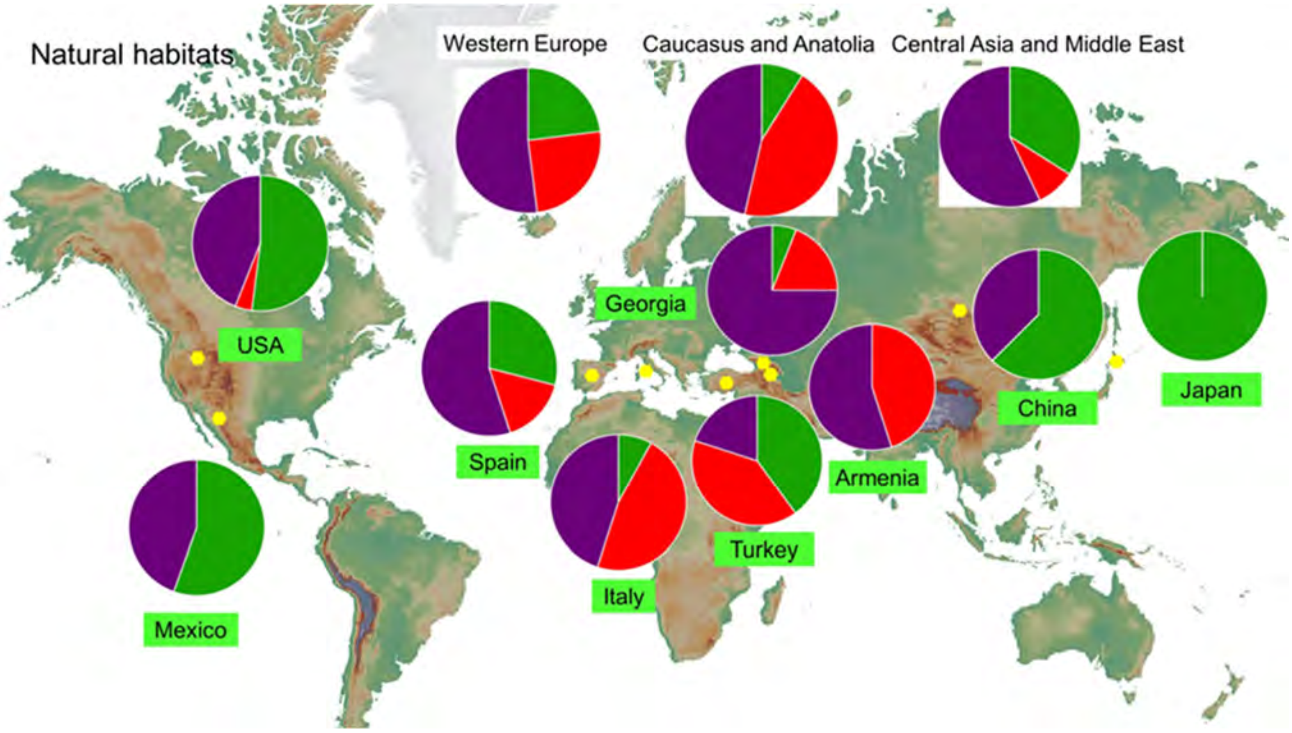

Figure 7. Proportion of domesticated, wild, and intermediate grapevine seed types within natural habitats, across selected countries and regions. Color codes: Red, domestic phenotype. Green, wild phenotype. Purple, intermediate or mixed phenotypes. The yellow dots mark the centroid corresponding to the nearest pie charts, which appear displaced for reasons of space.

The dispersal of pollen from cultivated vineyards in proximity may exert a substantial influence on the evolution of adjacent wild grapevine populations [60]. On the one hand, the low level of pollen-mediated gene flow from cultivated to wild vines could contribute to a risk of extinction for the wild component (i.e., the totality of wild individuals). Conversely, pollen dispersal within diminished wild populations could induce inbreeding depression among wild vines.

We must highlight the trade-off between domestication traits such as higher yield and larger berries, which often come at the expense of reduced resistance to environmental stresses [61].

4.2. Discussing the Importance of Finding Similar Samples in Both Vineyards and Natural Habitats

In general, it is apparent that the likelihood of encountering fruits of wild grapevine species, such as *Vitis sylvestris* and other taxa with similar seed characteristics, is significantly higher in natural habitats compared to vineyards (Figures 6–8), as indicated by an odds ratio of nearly 26 (26% in natural habitats versus 1% in vineyards). Conversely, the probability of encountering domesticated grapevines, specifically *Vitis vinifera*, is not so notably higher, with an odds ratio of 4 (92% in vineyards versus 23% in natural habitats) within vineyard settings (Figures 6 and 8), a relationship that is not intuitively expected, given that 92% of vineyard samples present a phenotype that is clearly domesticated. However, the probability of encountering domesticated grapevines in natural habitats, albeit lower, is still notable, at approximately 0.23.

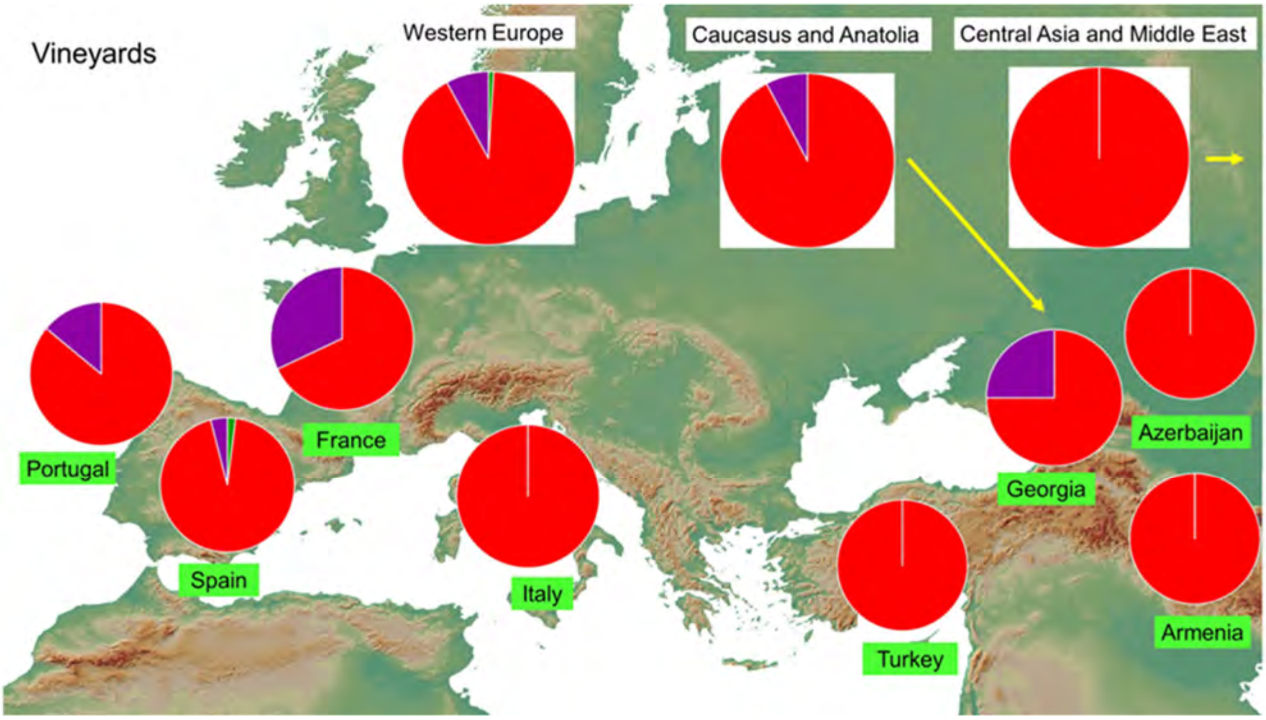

Figure 8. Proportion of domesticated, wild, and intermediate grapevine seed types within vineyards, across selected countries and regions. Color codes: Red, domestic phenotype. Green, wild phenotype. Purple, intermediate or mixed phenotypes. The yellow arrows mark the centroid corresponding to the nearest pie charts, which appear displaced for reasons of space.

Consequently, feral grapevines currently constitute a significant component of grapevine populations inhabiting natural habitats, while phenotypes resembling wild grapevines in their seed morphology are extremely rare in vineyards. Intermediate phenotypes appear in both vineyards and natural habitats, being predominant in the latter (Figures 6 and 7).

The presence of wild traits in vineyards is primarily associated with a select few "primitive" cultivars characterized by low domestication values, particularly evident in their seed morphology (Table 3). Conversely, domesticated traits within wild grapevine populations manifest in a diverse array of origins and types, aligning with Levadoux's [5] classification system. This spectrum spans from postcultural vines, which thrive in abandoned vineyards reclaimed by natural vegetation from nearby habitats, to wild sub-spontaneous lambruscae newly established in natural environments. Additionally, there are wild colonial vines, direct descendants of cultivars, which may have gradually lost some domestication traits over successive generations. Hybrid swarms of *Vitis vinifera*, exhibiting varying levels of introgression, further contribute to this complexity, mixing with preexisting wild autochthonous native *Vitis sylvestris.*

Table 3. Summary of descriptive parameters and indexes for various grapevine types: mean values and standard deviation [1].

Types According to Levadoux	Simplified	1 − DI	PW	WS/2	ME	SH	L (mm)	B (mm)	Stalk (mm)	Stummer Index
Wild autochthonous native	*Vitis sylvestris* modern	0.8 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	0.2 ± 0.2	2.1 ± 1.6	4.8 ± 0.4	3.6 ± 0.4	0.8 ± 0.2	76.1 ± 4.7
Asian *Vitis* spp.	Asian *Vitis*	0.7 ± 0.3	0.6 ± 0.4	0.6 ± 0.4	0.4 ± 0.3	3.5 ± 3.1	4.7 ± 1.0	3.6 ± 4.2	0.8 ± 0.3	77.5 ± 8.5
American *Vitis* spp.	American *Vitis*	0.7 ± 0.2	0.5 ± 0.4	0.6 ± 0.3	0.3 ± 0.2	3.1 ± 2.3	5.3 ± 0.8	4.0 ± 0.5	0.8 ± 0.2	77.1 ± 8.0
“Primitive” cultivars	*Vitis vinifera* “wild”	0.6 ± 0.2	0.3 ± 0.3	0.5 ± 0.2	0.3 ± 0.3	2.7 ± 2.4	5.1 ± 0.3	3.7 ± 0.3	1.1 ± 0.2	73.9 ± 3.8
Vitis caucasica	*Vitis caucasica*	0.5 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	5.4 ± 1.9	5.2 ± 0.6	4.0 ± 0.4	1.0 ± 0.2	77.1 ± 4.3
Wild mestizo (hybrids, different levels of introgression)	*Vitis* intermediate modern	0.4 ± 0.1	0.2 ± 0.2	0.3 ± 0.1	0.5 ± 0.3	4.5 ± 2.6	5.2 ± 0.4	3.8.01±	1.0 ± 0.2	72.2 ± 4.0
Wild colonial	Feral	0.3 ± 0.1	0.0 ± 00	0.1 ± 00	0.3 ± 0.3	2.9 ± 2.5	5.4 ± 3.7	3.7 ± 0.2	1 ± 0.1	69.5 ± 4.9
Cultivars	*Vitis vinifera* modern	0.1 ± 0.1	0.0 ± 00	0.0 ± 0.1	0.1 ± 0.2	1.0 ± 1.6	6.2 ± 0.7	3.9 ± 0.4	1.6 ± 0.3	63.2 ± 5.5
Postcultural vines in abandoned vineyards	Postcultural *Vitis vinifera*	0.1 ± 0.1	0.0 ± 00	0.0 ± 00	0.1 ± 0.1	0.8 ± 1.3	5.6 ± 0.3	3.8 ± 0.3	1.3 ± 0.1	68.0 ± 4.4
Wild sub-spontaneous lambruscae	Sub-spontaneous *Vitis vinifera*	0.1 ± 0.1	0.0 ± 00	0.1 ± 00	0.2 ± 0.3	2.2 ± 2.4	5.8 ± 4.4	3.9 ± 0.3	1.3 ± 0.2	67.3 ± 4.1

[1] Note: Descriptive parameters and indexes, including heterogeneity, are presented as mean values ± standard deviation for different types of grapevines, including wild, domesticated, and sub-spontaneous varieties. Abbreviations: *1 − DI*, wild index. PW, proportion wild, considering *DI* < 0.2. *WS/2*, wild sum/2. ME, mean equitability. SH, Shannon index estimates of heterogeneity within samples. L (mm), seed length in mm. B (mm), seed breadth in mm. Stalk (mm), seed-stalk length in mm.

The diversity within natural habitats is further compounded by distinctions between wild autochthonous native grapevines in Western Europe and those in the Caucasus (Table 2). Furthermore, the increasing presence of escaped American *Vitis* grapevines adds to this complexity, as they demonstrate seed morphology values akin to those of *Vitis sylvestris* (Table 3).

This phenomenon is interpreted as indicative of a substantial seed dispersal flux from cultivated fields to riparian forests and other natural habitats, where cultivars subsequently establish themselves as sub-spontaneous or feral populations. Repeatedly sowing a cultivar does not necessarily lead to convergence with *V. sylvestris*. Some recessive but homozygous traits of *V. vinifera* remain conserved. In cases of heterozygous cultivars, or when one of the ancestors possesses dominant traits like those of *V. sylvestris*, and in instances of multifactorial inheritance, descendants of one or more cultivars may, after a few generations, exhibit an increasing number of forms closely resembling the wild ones (Figure 1) [8].

Considering the observations, the prevalence of intermediate seed types, which are the primary group within natural habitats, reaching 41% (Figure 6), may be attributed to several factors. Firstly, it could reflect a process of partial regression through successive generations of feral grapevines. Additionally, it may result from continued introgression with preexisting native autochthonous wild grapevines. It is noteworthy to mention that samples exhibiting intermediate domestication values typically demonstrate significantly higher levels of heterogeneity or entropy compared to those representing pure wild or common domesticated categories (Table 2).

Throughout ampelographic history, various wild grapevine forms have been documented, often representing hybrid swarms with domesticated populations in different re-gions. In Andalusia, Simón de Roxas Clemente [62–64] identified six wild grapevine types, including Mollar negro bravío and Garabatona. Bronner [65,66], in the mid-19th century, distinguished 36 wild grapevine varieties in Alsace, some with oval seeds and a sweet taste, indicating a mix of true wild, feral, and possibly introgressed individuals. In Italy, authors like Mendola, Negri, and Franchino recognized wild grapevines under names such as Uzelina and Zampina. Uzelina is rustic with small black berries, while Zampina exhibits dioecious traits and foliar sexual dimorphism. Italian populations show considerable variability, with hybrid swarms and mixed populations due to the intentional grafting of cultivars on wild grapevines in natural habitats [67]. Scienza et al. [68,69], Anzani et al. [70,71], and Failla et al. [72] identified considerable variability in wild Italian grapevine populations. Scienza et al. [69] evidenced the presence of feral varieties. Eight plants from natural habitats with ampelographic traits of cultivated grapevine were discovered in Georgia by Kikvadze et al. 2024 [73].

Molecular analyses unveiled the potential introgression of *V. sylvestris* into certain cultivars in Italy and France [61], which could explain our detection of cultivars with prim-itive seed traits associated with low domestication markers (Table 3).

Biblical texts provide early documented evidence of vineyard heterogeneity and deterioration, notably in prophetic passages addressing the Israelites. This phenomenon may be attributed to propagation through seedlings rather than clonal reproduction. Two significant references illustrate this viticultural challenge: In Isaiah 5, the text employs the metaphor of a carefully tended vineyard that produces inferior fruit, stating, “He waited for the vineyard to yield good grapes, but the fruit it produced was sour.” Similarly, Jeremiah 2 extends this metaphor, lamenting, “Yet I planted you as an elect vineyard, with only true seed. Then how have you been turned away from me, toward that which is depraved, O strange vineyard?” These passages not only serve as religious allegories but also potentially document early observations of genetic drift in grapevine cultivation. Such deterioration may have manifested particularly in female raisin and table grape cultivars exposed to cross-pollination from nearby wild grapevine populations, resulting in offspring with undesirable characteristics.

While *V. vinifera* cultivars cultivated in Germany, propagated solely vegetatively, ex-hibit hermaphroditic traits, both female and male forms emerge in their crosses with di-oecious wild grapevines (*V. sylvestris*). Consequently, feral hybridogenic populations may manifest dioecy, as highlighted by Wagner in 1960 [74]. Avramov et al. [75] initially pro-posed a two-allele model for controlling flower type in *V. vinifera* L. grape varieties, while Antcliff’s model introduced a single genetic determinant with three alleles for sex deter-mination, and subsequent research identified two markers linked to *Vitis* species flower sex loci on chromosome 2 [76–80].

Regardless of the explanatory model adopted to interpret dioecy control in *Vitis*, it is evident that dioecy, while a prerequisite for considering the possibility of a specific vine belonging to the species *V. sylvestris*, is not a sufficient characteristic. In natural habitats across Eurasia, dioecious vines can arise from the introgression of wild vines with cultivated varieties from nearby vineyards. Additionally, they may originate from American or Asian vines that have escaped cultivation or from direct dioecious hybrid producers.

Consequently, the occurrence of dioecious individuals across natural habitats cannot be exclusively attributed to *V. sylvestris* populations. These observations demonstrate the intricate interactions between natural evolutionary processes and anthropogenic selection pressures in determining genetic structure and population dynamics across diverse geographical distributions. Further research is warranted to elucidate the mechanisms underlying dioecy expression and its ecological implications within wild *Vitis* populations.

4.3. Cultivar Origins Unveiled: Exploring the Influence of Hybridization on the Evolution of Modern Grapevine Varieties

The analysis by Riaz et al. [77] of genetic relationships among selected grapevine genotypes provided evidence of genetic relationships between wild and cultivated samples from the Mediterranean basin to Central Asia. The genetic structure indicated a con-siderable amount of gene flow, which limited the differentiation between *Vitis vinifera* and *V. sylvestris*. Georgian wild grapevine accessions showed genetic clustering not only with local cultivars (classified under proles Pontica according to Negrul's system) but also with Western European varieties (proles Occidentalis), lending further support to Georgia's status as an ancient center of grapevine domestication. This may provide an explanation for the high degree of admixture detected in our analyses of samples from natural habitats in Georgia and, to a lesser extent, Armenia, where the presence of feral vines in those habitats is extremely high (Figure 7). Cluster analysis revealed that Western European wild grapevines grouped with cultivated varieties from the same region, suggesting that the local proles Occidentalis played a more significant role in Western European viticulture development than the introduction of wild or proto-domesticated vines from Eastern Europe [77]. The results also indicated that grapes with mixed ancestry occur in regions where wild vines were domesticated. This may be at the origin of the high degree of mix-ing and heterogeneity detected in the samples of the cultivars analyzed from Georgia, but also from France and Portugal, and to a lesser extent Spain (Figure 8).

Cunha et al. [81] in their study on local grapevine varieties grown in Portugal showed that part of the diversity recorded was mostly local in some cases, as demonstrated by the proximity of several varieties (Vinhos Verdes) to the wild cluster in different analyses. All these findings, together with the known pairing between the wild maternal lineage of the Iberian Peninsula and an important number of Portuguese grapevine varieties (with chlorotype A), point to the fact that some of these varieties derive, directly or indirectly, from originally local wild populations, supporting the possible occurrence of an introgression process from wild to cultivated grapevine. The predominantly local genetic diversity pat-terns and the east-to-west decline in diversity could alternatively suggest that local pop-ulations likely developed distinct sub-gene pools through time, primarily due to gene flow from domesticated to wild populations, rather than supporting wild-to-cultivated introgression.

4.4. Unraveling the Geographical Gradient of Phenotypic Characteristics in Western Europe

The spatial gradient from western to eastern regions of Spain exemplifies, on a reduced scale, the broader phenomenon of a higher prevalence of mixed populations or predominance of feral cultivars in the eastern portions of the species' range. Initial field sampling efforts aimed to collect morphologically identifiable fruits of the wild grape *Vitis sylvestris*, while attempting to exclude those from feral, rootstock, and recognizable hybrid individuals. However, subsequent analysis revealed notable differences when the data were organized by major river basins, providing overwhelming evidence of intermediate, mixed, or domesticated trait expressions (Figure 9).

The Guadiana River basin stands out, with a significant proportion of samples exhibiting wild characteristics (Cabañeros, Badajoz, and Chanza in Figure 9) and a relatively low degree of introgression. Conversely, the Ebro River basin shows a troubling situation, where nearly all samples are feral, domesticated, or intermediate, suggesting wide introgression processes from vineyards to natural habitats, seemingly replacing wild populations, except for one sample of Erro River with typical wild traits. However, other samples from the same river Erro, which is a tributary of the Irati, showed intermediate or clearly domesticated values. In all the samples from the banks of the Erro, a relatively high heterogeneity was observed, ranging between 3 and 5, when in pure populations of wild vines or in the fields of cultivated vines the heterogeneity varies between 0 and 2.

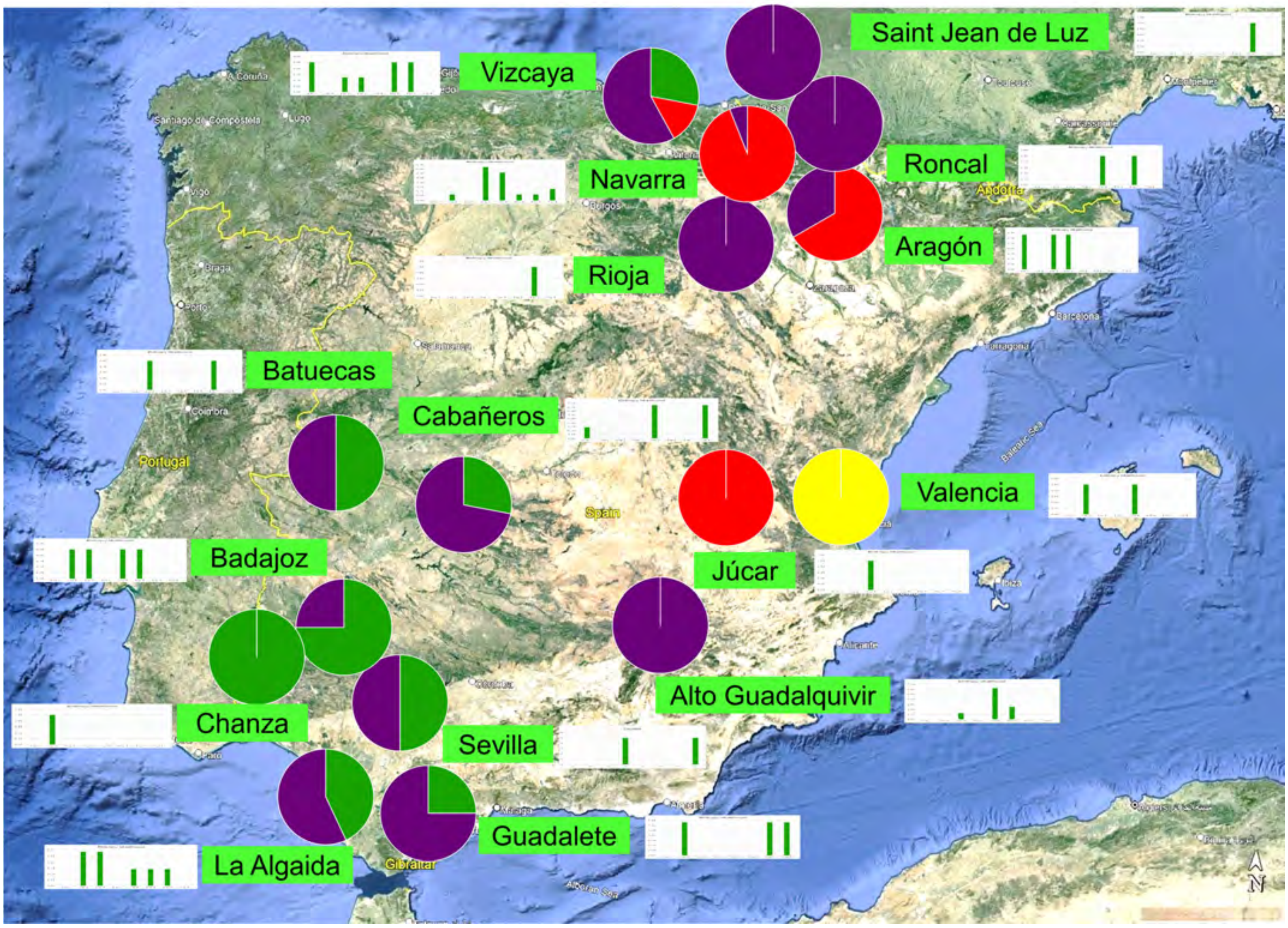

Figure 9. Analysis of the proportions of domesticated, wild, and intermediate seed types in selected Spanish populations from natural habitats and the heterogeneity within samples. Color codes: Red, domestic phenotype *V. vinifera*. Green, wild phenotype *V. sylvestris*. Purple, intermediate or mixed phenotypes. Yellow, American rootstocks. Note: the number of sampled localities in each river basin, ranges 1 to 17. Bar charts represent the proportion of heterogeneity within zones, where x is the heterogeneity class, ranging from 0 to 8 and y is the likelihood of each class. Image map: Diego Rivera. Background: Google Earth.

The small basins draining into the Cantabrian Sea primarily display pure wild or mixed characteristics, with only one population classified as feral with domestication traits (at Monchué). The mixed condition of populations in Urdaibai (Vizcaya, Spain) was analyzed by Albizuri et al. [82], where they recorded native wild Eurasian grapevines co-existing with feral *V. vinifera* varieties, feral rootstocks resembling *Vitis rupestris* or *V. ri-paria*, and direct producer hybrid grapevines. Our sample from Urdaibai has wild charac-teristics with heterogeneity values less than 1; thus, it is attributed to *V. sylvestris*.

In the few sampled localities along the western Tagus River, only intermediate or wild vines were found (Figure 8). In the Jucar and Turia basins, feral *V. vinifera* cultivars and American rootstock grapevines were identified, as previously reported by Laguna on an ampelographic basis [83–85].

In the study by De-Andrés et al. [86] on a molecular basis, in the structure model, depending on the number of clusters identified by adjusting the parameter K of Evano, individuals from wild populations of Spanish grapevines increasingly differentiate from cultivated ones as the number of clusters grows. However, traces of introgression persist in both cases, while a notable difference between wild populations from the northern and southern regions of the Peninsula is distinguished. A portion of these populations has also been studied in the present work, where the north–south gradient is detected but is partly masked by a notable east–west difference (Figure 9).

Regarding heterogeneity within each sample (Figure 9), we observe the lowest values in Chanza, associated with wild vines, and the highest values in La Rioja and along the banks of the Bidasoa River in Saint Jean de Luz, associated in this case with mixed or intermediate populations, with a notable presence of "hybrids" or "mestizos" in the Levadoux sense [8]. In the rest of the areas, we find significant variability among samples, ranging from low to very high in Cabañeros, Biscay, and Guadalete, intermediate in Aragón, Valencia, Jucar, La Algaida, Upper Guadalquivir, Badajoz, or Batuecas, and from moderate to very high in Navarra and El Roncal.

The analyzed samples collected in natural habitats from France and Switzerland are limited in number, hindering a comprehensive understanding of the issue. In the case of Saint Jean de Luz (France), there appears to be an intermediate grapevine population with a significant feral influence. Ocete et al. [87] described a wide range of Stummer's index values for the Saint Jean de Luz population, varying between 0.5 and 0.8, with a mode below 0.75, supporting the notion of a wide introgression with a substantial feral influ-ence. Our results coincide, showing an intermediate mean domestication probability of 0.56 associated with a high heterogeneity above 6 which is characteristic of mixed indi-viduals and populations, which may imply the presence in the population of sub-sponta-neous wild vines, colonial or feral, and of crossbreeds (mestizos in the sense of Levadoux) with varying degrees of introgression between the native wild autochthonous and the re-maining. Conversely, the population in the Rhône Valley, Mont d'Autan, Valais, in Swit-zerland, with a mean domestication probability of only 0.1 and heterogeneity index below 2, seems unequivocally native wild (*V. sylvestris*).

In Sardinia, the situation is even more complex (Figure 10), since only one locality, Montresta, shows clearly wild characteristics, with a domestication probability of only 0.07, although with heterogeneity values above 2. In Santadi, in the Pula area, one sample was identified as wild, with a very low probability of domestication, 0.12, and a heterogeneity below 2. But other samples from the area were clearly intermediate (with a domestication probability of 0.67, but extremely high heterogeneity above 7) or domesticated (with a domestication probability of 0.97, and a heterogeneity index value of only 1.3). A third sample, among the 31 analyzed from Sardinia, could eventually be identified as wild, collected at Flumini Maggiore–Malacalzetta, but with a very high heterogeneity within the sample, close to 6, and a probability of domestication higher than 0.2; we should consider it a result of successive introgressions with domesticated vines. Regarding the heterogeneity within each sample, it is highest in the localities of Padria and Talana, and lowest in Tiana. Meanwhile, intermediate values are observed in Montresta, Santu Lussurgiu, Domusnovas, Nuoro, and partly in Urzulei. There are populations with samples showing varied levels of heterogeneity, typically ranging from intermediate to high, such as Malacalzetta, Uta, Pula, Villanova Tulo, Mamoiada, and Orgosolo (Figure 10).

Using molecular markers, phenomena of introgression were detected by Zecca et al. [88] in the wild grapevine populations of Sardinia, originating from nearby vineyards. Contrary to our findings, the authors emphasize the predominantly wild nature of the sampled individuals. We propose that the molecular markers currently utilized to differ-entiate between wild and domesticated grapevines should be validated through compre-hensive phenotypic analyses of voucher specimens.

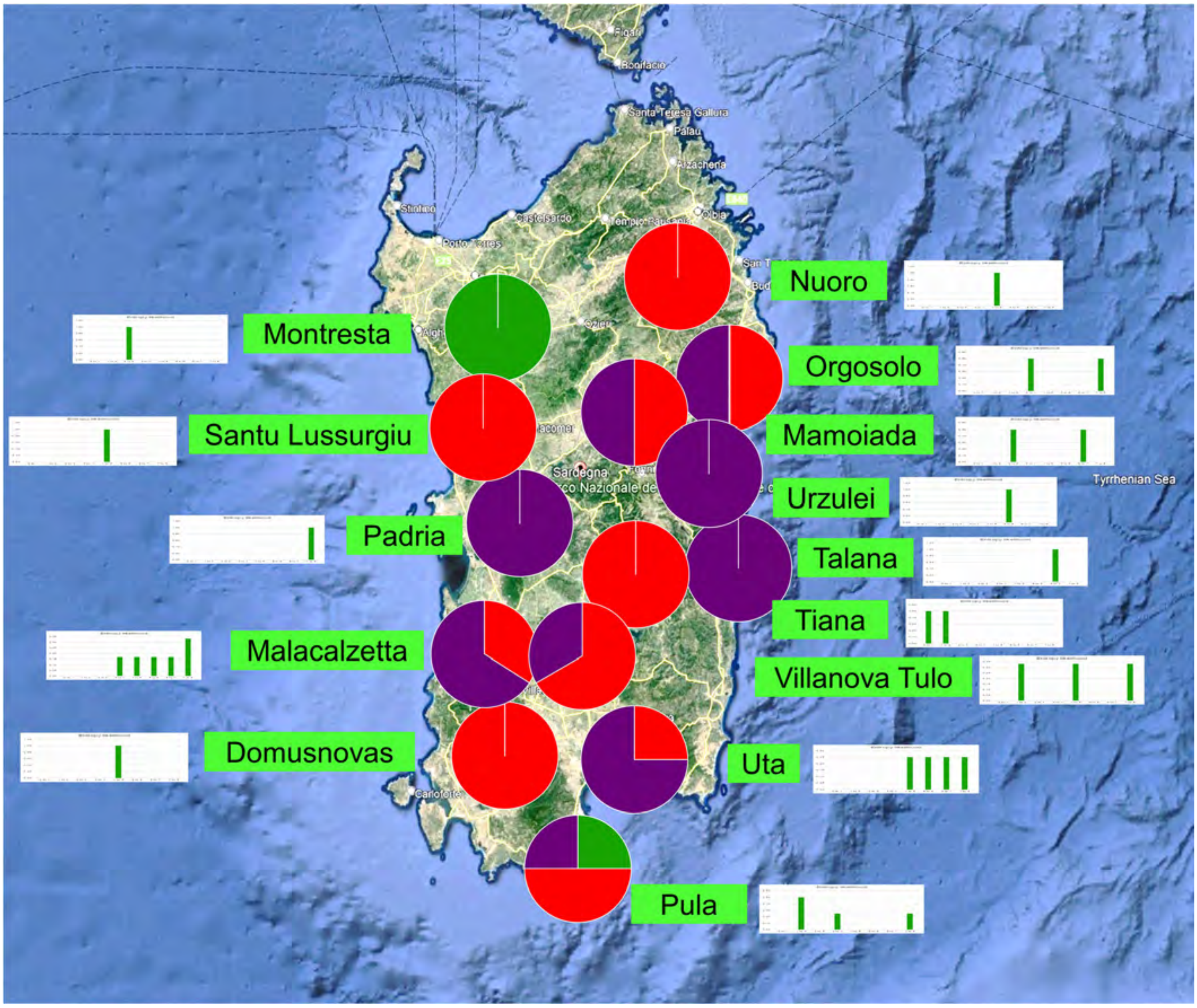

Figure 10. Analysis of the proportions of domesticated, wild, and intermediate seed types in selected Sardinian populations (Italy) from natural habitats and their heterogeneity within samples. Color codes: Red, domestic phenotype *V. vinifera*. Green, wild phenotype *V. sylvestris*. Purple, intermediate or mixed phenotypes. Bar charts represent the proportion of heterogeneity within zones, where *x* is the heterogeneity class, ranging from 0 to 8 and *y* is the likelihood of each class. Image map: Diego Rivera. Background: Google Earth.

Our findings suggest an alternative interpretation to the proposed secondary center of grapevine domestication in Sardinia by Grassi et al. in 2003 [89]. While the genetic proximity between wild grapevine specimens from Nuoro and local Sardinian cultivars (Bo-vale Murru and Bovale Muristellu) has been interpreted as evidence of local domestication, our data support an alternative hypothesis. The presence of exclusively domesticated-type seeds in our Nuoro wild grapevine samples suggests these populations may represent naturalized or feral vines rather than truly wild specimens, thus challenging the local domestication theory (Figure 10).

In summary, the examination of wild grapevine populations in Western Europe, particularly in Spain and Sardinia (Italy), reveals a substantial degree of introgression or hy-bridization, with seeds exhibiting domesticated traits that can be interpreted as either re-peated introgressions or feral occurrences. This phenomenon is not confined to Western Europe, as similar patterns are observed in the Caucasus, where the degree of domestication in "wild" grapevines is higher [90] (Figure 7). Riaz et al. [61] analyzed the relation-ships between 1378 wild and cultivated grapevine genotypes as represented by the first two principal coordinates of a PCoA using allelic profiles from 20 SSR molecular markers. The figures clearly indicate the existence of two well-differentiated groups within *Vitis sylvestris:* one centered in the Caucasus (Armenia, Azerbaijan, and Georgia) and another in Western Europe (Spain, Croatia, France, and Italy), separated by the core of cultivars originating from France, Spain, Georgia, Italy, Pakistan, and Turkmenistan. This provides us with an alternative perspective on the anomalies detected in the phenotypes of wild vines from the South Caucasus with a higher degree of domestication.

4.5. Highlighting Conservation Significance: Recognizing Mixed Grapevine Populations as Crucial Repositories of Genetic Diversity

The relatively high stability of natural riparian habitats over the centuries until the mid-20th century made them an optimal refuge for escaped cultivars, whose seeds were transported from the vineyards by fruit-eating birds and mammals. In addition, there is evidence of the occasional but intentional practice of grafting cultivars onto wild grape-vine individuals in riparian forests, thus increasing the presence of domesticated grape-vines among wild plants. Terpó [21,22,91,92] described examples from a Hungarian beech forest of grapevine populations with 80% male individuals, including feral forms of *V. riparia* and escaped *V. vinifera* cultivars.

This continuous process of introgression into wild populations is not merely recent, since it was noted by authors in the nineteenth and twentieth centuries before and after the introduction of phylloxera and other grapevine pests, which led to the abandonment of European vineyards, followed by their replantation using American grapevines and their hybrids as rootstocks. It underscores the gene reservoir role of populations in natural habitats. This reservoir extends beyond wild grapevines to include cultivated grapevines, offering the potential discovery of ancient cultivars lost from vineyards, as previously noted by De Andrés et al. [86].

Sometimes no relationships between wild populations and nearby vineyards are detected, which may be due to recent and profound changes in the repertoire of cultivars growing in the vineyards. Molecular marker studies suggest no genetic contribution from the autochthonous vines of the Iregua River, a tributary of the Ebro, to the current local varieties, and vice versa [93]. Notably, despite an *HI* value of 0.31 and *DI* value of 0.62 (Supplementary Table S1, Sample 254), the seeds studied suggest introgression, possibly with varieties that have not been grown in the area for a century.

The adverse effects of anthropogenic habitat alteration on riparian forests extend beyond the mere destruction of tree cover. The introduction of invasive species and pathogens further exacerbates these impacts, potentially posing significant consequences for biodiversity conservation and overall ecosystem survival.

The invasion of phylloxera, powdery mildew, and downy mildew in 1850 caused the disappearance, in only half a century, of most vineyards and numerous wild grapevines, in France and other countries of Europe [8]. This resulted in significant transformations in the structure of grapevine populations, both within vineyards and in natural habitats.

Given the great diversity of wild populations of *Vitis* found in riparian forests and other natural habitats, from the Iberian Peninsula to Central Asia, which harbor not only wild vines but also modern and ancient feral cultivars, as well as a complex network of introgressions, the systematic "cleaning and rearrangement" of riverbanks with the elimination of existing vegetation means the destruction and loss of this diversity of great interest for agriculture and food.

Another aspect to consider is the growing presence of feral American vines in the natural habitats of Eurasia because of the massive use of these species and their hybrids as rootstocks, of which we have an example in Valencia (Spain) [83–85]. This phenomenon leads to the substitution of the hybrid swarm *Vitis vinifera–Vitis sylvestris* by American vines with lesser aptitudes to produce grapes of interest for table consumption or wine-making. The remarkable presence of feral rootstocks along both banks of the Danube River between Esztergom and Budapest stands out. In this area, true wild specimens of *V. sylvestris* are only preserved on certain islets, as documented by Popescu et al. in 2013 [94]. Feral individuals of American rootstocks and their hybrids have also been identified in Italy and Spain [40,41,95,96]. American rootstock genotypes now constitute the predomi-nant wild *Vitis* germplasm in several European viticultural regions. This is particularly evident in areas such as Campania, Italy, where they have largely displaced native wild *Vitis* populations—if these populations had not already been lost due to phylloxera [97].

Recent morphometric approaches to grapevine seed analysis have yielded valuable insights. Karasik et al. [98] demonstrated clear varietal differentiation using Fourier coefficient analysis, though they did not explore intra-sample heterogeneity or wild–cultivated relationships. More comprehensively, Bouvy et al. [99] analyzed over 19,000 archaeological grape pips, revealing a transition from wild to domestic varieties in France around 600–500 BCE, coincident with Mediterranean influences and eastern table cultivar introduction. Their discovery of abundant wild-like morphologies suggests both early-stage domestication and significant gene flow between the introduced and wild popula-tions, supporting the concept of continuous genetic exchange during domestication.

Riaz et al. [61] emphasized the importance of exploring wild relatives of crops to identify genetic factors associated with stress resistance. Specifically, they mention the presence of salt-tolerant grape accessions in certain *V. sylvestris* populations and the recent discovery of wild and cultivated accessions from Germany, Iran, and Georgia with tolerance to mildew diseases. This stresses the potential of wild ancestors as genetic resources for breeding disease-resistant varieties.

In summary, we must underscore the significance of identifying, preserving, and characterizing wild grapevine germplasm (including true wild, feral, and intermediate), for their potential contributions to disease and stress resistance, as well as berry quality traits, in the wine and grape industry's future.

Further research is needed to assess the extent to which American rootstock genotypes have displaced both naturalized *Vitis vinifera* populations and wild *Vitis sylvestris* communities in natural and semi-natural habitats. Additionally, it is crucial to evaluate the conservation status and potential threats to remnant Eurasian grapevine populations in their native ecosystems.

5. Conclusions

The coexistence of mixed populations, comprising dioecious autochthonous wild subpopulations, feral hermaphroditic cultivars, and hybrids, presents opportunities for genetic diversification in both cultivated crops and wild populations. Moreover, these mixed populations serve as potential repositories for ancient varieties that are currently absent from cultivation. However, the drastic intervention in natural habitats, particularly riparian forests, poses a significant threat. Activities such as riverbank channeling, improper clearing of waterways, and large-scale public infrastructure projects have resulted in the burial of numerous pure or hybrid wild grapevine populations.

Following such interventions, secondary colonization by modern cultivars from nearby vineyards or by rootstocks that produce seeds in abandoned vineyards often occurs. In this process, birds play a crucial role in facilitating the recolonization of altered habitats by seedlings from cultivated or abandoned vineyards.

It is imperative to raise awareness about the fragility of grapevine natural habitats, the ongoing reduction in their area in Europe, and the anticipated impact of climate change. These factors underscore the necessity to protect and preserve these habitats as repositories of significant diversity legally and practically. By doing so, we can safeguard the genetic resources essential for the sustainability and resilience of grapevine cultivation in the face of environmental challenges.

Potential directions for future research should encompass aspects such as the following:

Investigating the specific mechanisms driving secondary colonization by modern cultivars and rootstocks in altered habitats could provide insight into the dynamics of grapevine population shifts. Assessing the effectiveness of different conservation strategies would be crucial for preserving wild grapevine populations and their genetic diversity. Exploring the potential for utilizing mixed populations as sources of genetic material could enable breeding programs aimed at improving crop resilience and adaptability. Additionally, examining the role of other wildlife, in addition to birds, in facilitating the dispersal and colonization of grapevines in altered habitats may reveal important ecological interactions. Conducting parallel studies to quantify phenotypic and genetic diversity in mixed populations could shed light on their potential for enhancing grapevine resilience to environmental stressors.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae11010092/s1, Table S1: Samples analyzed organized by decreasing heterogeneity and increasing proportion of seeds with wild traits; Table S2: Samples analyzed organized by hybridization index, domestication index values, and geographical origins; Methods S1: Standard methods.

Author Contributions: Conceptualization, D.R. and C.O.; Data curation, J.V., D.M., M.K., A.N., E.L., F.A., D.J.R.-O., G.L. and M.F.; Formal analysis, D.J.R.-O.; Investigation, D.R., J.V., M.K., A.N., R.O. and M.F.; Methodology, D.R., F.A. and D.J.R.-O.; Resources, D.R., D.M., A.N., R.O., C.Á.O., C.A., E.L. and G.L.; Software, F.A. and D.J.R.-O.; Validation, R.O. and C.O.; Visualization, D.R.; Writing—original draft, D.R.; Writing—review and editing, D.R., D.M., E.L. and C.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, and further inquiries can be directed to the corresponding author/s.

Acknowledgments: We extend our gratitude to Erika Maul from The Institute for Grapevine Breeding Geilweilerhof, Germany, for generously providing samples of grapes from contemporary Near Eastern varieties. Additionally, we express our appreciation to Gayane Melyan from the Center of Agrobiotechnology of the Armenian National Agrarian University in Etchmiadzin, Armenia, for sending wild grape samples from Armenia. We would also like to acknowledge the contributions of several institutions and individuals. Specifically, we thank the U.S. National Plant Germplasm System, Rancho Santa Ana, Istituto ed Orto Botanico di Palermo, Botanisches Garten Johannes Gutenberg—Universität Mainz, Smithsonian Institution, and Giardino Botanico di Padova for supplying us with seeds of American and Asian Vitis species and related genera. Furthermore, our gratitude extends to the curators of grapevine collections, including those from the Rioja region (Spain) in Mendavia, La Casa de las Vides nurseries in Agullent (Valencia, Spain), Rojas Clemente grapevines from the Royal Botanical Garden of Madrid (Spain), and the Istituto Agrario di San Michele all'Adige (Trentino, Italy), for granting us access to leaves, shoots, and grapes for our research. Wild populations were sampled by the authors and Encarna Carreño.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Magurran, A.E. *Ecological Diversity and Its Measurement*; Springer Science & Business Media: Secaucus, Spain, 2013.
2. Rivera, D.; Alcaraz, F.; Rivera-Obón, D.J.; Obón, C. Phenotypic Diversity in Wild and Cultivated Date Palm (*Phoenix*, Arecaceae): Quantitative Analysis Using Information Theory. *Horticulturae* **2022**, *8*, 287. [CrossRef]
3. Dong, Y.; Duan, S.; Xia, Q.; Liang, Z.; Dong, X.; Margaryan, K.; Musayev, M.; Goryslavets, S.; Zdunić, G.; Bert, P.F.; et al. Dual domestications and origin of traits in grapevine evolution. *Science* **2023**, *379*, 892–901. [CrossRef] [PubMed]
4. Grassi, F.; De Lorenzis, G. Back to the origins: Background and perspectives of grapevine domestication. *Int. J. Mol. Sci.* **2021**, *22*, 4518. [CrossRef] [PubMed]
5. Arnold, C. Ecologie de la vigne sauvage, *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, dans les forêts alluviales et colluviales d'Europe. *Geobot. Helv.* **2002**, *6*, 1–256.
6. Arnold, C.; Gillet, F.; Gobat, J.M. Occurrence of the wild grapevine *Vitis vinifera* ssp. *sylvestris* in Europe. *Vitis* **1998**, *37*, 159–170.
7. Martínez de Toda, F. *Biología de la Vid*; Mundi-Prensa: Madrid, Spain, 1991; pp. 1–346.
8. Levadoux, L. Les populations sauvages et cultivées de *Vitis vinifera* L. *Ann. Amélior. Plant.* **1956**, *1*, 59–118.
9. Mercuri, A.M.; Torri, P.; Florenzano, A.; Clò, E.; Mariotti Lippi, M.; Sgarbi, E.; Bignami, C. Sharing the Agrarian Knowledge with Archaeology: First Evidence of the Dimorphism of *Vitis* Pollen from the Middle Bronze Age of N Italy (Terramara Santa Rosa di Poviglio). *Sustainability* **2021**, *13*, 2287. [CrossRef]
10. Gallardo, A.; Ocete, R.; López Martínez, M.A.; Lara, M.; Rivera, D. Assesment of pollen dimorphism in populations of *Vitis vinifera* L. subsp. *sylvestris*(Gmelin) Hegi in Spain. *Vitis* **2009**, *48*, 59–62.
11. This, P.; Lacombe, T.; Thomas, M.R. Historical origins and genetic diversity of wine grapes. *Trends Genet.* **2006**, *22*, 511–519. [CrossRef]
12. Stummer, A. Zur Urgeschichte der Rebe und des Weinbaumes. *Mitt. Anthrop. Ges. Wien* **1911**, *41*, 283–296.
13. Ocete, C.A.; Martínez-Zapater, J.; Ocete, R.; Lara, M.; Cantos, M.; Arroyo, R.; Morales, R.; Iriarte-Chiapusso, M.; Hidalgo, J.; Valle, J.M.; et al. La vid silvestre euroasiática; un recurso fitogenético amenazado ligado a la historia de la humanidad. *Enoviticultura* **2018**, *50*, 1–16.
14. Ocete, R.; Arnold, C.; Failla, O.; Lovicu, G.; Biagini, B.; Imazio, S.; Lara, M.; Maghradze, D.; Lopez, M.A. Considerations on the European wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) and Phylloxera infestation. *Vitis* **2011**, *50*, 97–98.
15. Turkovic, Z. Untersuchungs ergebnisse über *Vitis sylvestris* Gmelin im Jahre 1954. *Weinb. Keller* **1955**, *2*, 74–79.
16. Bitsadze, N.; Kikilashvili, S.; Chipashvili, R.; Mamasakhlisashvili, L.; Maghradze, T.; Kikvadze, M.; Toffolatti, S.L.; De Lorenzis, G.; Failla, O.; Ocete, R.; et al. Resistance to downy mildew in wildly growing Eurasian *Vitis vinifera* L. grapevines. *J. Plant Pathol.* **2024**, *106*, 1759–1771. [CrossRef]
17. Campostrini, F.; Anzani, R.; Failla, O.; Iacono, F.; Scienza, A.; de Micheli, L. Application of the phyllometric analysis to the geographic classification of Italian population of wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* Hegi). *J. Int. Sci. Vigne Vin* **1993**, *27*, 255–262.
18. Rathay, E. *Die Geschlechtsverhältnisse der Reben und ihre Bedeutung für den Weinbau*; K.K. Hofbuchhandlung Wilhem Frick: Wien, Austria, 1889; Volume 2.
19. Zdunić, G.; Maul, E.; Eiras Dias, J.; Muñoz-Organero, G.; Carka, F.; Maletić, E.; Savvides, S.; Jahnke, G.; Nagy, Z.; Nikolić, D.; et al. Guiding principles for identification, evaluation and conservation of *Vitis vinifera* L. subsp. *sylvestris*. *Vitis* **2017**, *56*, 127–131. [CrossRef]
20. Facsar, G. Összehasonlitö morfolögiai vizsgälatok kerti szölöfajtäk magjain I (Vergleichende morphologische Unter-suchungen der Samen von Gartenrebensorten. I.). *Bot. Közlemenyek* **1970**, *57*, 221–231.
21. Terpó, A. The carpological examination of wild growing grapevine species of Hungary. I. Qualitative and quantitative characteristics of grapevine seeds. *Acta Bot. Hung.* **1976**, *22*, 209–247.
22. Terpó, A. The carpological examination of wild growing grapevine species of Hungary. II. Qualitative and quantitative characteristics of grapevine seeds. *Acta Bot. Hung.* **1977**, *23*, 247–273.
23. Facsar, G.; Jerem, E. Zum urgeschichtlichen Weinbau in Mitteleuropa. Rebkernfunde von *Vitis vinifera* L. aus der urnenfelder-, hallstatt-und latenezeitlichen Siedlung Sopron-Krautacker. *Wiss. Arb. Burgenland* **1985**, *71*, 121–144.
24. Rivera, D.; Walker, M. A review of paleobotanical findings of early *Vitis* in the Mediterranean and of the origins of cultivated grape-vines, with special reference to prehistoric exploitation in the Western Mediterranean. *Paleobot. Palynol.* **1989**, *61*, 205–237. [CrossRef]
25. Mangafa, M.; Kotsakis, K. A new method for the identification of wild and cultivated charred grape seeds. *J. Archaeol. Sci.* **1996**, *23*, 409–418. [CrossRef]
26. Perret, M. *Caractérisation et Evaluation du Polymorphisme des Génotypes Sauvages et Cultivés de Vitis vinifera L. à L'aide de Marqueurs RAPD et de Certains Traits Morphologiques*; Travail de Diplôme, Université de Neuchâtel: Neuchâtel, Switzerland, 1997.
27. Rivera, D.; Miralles, B.; Obón, C.; Carreño, E.; Palazón, J.A. Multivariate analysis of *Vitis* subgenus *Vitis* seed morphology. *Vitis* **2007**, *46*, 158–167.

28. Obón, C.; Rivera, D.; Carreño, E.; Alcaraz, F.; Palazón, J.A. Seed Morphology of *Vitis vinifera* and Its Relationship to Ecogeographical Groups and Chlorotypes. *Acta Hortic.* **2008**, *799*, 51–69. [CrossRef]
29. Orrù, M.; Grillo, O.; Lovicu, G.; Venora, G.; Bacchetta, G. Morphological characterisation of *Vitis vinifera* L. seeds by image analysis and comparison with archaeological remains. *Veg. Hist. Archaeobot.* **2012**, *22*, 231–242. [CrossRef]
30. Orrù, M.; Grillo, O.; Venora, G.; Bacchetta, G. Computer vision as a method complementary to molecular analysis: Grapevine cultivar seeds case study. *C. R. Biol.* **2012**, *335*, 602–615. [CrossRef]
31. Orrù, M.; Grillo, O.; Venora, G.; Bacchetta, G. Seed morpho-colotimetric análisis by computer vision: A helpful tool to identify grapevine (*Vitis vinifera* L.) cultivars. *Aust. J. Grape Wine Res.* **2015**, *21*, 508–519. [CrossRef]
32. Ucchesu, M.; Orrù, M.; Grillo, O.; Venora, G.; Usai, A.; Serreli, P.F.; Bacchetta, G. Earliest evidence of a primitive cultivar of *Vitis vinífera* L. during the Bronze Age in Sardinia (Italy). *Veg. Hist. Archaeobot.* **2015**, *24*, 587–600. [CrossRef]
33. Ucchesu, M.; Orrò, M.; Grillo, O.; Venora, G.; Pagietti, G.; Ardu, A.; Bacchetta, G. Predictive method for correct identification of archaeological charred grape sedes: Support for advances in knowledge of grape domestication process. *PLoS ONE* **2016**, *11*, e0149814. [CrossRef]
34. Bonhomme, V.; Ivorra, S.; Lacombe, T.; Evin, A.; Figueiral, I.; Maghradze, D.; Marchal, C.; Pagnoux, C.; Pastor, T.; Pomarèdes, H.; et al. Pip shape echoes grapevine domestication history. *Sci. Rep.* **2021**, *11*, 21381. [CrossRef]
35. Obón, C.; Rivera-Obón, D.J.; Valera, J.; Matilla, G.; Alcaraz, F.; Maghradze, D.; Kikvadze, M.; Ocete, C.A.; Ocete, R.; Nebish, A.; et al. Is there a domestication syndrome in *Vitis* (Vitaceae) seed morphology? *Genet. Resour. Crop Evol.* **2024**, 1–25. [CrossRef]
36. POWO. Plants of the World Online. Available online: https://powo.science.kew.org/ (accessed on 15 February 2024).
37. GRIN. Search Taxonomy Data in GRIN-Global. Available online: https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch (accessed on 15 February 2024).
38. Facsar, G.; Udvardy, L. Adventive grapevine species (*Vitis*-hybrids). In *The Most Important Invasive Plants in Hungary*; Botta-Dukát, Z., Balogh, L., Eds.; Institute of Ecology and Botany, Hungarian Academy of Sciences: Vácrátót, Hungary, 2008; pp. 47–54.
39. Schnitzler, A.; Arnold, C.; Guibal, F.; Walter, J.M. Histoire de la vigne sauvage, *Vitis vinifera* ssp. *sylvestris*, en Camargue / Wild grapevine *Vitis vinifera* ssp. *sylvestris* in Camargue, southern France. *Ecol. Mediterr.* **2018**, *44*, 53–66. [CrossRef]
40. Ardenghi, N.M.; Galasso, G.; Banfi, E.; Zoccola, A.; Foggi, B.; Lastrucci, L. A taxonomic survey of the genus *Vitis* L. (Vitaceae) in Italy, with special reference to Elba Island (Tuscan Archipelago). *Phytotaxa* **2014**, *166*, 163–198. [CrossRef]
41. Vázquez, F.M.; García Alonso, D. Aproximación al conocimiento de los táxones del género *Vitis* L. (Vitaceae) que viven silvestres en Extremadura (España). *Folia Bot. Extrem.* **2017**, *11*, 5–37.
42. Valera, J.; Matilla-Seiquer, G.; Obón, C.; Alcaraz, F.; Rivera, D. Grapevine in the ancient Upper Euphrates: Horticultural implications of a Bayesian morphometric study of archaeological seeds. *Horticulturae* **2023**, *9*, 803. [CrossRef]
43. Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; et al. Fiji: An Open-Source Platform for Biological-Image Analysis. *Nat. Methods* **2012**, *9*, 676–682. [CrossRef]
44. Hosmer, D.W., Jr.; Lemeshow, S.; Sturdivant, R.X. *Applied Logistic Regression*; John Wiley & Son: Hoboken, NJ, USA, 2013.
45. Couronné, R.; Probst, P.; Boulesteix, A.L. Random forest versus logistic regression: A large-scale benchmark experiment. *BMC Bioinform.* **2018**, *19*, 1–14. [CrossRef]
46. randomForest. randomForest: Breiman and Cutler's Random Forests for Classification and Regression. Available online: https://cran.r-project.org/web/packages/randomForest/index.html (accessed on 18 December 2023).
47. Breiman, L. Random Forests. *Mach. Learn.* **2001**, *45*, 5–32. [CrossRef]
48. Breiman, L.; Cutler, A.; Liaw, A.; Wiener, M. Package 'randomForest' 2022. Available online: https://cran.r-project.org/web/packages/randomForest/randomForest.pdf (accessed on 18 December 2023).
49. Rossiter, D.G. Random Forests Two Ways. Available online: https://www.css.cornell.edu/faculty/dgr2/_static/files/R_PDF/CompareRandomForestPackages.pdf (accessed on 18 December 2023).
50. R Project. The R Project for Statistical Computing. Available online: https://www.r-project.org/ (accessed on 18 December 2023).
51. RStudio. RStudio Desktop. Available online: https://posit.co/download/rstudio-desktop/ (accessed on 18 December 2023).
52. Liu, Y.; Wang, Y.; Zhang, J. New Machine Learning Algorithm: Random Forest. In *ICICA 2012, LNCS 7473*; Liu, B., Ma, M., Chang, J., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 246–252.
53. R-Bloggers. Random Forest in R. 2023. Available online: https://www.r-bloggers.com/2021/04/random-forest-in-r/ (accessed on 18 December 2023).
54. Negrul, A.M. Origin of the cultivated grapevine and its classification. In *Ampelografiia SSSR [Ampelography of the USSR]*; Varanov, A., Ed.; Pischepromizdat: Moscow, Russia, 1946; Volume 1, pp. 133–216. (In Russian)
55. La Mancha Denominación de Origen. Available online: https://lamanchawines.com/denominacion-de-origen/mapa-y-estadisticas/ (accessed on 3 September 2024).
56. Manchuela Denominación de Origen. Available online: https://www.manchuela.wine/cifras-y-anadas/ (accessed on 3 September 2024).

57. Almansa Denominación de Origen. Available online: https://denominacion-origen-almansa.com/zona-de-produccion/ (accessed on 3 September 2024).
58. Jumilla Denominación de Origen. Available online: https://jumilla.wine/municipios-de-la-dop/ (accessed on 3 September 2024).
59. D'Onofrio, C. Introgression among cultivated and wild grapevine in Tuscany. *Front. Plant Sci.* **2020**, *11*, 202. [CrossRef]
60. Di Vecchi-Staraz, M.; Laucou, V.; Bruno, G.; Lacombe, T.; Gerber, S.; Bourse, T.; Boselli, M.; This, P. Low level of pollen-mediated gene flow from cultivated to wild grapevine: Consequences for the evolution of the endangered subspecies *Vitis vinifera* L. subsp. *silvestris*. *J. Hered.* **2009**, *100*, 66–75. [CrossRef]
61. Riaz, S.; De Lorenzis, G.; Velasco, D.; Koehmstedt, A.; Maghradze, D.; Bobokashvili, Z.; Musayev, M.; Zdunic, G.; Laucou, V.; Andrew Walker, M.; et al. Genetic diversity analysis of cultivated and wild grapevine (*Vitis vinifera* L.) accessions around the Mediterranean basin and Central Asia. *BMC Plant Biol.* **2018**, *18*, 137. [CrossRef] [PubMed]
62. Clemente, S. *Ensayos Sobre las Variedades de vid que Vegetan en Andalucia*; Villalpando: Madrid, Spain, 1807.
63. Clemente, S. *Essai sur les Variétés de la Vigne qui Végétent en Andalousie*; Poulet: Paris, France, 1814.
64. Clemente, S. *Ensayo Sobre las Variedades de la vid Común que Vegetan en Andalucía*; Estereotipia Perojo: Madrid, Spain, 1879.
65. Bronner, J.P. *Die Wilden Trauben des Rheinthales*; Buchdruckerei von Georg Mohr: Heidelberg, Germany, 1857.
66. Bronner, J.P. Description des vignes sauvages de la vallée du Rhin. *La Bourgogne* **1859**, *1*, 97–110.
67. Scossiroli, R.E. Origine ed evoluzione della vite. *Atti Ist. Bot. e Lab. Critt.* **1988**, *7*, 35–55.
68. Scienza, A.; Failla, O.; Anzani, R. Wild grapevine (Vitis vinifera L. *sylvestris* C.C.Gmel.) in Italy: Diffusion, characteristics and germplasm preservation. In Proceedings of the Germplasm Preservation of Horticultural Crops: Wild and·Cultivated Plants, Bejing, China, 1–3 September 1988.
69. Scienza, A.; Protii, A.; Conca, E.; Romano, F. Diffusione e caratteristiche della *Vitis vinifera sylvestris* Gmelin in ltalia. In Proceedings of the Compte Rendu IV Symposium Lntern. de Génétique de la Vigne, Verona, Italy, 13–18 April 1986; pp. 86–95.
70. Anzani, R.; Failla, O.; Scienza, A.; Campostrini, E. Wild grapevine (Vitis vinifera ssp. *sylvestris*) in Italy: Distribution characteristics and germplasm preservation. In Proceedings of the 5th International Symposium on Grape Breeding, St. Martin/Pfalz, Germany, 12–16 September 1989; pp. 97–113.
71. Anzani, R.; Failla, O.; Scienza, A.; De Micheli, L. Individuazione e conservazione del germoplasma di vite selvatica (*Vitis vinifera sylvestris*) in Italia. *Vignevini* **1993**, *6*, 51–60.
72. Failla, O.; Anzani, R.; Scienza, A. La vite selvatica in Italia: Diffusione, caratteristiche e conservazione del germoplasma. *Vignevini* **1992**, *19*, 37–46.
73. Kikvadze, M.; Kikilashvili, S.; Bitsadze, N.; Maghradze, T.; De Lorenzis, G.; Rubio, R.O.; Rivera, D.; Bacilieri, R.; Failla, O.; Maghradze, D. Wildly growing Eurasian grapevine (*Vitis vinifera* L.) in Georgia: Composition, research and efforts for the preservation. *Acta Hortic. ISHS* **2024**, *1385*, 19–24. [CrossRef]
74. Wagner, E. Die Feststellung des Geschlechts bei Rebensämlingen. *Vitis* **1960**, *2*, 190–197.
75. Avramov, L.; Jelenkovi, G.; Jovanovi, M.; Rodic, Z. Inheritance of flower type in some grape varieties (*Vitis vinifera* L.). *Vitis* **1967**, *6*, 129–135.
76. Dalbo', M.A.; Ye, G.N.; Weeden, N.G.; Steinkellner, H.; Sefc, K.M.; Reisch, B.I. A gene controlling sex in grapevines placed on a molecular-based genetic map. *Genome* **2000**, *43*, 333–340. [CrossRef]
77. Riaz, S.; Krivanek, A.F.; Xu, K.; Walker, M.A. Refined mapping of the Pierce's disease resistance locus, PdR1, and Sex on an extended genetic map of *Vitis rupestris* × *V. arizonica*. *Theor. Appl. Genet.* **2006**, *113*, 1317–1329. [CrossRef]
78. Marguerit, E.; Boury, C.; Manicki, A.; Donnart, M.; Butterlin, G.; Ne'morin, A.; Wiedemann-Merdinoglu, S.; Merdinoglu, D.; Ollat, N.; Decroocq, S. Genetic dissection of sex determinism, inflorescence morphology and downy mildew resistance in grapevine. *Theor. Appl. Genet.* **2009**, *118*, 1261–1278. [CrossRef] [PubMed]
79. Coito, J.L.; Silva, H.G.; Ramos, M.J.; Cunha, J.; Eiras-Dias, J.; Amâncio, S.; Costa, M.M.; Rocheta, M. *Vitis* flower types: From the wild to crop plants. *PeerJ* **2019**, *7*, e7879. [CrossRef] [PubMed]
80. Fechter, I.; Hausmann, L.; Daum, M.; Sörensen, T.R.; Viehöver, P.; Weisshaar, B.; Töpfer, R. Candidate genes within a 143 kb region of the flower sex locus in *Vitis*. *Mol. Genet. Genom.* **2012**, *287*, 247–259. [CrossRef]
81. Cunha, J.; Ibáñez, J.; Teixeira-Santos, M.; Brazão, J.; Fevereiro, P.; Martínez-Zapater, J.M.; Eiras-Dias, J.E. Genetic relationships among Portuguese cultivated and wild *Vitis vinifera* L. germplasm. *Front. Plant Sci.* **2020**, *11*, 127. [CrossRef]
82. Albizuri, A.A.; Cano, A.G.; López, M.; Pérez, M.; Ocete, R. Distribución, ecología y caracterización in situ de la vid silvestre en la Reserva de la Biosfera de Urdaibai (Bizkaia). *Munibe* **2009**, *57*, 15–34.
83. Laguna, E. Sobre las formas naturalizadas de *Vitis* en la Comunidad Valenciana. I. Las especies. *Flora Montiberica* **2003**, *23*, 46–82. Available online: https://dialnet.unirioja.es/descarga/articulo/1394799.pdf (accessed on 7 January 2025).
84. Laguna, E. Datos foliares de las especies e híbridos alóctonos de vides (género *Vitis*) en el territorio valenciano. *Toll Negre* **2004**, *3*, 11–25.

85. Laguna, E. American and hybrid grapevines (*Vitis* spp.): A new concept of invasive plants to Europe. In Proceedings of the 4th European Conference on the Conservation of the Wild Plants—A Workshop on the Implementation of the Global Strategy for Plant Conservation in Europe, Valencia, Spain, 17–20 August 2004; Aguilella, A., Ibars, A., Laguna, E., Pérez-Rocher, B., Eds.; CD Rom. Generalitat Valenciana: Valencia, Spain, 2006.
86. De Andrés, M.T.; Benito, A.; Pérez-Rivera, G.; Ocete, R.; Lopez, M.A.; Gaforio, L.; Muñoz, G.; Cabello, F.; Martínez, J.; Arroyo-García, R. Genetic diversity of wild grapevine populations in Spain and their genetic relationships with cultivated grapevines. *Mol. Ecol.* **2012**, *21*, 800–816. [CrossRef]
87. Ocete, R.; López, M.Á.; Gallardo, A.; Arnold, C. Comparative analysis of wild and cultivated grapevine (*Vitis vinifera*) in the Basque Region of Spain and France. *Agric. Ecosyst. Environ.* **2008**, *123*, 95–98. [CrossRef]
88. Zecca, G.A.; De Mattia, F.; Lovicu, G.; Labra, M.; Sala, F.; Grassi, F. Wild grapevine: Silvestris, hybrids or cultivars that escaped from vineyards? Molecular evidence in Sardinia. *Plant Biol.* **2010**, *12*, 558–562. [CrossRef]
89. Grassi, F.; Labra, M.; Imazio, S.; Spada, A.; Sgorbati, S.; Scienza, A.; Sala, F. Evidence of a secondary grapevine domestication centre detected by SSR analysis. *Theor. Appl. Genet.* **2003**, *107*, 1315–1320. [CrossRef] [PubMed]
90. Kikvadze, M.; Valera, J.; Obón, C.; Ocete, R.; Ocete, C.A.; Kikilashvili, S.; Maghradze, D.; Rivera-Obón, D.J.; Rivera, D. Wild grapevines of Georgia and their relationship with local cultivars: A Bayesian analysis of seed morphology. *Vitis* **2024**, *63*, 9. [CrossRef]
91. Terpó, A. A *Vitis sylvestris* C.C.Gmel. Magyar középhegy ségi termőhelyi viszonyainak vizsgálata [Investiga tions on ecological conditions of habitats of *Vitis sylvestris* C.C.Gmel. in Central Hungarian Mountains]. *Bot. Közlem.* **1969**, *56*, 27–35.
92. Terpó, A. Origin and distribution of *Vitis sylvestris* C.C.Gmel. In Proceedings of the II International Symposium on the problems of Balkan Flora and Vegetation, Istanbul, Turkey, 3–10 July 1978; pp. 1–5.
93. Ocete, R.; Arroyo-Garcia, R.; Morales, M.L.; Cantos, M.; Gallardo, A.; Pérez, M.A.; Gómez, I.; López, M.A. Characterization of *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi in the Ebro River Basin (Spain). *Vitis* **2011**, *50*, 11–16.
94. Popescu, C.; Dejeu, L.; Ocete, R. Preliminary Characterization of Wild Grapevine Populations (*Vitis vinifera* ssp. *sylvestris*) Grown Along the Danube River. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2013**, *41*, 472–477. [CrossRef]
95. Ardenghi, N.M.; Galasso, G.; Banfi, E.; Cauzzi, P. *Vitis* × *nova-angliae* (*Vitaceae*): Systematics, distribution and history of an "illegal" alien grape in Europe. *Wiildenowia* **2015**, *45*, 197–207. [CrossRef]
96. Ardenghi, N.M.; Banfi, E.; Galasso, G. A taxonomic survey of the genus *Vitis* L. (Vitaceae) in Italy, part II: The 'Euro-American' hybrids. *Phytotaxa* **2015**, *24*, 232–246. [CrossRef]
97. Stinca, A. The Genus *Vitis* L. (Vitaceae) in Campania (Southern Italy), with emphasis on alien units. *Ann. Bot.* **2019**, *9*, 107–112. [CrossRef]
98. Karasik, A.; Rahimi, O.; David, M.; Weiss, E.; Drori, E. Development of a 3D seed morphological tool for grapevine variety identification, and its comparison with SSR analysis. *Sci. Rep.* **2018**, *8*, 6545. [CrossRef]
99. Bouby, L.; Bonhomme, V.; Ivorra, S.; Bacilieri, R.; Ben Makhad, S.; Bonnaire, E.; Cabanis, M.; Derreumaux, M.; Dietsch-Sellami, M.; Durand, F.; et al. Seed morphometrics unravels the evolutionary history of grapevine in France. *Sci. Rep.* **2024**, *14*, 22207. [CrossRef]