

New insights into the population genetics of partially clonal organisms: when seagrass data meet theoretical expectations

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Abstract

Seagrass meadows are among the most important coastal ecosystems in terms of both spatial extent and ecosystem services but are declining worldwide. Understanding the drivers of seagrass meadow dynamics is essential for designing sound management, conservation and restoration strategies. However, the effect of clonality on the population genetics of natural populations understood, severely limiting our comprehension of the dynamics and connectivity of meadows. Here, we revisited population genetics data on 165 meadows of four seagrass species in light of recent theoretical developments, which provide new expectations for the distributions of genotypic and genetic polymorphism under increasing clonal rates. Contrasting shoot life span and rhizome turnover lead to the prediction of a decreasing influence of asexual reproduction along a gradient from *Posidonia oceanica* to *Cymodocea nodosa*, *Zostera marina* and *Zostera noltii*, which should be reflected by decreasing departure from Hardy-Weinberg equilibrium (F_{is}) and increasing genotypic diversity (R). This meta-analysis provides a nested validation of this hypothesis at the species scale but also at the meadow scale through the emergence of a significant relationship between F_{is} and R within each species. By empirically demonstrating the theoretical expectations derived from recent modelling approaches, this work calls for the use of Hardy-Weinberg equilibrium (F_{is}) rather than the strongly sampling-sensitive genotypic index R in studies aiming at understanding the importance of clonal reproduction. We also emphasize the need to revise our perception of the influence of clonality on the dynamics, connectivity and evolutionary trajectory of seagrass meadows to develop the most accurate conservation strategies.

Introduction

Clonality is a life history trait spread across the Tree of Life (Halkett et al., 2005) and characterizes species forming the basis of most important terrestrial and marine ecosystems. The drastic decline in many ecosystems engineered by partially clonal species (Carlsson & Callaghan, 1994; Carpenter et al., 2008; Polidoro et al., 2010; Waycott et al., 2009) emphasizes the need to identify the drivers and life history traits, including reproductive strategy, underlying demographic decline or the colonization of new areas during range collapse, shift or expansion (Aitken et al., 2008; Callaghan et al., 1992; Cornelissen et al., 2014; Pecuchet et al., 2018; Yu et al., 2016). Although population genetics can provide essential indirect evidence, its use has been limited thus far by the lack of clear theoretical predictions as to the effect of partial asexuality on the distribution of genetic polymorphism, hampering the comprehensive analysis and interpretation of population genetics data.

The use of molecular markers has led to major improvements in the study of the architecture  dynamics and evolution of clonal organisms, particularly since the development of molecular and analytical methods to detect clonality (Halkett et al., 2005; Tibayrenc et al., 1990) and to assess clonal membership (Arnaud-Haond, Duarte, et al., 2007; Tibayrenc et al., 1990). These improvements allowed the recognition of clonal lineages and estimation of not only genetic but also genotypic diversity. The simplest index of genotypic richness, G , corresponds to the number of multi-locus genotypes (MLGs) or multi-locus lineages (MLLs) in the population (Arnaud-Haond, Duarte, et al., 2007). Since this specific unit of evolution (i.e., the genotype: Ayala, 1998) became accessible, genotypic diversity has been the subject of a growing number of studies aiming to investigate the dynamics and evolution as well as resistance of natural populations in a diversity of environmental conditions (Hughes et al., 2008; Massa et al., 2013; Reusch et al., 2005). Such information is essential for understanding the evolution and dynamics of natural populations, including environmental engineers (Callaghan et al., 1992; Cornelissen et al., 2014), such as corals and seagrasses, that form the basis of essential and declining coastal ecosystems (Carpenter et al., 2008; Hughes & Stachowicz, 2009; Orth et al., 2006). However, G naturally increases with sample size, as does the clonal richness index R (Ellstrand & Roose 1987; Dorken & Eckert 2001 ), thus, the ratio of different genotypes to the sample size is the most widely used index of genotypic diversity (Arnaud-Haond, Duarte, et al., 2007). Initially thought to allow comparative studies, R was even considered as a *proxy* with which to infer the relative influence of sexual *versus* clonal reproduction and the consequences of such a reproductive strategy for the dynamics of natural populations, the models of mutation, the unit mainly targeted by natural selection and drift (genotype or alleles), and the main drivers of migrations (propagules or adults/fragments for plants, fungi and several invertebrates such as corals). Unfortunately, the strong dependence of the R index on sampling strategy and density  aud-

Haond, Migliaccio, et al., 2007; Gorospe et al., 2015) results in an inability to derive any equivalence between its value and the extent of clonal reproduction, c , thus jeopardizing the management and conservation strategies for populations of partially asexual organisms and the ecosystems they support. In fact, only two recent programs allow us to compute c from genetic data, and their use relies on the important but seldomly fulfilled requirement of temporal samples: CloNcaSe (Ali et al., 2016), a method used for organisms with cyclical parthenogenesis, and ClonEstiMate (Becheler et al., 2017), a Bayesian method ideal for samplings separated by exactly one generation.

Pioneer mathematical models suggested a very limited influence of clonal rate on the genetic composition of populations reported through F_{is} (de Meeûs et al., 2007) and linkage disequilibrium (Navascues et al., 2010) indexes. Almost exclusively clonal lineages are exceptions, resulting in the interpretation of negative F_{is} values (heterozygous excesses), when not overlooked or hastily discarded through over-conservative corrections for multiple tests (Bonferroni correction, Rice, 1989), as indicative of high clonality rates (Halkett et al., 2005). More recent mathematical models suggested larger inter-locus variance in F_{is} values at equilibrium as the rate of clonality increases (Stoeckel, S. & Masson, 2014) and expected departure from Hardy-Weinberg equilibrium (HWE), even for modest rates of clonality (Reichel et al., 2016; Stoeckel, S. et al. 2019).

The combination of sampling bias seriously limiting the interpretation of R and the expected influence of c on genetic parameters may explain the paradoxical observation of heterozygote excess in partially clonal organisms (Reichel et al., 2016) despite often elevated values for clonal richness, possibly partly due to sampling density (Arnaud-Haond, Migliaccio, et al., 2007; Gorospe et al., 2015). Seagrasses are a good example of this paradox. On the one hand, moderate to high levels of clonal richness, R , together with limited but significant values of genetic differentiation (F_{st}), led some authors to propose a strong influence of sexual reproduction on the dynamics and evolution of seagrass meadows, implying important recombination rates and large-scale dispersal (Kendrick et al., 2012; McMahon, K. et al., 2014). On the other hand, the maximum values that F_{st} can reach are strongly limited by the use of highly polymorphic molecular markers (Hedrick, P. W., 1999; Hedrick, 2005; Jost, 2008), and those elevated values for clonal richness often appear with significant but often overlooked heterozygote excesses, contrastingly suggesting a high incidence of clonality (Reichel et al., 2016; Stoeckel, S. et al. 2019).

To resolve this paradox and better understand the meaning of R and F values as they relate to the extent of clonal reproduction and the importance of dispersal, we propose here a re-analysis of previously published seagrass data in light of recent modelling developments (Stoeckel, S. et al., 2019). These four seagrasses are the main species structuring coastal ecosystems along the Atlantic

and Mediterranean coasts and exhibit a gradient from long-lived species exhibiting slow turnover of shoots and low to moderate reproductive output, namely, *Posidonia oceanica* and *Cymodocea nodosa*, to shorter-lived ones, namely, *Zostera marina* and *Zostera noltii*, which exhibit higher shoot turnover and higher allocation to sexual reproduction (see Material and Methods section and Table 1). Considering together the expected influence of clonality based on knowledge of clonal growth and allocation in those four species and more recent theoretical predictions of increasing heterozygote excess with higher levels of clonality, we expected a progressive increase in clonal diversity and a progressive decrease in heterozygosity along this gradient from *P. oceanica* to *Z. noltii*, starting with negative F_{is} values (heterozygote excess) and moving towards null (or slightly negative if heterozygote deficiency occurs due to inbreeding) F_{is} values for the shortest-lived species.

Here, we aimed to test these theoretical expectations by using a meta-analysis of population genetics data published on these four seagrass species to answer the following questions:

1) Can the estimate of genotypic diversity deliver informative values with which to assess comparative investment in clonal *versus* sexual reproduction among species submitted to a similar sampling scheme?

2) Does departure from HWE reflect the distance from equilibrium? Does the genetic composition of natural meadows provide a reliable assessment of the relative clonal rates among species with different investments in clonal growth and different life spans?

3) Do the two families of parameters (genotypic and genetic) provide congruent qualitative and quantitative estimates at both the inter-specific and intra-specific levels?

Material and methods

Studied species

Seagrasses reproduce clonally through rhizome elongation, the rhythm and shape of which differ among the studied species and largely scale with plant size (Duarte, 1991a; Marba & Duarte, 1998). *P. oceanica* and *C. nodosa* are known to exhibit more stable rhizome connections, a longer shoot life span and a lower shoot turnover (Diaz-Almela et al., 2008; Duarte, 1991a; Marba et al., 1996; Marba & Duarte, 1998). In contrast, *Zostera* species exhibit the fastest growth but a more limited occupation of space (Marba & Duarte, 1998; Sintes et al., 2006) as well as a much shorter life span.

Plant size thus appears to be strongly related to turnover time and modules' (i.e., rhizome internodes, leaf clusters and roots) longevity (Duarte, 1991a). These parameters of clonal growth are accompanied by a relatively parallel trade-off between clonal architecture and sexual reproductive

output. The events and success of flowering are highly temporally and spatially heterogeneous for *P. oceanica* and *C. nodosa*, for which the number of seeds produced seldomly exceeds several tens per square metre, whereas highly profuse episodes of annual flowering for *Zostera* can lead to the production of thousands of seeds per square metre (Marba et al., 1996; Marba et al., 2004).

These descriptions of clonal architecture and sexual reproduction features allow us to derive expectations of a decreasing influence of clonality for the four species studied here from *P. oceanica* to *C. nodosa*, *Z. marina* and *Z. noltii* (see Table 1). Although no numerical estimates of shoot life span and rhizome turnover are available for the smallest and shorter-lived species *Z. noltii*, it is expected to exhibit a much higher turnover, in line with its habitat characterized by less stable environmental conditions (Duarte, 1991b).

Interestingly, these species also most often exhibit a gradient on the shore (Duarte, 1991b). *P. oceanica* and *C. nodosa* reach 40 to 50 metres in depth (with *P. oceanica* dominating at greater depths, den Hartog, 1970), while the two *Zostera* species are most often encountered in intertidal areas, with *Z. noltii* sometimes exposed to desiccation and stressful conditions (Massa et al., 2009). All four species have been the focus of large-scale genetic surveys for phylogeographic studies in the past few decades. Datasets are thus available that can be used to test expectations about their respective investment in clonal reproduction and to screen for signatures of clonal reproduction effects on their genetic composition.

Genetic datasets

The *P. oceanica* dataset contains 36 samples of approximately 40 units each, for a total of 1424 shoots or connected shoots representing sampling units (SUs) from Spain to Cyprus, over 4000 km of coastline. For each sampling site, the SUs were collected according to randomly defined coordinates in a quadrat 80 metres long and 20 metres wide. Seven microsatellite markers were used: Po15, Po5, Po5-40, Po5-49, Po5-10, Po4-3 and Po5-39. The database of genotypes was constructed in a previous meta-analysis (Arnaud-Haond et al., 2014). Three meadows that were initially studied for the impact of farms on the genetic composition of the species (Amathous, Acqua Azzura and Agias Nicholas; Diaz Almela et al., 2007) were discarded to avoid introducing bias.

The *C. nodosa* dataset (Alberto et al., 2008) consisted of 47 meadows of approximately forty SUs each, for a total of 1586 from Cyprus to the Canary Islands and Madeira. For each meadow, the SUs were selected randomly in a quadrat 60 meters long by 14 meters wide. Eight microsatellite markers were used: Cn2-38, Cn2-14, Cn2-24, Cn4-19, Cn2-16, Cn2-18, Cn4-29 and Cn2-45 (Arnaud-Haond et al., 2014).

The first dataset for *Z. marina* consisted of 13 quadrats sampled in 7 meadows of 30 SUs each, for a total of 390, on the Brittany coast from Saint-Malo to Arradon. For each sampling site,

SUs were randomly selected in two quadrats 20 meters long by 30 meters wide in 2009 (Becheler et al., 2013; the same sampling was performed in 2011, but to avoid partially duplicating information, only the first time series was included). A total of 9 microsatellite markers were used: GA35, GA2, GA17H, GA23, GA12, GA19, GA20, GA16 and GA17D {Becheler, 2014 #50; Becheler, 2010 #74}. The second dataset consisted of 14 meadows from Greenland to Southern Iberia, where 427 SUs were collected haphazardly but at least 1–1.5 m apart from each other (Diekmann, O. E. & Serrão, E. A., 2012) and genotyped with 8 microsatellite markers: GA6, GA3, CT17H, CT19, CT3, CT20, GA2 and CT35 (Diekmann, O.E. & Serrão, E.A., 2012). We removed genotypes with missing loci and populations with few SUs (for Greenland: Kobbefjord, with one SU; for Southern Iberian: Arrabida, with 4 SU, and Sado, with 6 SUs), leaving 11 meadows and 403 SUs.

The *Z. noltii* dataset included 33 meadows sampled across the entire geographic range of the species and genotyped with 9 microsatellite markers: ZnB1, ZnH10, ZnB3, ZnB8, ZnH8, ZnD6, ZnE7, ZnF8 and ZnF11 (Coyer et al., 2004). Different sampling protocols were used and are detailed in the original publication, including the random collection of SUs within quadrats 60 metres long by 20 metres wide, 10 metres long by 15 metres wide and 10 metres long by 25 metres wide, as well as sampling along a linear transect every metre. One sampling site (BSea3) represented by a single SU was excluded from the initial dataset; thus, 32 meadows were kept for a total of 1117 SUs.

Genetic and genotypic indexes

RClone 1.0.2 (Bailleul et al., 2016) and GenClone (Arnaud-Haond & Belkhir, 2007) in R statistical software (R Core Team, 2015) were used to manage the different datasets and to compute the genetic and genotypic indexes of interest. We chose to examine the F_{is} genetic index, for which models delivered several theoretical predictions, and the genotypic clonal richness index R, the supposed inferred sexual reproduction rate. R was computed for each population, and the F_{is} was computed for each population with (ramet level) and without (genet level) replicates.

The relationship between R and the F_{is} was considered at both the intra- and inter-species levels. The Pearson correlation index between R and the F_{is} was computed, and the significance was assessed. Linear models predicting the F_{is} based on R were constructed, and the determination coefficient R^2 was used to assess the quality of each model and the F-test. R^2 represents the proportion of the F_{is} variability explained by the model; R^2 values are between 0 (the F_{is} values are not at all explained by the model) and 1 (the F_{is} values are very well predicted by the model). The F-test compares a model with no predictors (intercept-only model) to the specified model. If the p-value of the F-test is significant, the regression model predicts the response variable better than the mean of the response does.

Results

Clonal richness, R

The mean genotypic index R increased regularly from *P. oceanica* to *C. nodosa*, *Z. marina* and *Z. noltii* (Table 1, Figure 1), with the variance (reflected by the maximal and interquartile ranges) of these values decreasing from *P. oceanica* to *Z. noltii*. Only *C. nodosa* slightly departed from this trend, with mean lower than that of *P. oceanica* and the greatest maximal ranges of R values among the seagrasses.

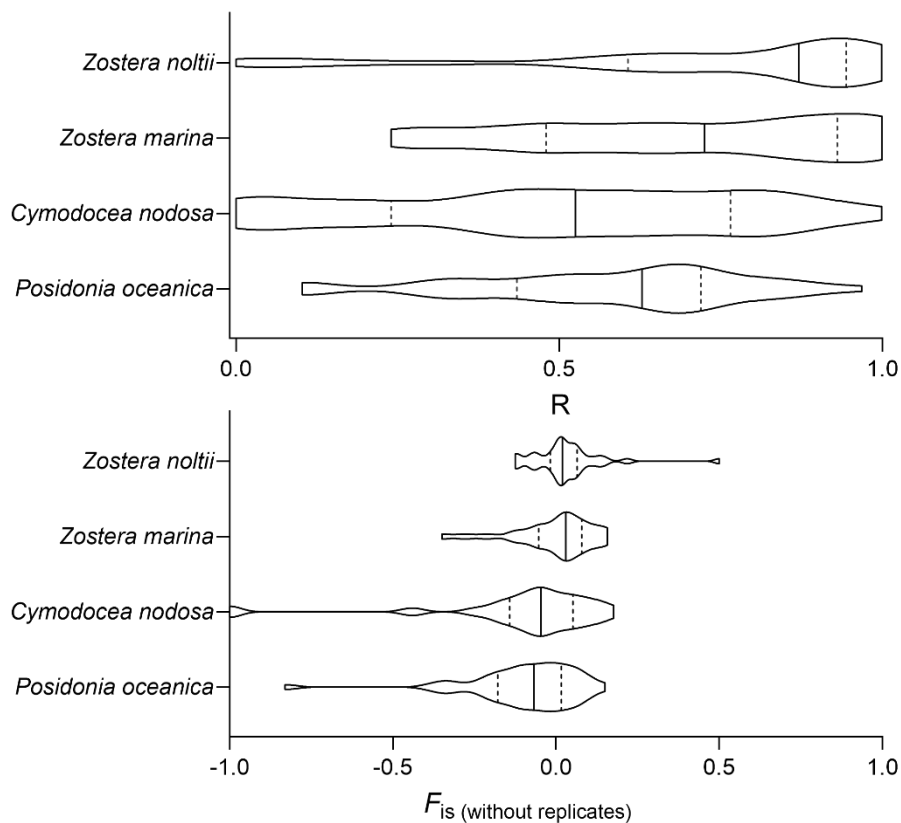


Figure 1: Boxplot showing the average departure from Hardy-Weinberg equilibrium (F_{is} , with replicates) and genotypic richness (R) values over all studied meadows for each of the four seagrass species: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Z. noltii*
Inbreeding coefficient, F_{is}

The F_{is} values and their means progressively increased from *P. oceanica* to *Z. noltii* (Table 1; Figure 1). Only *Z. noltii* showed a slightly positive means F_{is} . The interquartile values were nearly strictly negative for *P. oceanica* and *C. nodosa*, while they included increasingly positive F_{is} values from *Z. marina* to *Z. noltii*. This progression of F_{is} interquartile values was even clearer when the datasets were analysed without replicates (Figure 1).

with replicates

without replicates

	rhizome turnover	shoot lifespan	R	F_{is}	correlation with R	F_{is}	correlation with R
<i>Posidonia oceanica</i>	0.09	11.98	0.667	-0.085	0.63***	-0.108	0.73***
<i>Cymodocea nodosa</i>	0.14	2.40	0.540	-0.075	0.76***	-0.002	0.48***
<i>Zostera marina</i>	2.19	1.52	0.828	-0.105	0.46***	-0.091	0.39**
<i>Zostera noltii</i>			1.000	0.012	0.17	0.015	0.14

Table 1: Summary of the clonal growth features (Duarte, 1991) for each of the four seagrass species, the average compiled values of genotypic diversity (R) and departure from Hardy-Weinberg equilibrium (F_{is}) and the correlations between these values (*: $p < 0.05$; **: $p < 0.01$; ***: < 0.001), obtained from the dataset including all ramets genotyped (with replicates) and that including only multi-locus lineages, or genets (without replicates).

Relationship between R and F_{is}

At the intra-species level (Figure 2, Table 1), the relationships between R and F_{is} were also positive and significant for all but the *Z. noltii* datasets. The relationships spanned from highly positive (*P. oceanica* and *C. nodosa*) to slightly positive (*Z. marina*) and nearly null (*Z. noltii*). Similar results, though slightly weaker, were obtained without replicates (Figure S2, Table 1), with the exception of a stronger correlation for *P. oceanica* without replicates.

In analyses both with and without replicates, R was a good predictor of F_{is} in linear models (Table 1), with the exception of *Z. marina* when replicates were excluded, and *Z. noltii* for datasets either with or without replicates. The F-tests were significant, with relatively high and significant r^2 indexes for the three other species. At the inter-species level (Figure 3), the relationships between R and F_{is} were always positive and strong (Table 1). When considering only the genets (i.e., no replicates), the results were similar (Figure S3), but the correlation and the linear model were weaker than those obtained at the genet level (Table 1).

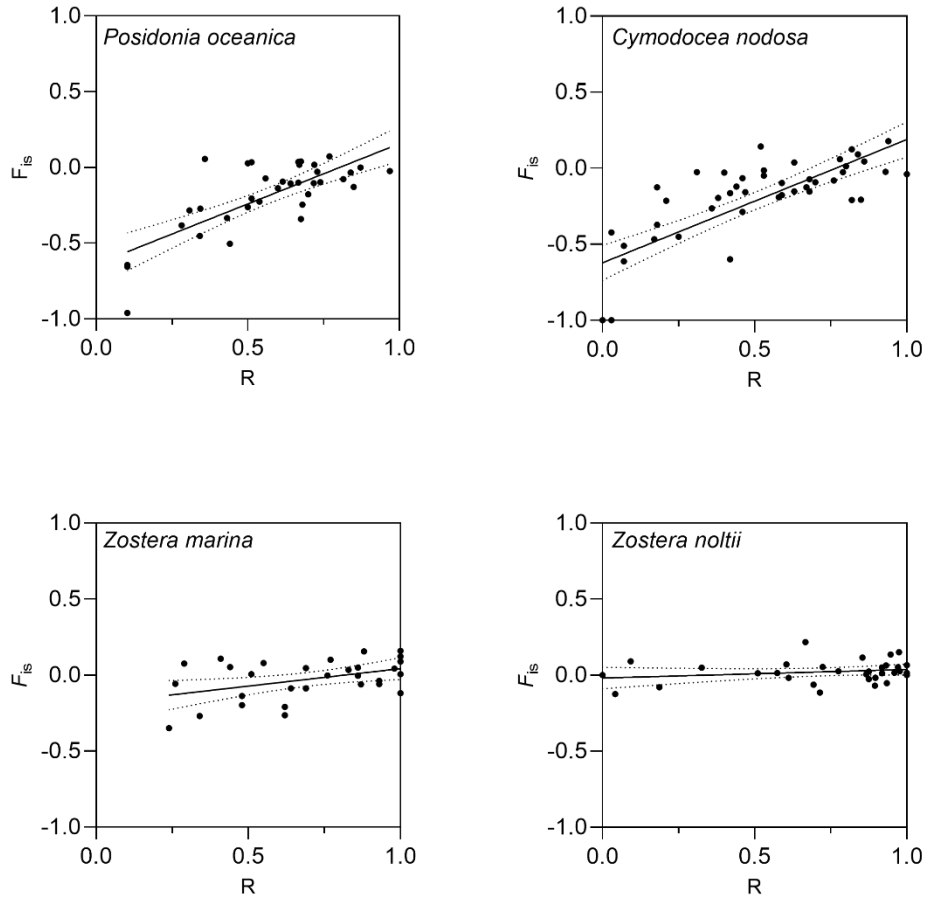


Figure 2: Relationships between the level of genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is} , at the genet level, i.e., without replicates) at the meadow scale for each of the four seagrass species

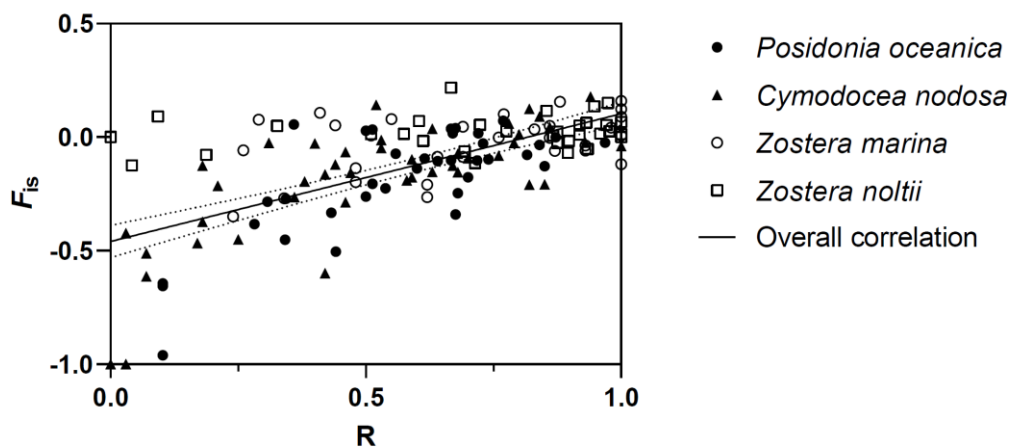


Figure 3: Overall relationships between the level of genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is} , at the genet level, i.e., without replicates) at the meadow scale for each of the four seagrass species

Discussion

Here, we conducted a comparative re-analysis of genetic data from four species for which a good ecological knowledge of clonal architecture allowed testing and validating the predictions obtained from recent mathematical developments. Our analyses confirmed nearly all expectations derived from those recent mathematical predictions about the relationships between 1) the turnover and longevity of shoots, 2) the prevalence of clonality effects on demography, and 3) the consequent signatures in the genotypic and genetic composition of natural meadows. By giving more weight to the signature of genetic composition (F_{is}) than to the index highly sensitive to sampling, i.e., genotypic richness (R), in assessing the balance between clonal and sexual reproduction, this analysis allowed an apparent paradox reported in the literature to be unravelled: the frequent observation of apparently high genotypic richness, suggesting a high rate of sexual input, and systematic departure from HWE towards heterozygote excess, suggesting a very high rate of asexual reproduction. This trend was clear at both the inter-species and intra-species (among-meadow) levels, showing a nested pattern at both the inter- and intra (population)-species scales. Consequently, these results illustrate the need for a revised framework for the interpretation of molecular data from partial asexuals such as seagrasses, which may have important consequences for conservation and management strategies in the context of global change and increasing restoration needs (Carpenter et al., 2008; Hughes et al., 2008; Orth et al., 2006).

Fitting empirical data to model predictions

Our results support the ecological hypothesis that the longer the module's lifespan is, the more important the vegetative multiplication of ramets through clonal growth compared to sexual input through seedlings is for the growth and maintenance of the population.

First, the mean genotypic richness R gradually increases with decreasing longevity and increasing turnover of modules, i.e., from *P. oceanica* to *Z. noltii* (Figure 1). Although unable to provide a reliable quantitative assessment of the rates of clonality due to large subsampling bias (Arnaud-Haond, Duarte, et al., 2007; Becheler et al., 2017; Gorospe et al., 2015; Stoeckel, S. et al. 2019), R remains useful in comparative studies to assess the relative importance of clonality among natural populations if the sampling scheme and effort are comparable. Such comparability was true here for *P. oceanica* and *C. nodosa* and partly for the *Zostera* species. It is thus important to bear in mind that the use of R here was by no means an attempt to estimate c but to compare the values among meadows sampled with similar sampling scales and strategies.

Thus, the high diversity of *Z. noltii* in this analysis may be influenced by both its short life span (and high turnover) of shoots and the slightly different sampling designs used to gather the data

analysed for the species. Indeed, whereas the other three datasets followed a random or haphazard sampling scheme in a standardized area appropriate for the clonal architecture of the species, which should minimize bias (Pielou, 1966) and allow comparative analysis, part of the *Z. marina* data were collected haphazardly, but more importantly, the *Z. noltii* datasets were collected by sampling along transects. Linear transects result in significantly higher genotypic diversities due to a strong edge effect (Arnaud-Haond, Duarte, et al., 2007). It is thus not possible to rigorously disentangle the effect of high rhizome turnover and low shoot longevity from that of putative edge effects on the ranking of *Z. noltii* in terms of genotypic richness. However, the expected ranking for F_{is} stands (Figure 1), suggesting a stronger influence of life history traits than sampling bias on these results.

This importance of sampling is reflected in a different way by the slight deviation from this general trend for *C. nodosa*. Despite having a slightly higher rhizome turnover than *P. oceanica* (Duarte, 1991a), *C. nodosa* shows lower mean R values (Figure 1). Several hypotheses can explain this result. First, despite a slightly higher rhizome turnover and a much shorter shoot life span, the establishment and dynamics of *C. nodosa* meadows may rely more on clonal reproduction than those of *P. oceanica* meadows. Indeed, *C. nodosa* similarly exhibits irregular sexual reproductive events dependent on environmental conditions but also a much faster clonal extension rate (Marba et al., 1996; Marba et al., 2004). Moreover, despite being collected with an identical sampling strategy, the *C. nodosa* dataset contained more highly clonal meadows, which may be partly due to the inclusion of sampling sites located at the limit of its distribution (Billingham et al., 2003). In fact, repeating the same analysis in Mediterranean populations delivered higher mean R values comparable only to those of *P. oceanica* and a slightly less negative F_{is} , providing a better fit to the initial predictions (Supplementary Materials S1).

Second, the increasing departure from HWE towards negative F_{is} (heterozygote excess, Figure 1 and 2) from *Z. noltii* to *P. oceanica* also supports the prediction of mathematical models (Stoeckel, S. & Masson, 2014). As the prevalence of clonality increases, the trajectory of the population towards equilibrium slows down for both positive and negative F_{is} values, with less positive than negative F_{is} values (Reichel et al., 2016). In fact, F_{is} values are usually negative for *P. oceanica* and *C. nodosa*, and an increasing proportion of positive values are observed for *Z. marina* and *Z. noltii*. The results are even clearer for the two former species when the data are considered at the genet (without replicates, Figure 2) rather than the ramet scale (Figure S2), with only interquartile F_{is} values equal to or greater than 0.

Interestingly, this inter-species pattern is also observed at the nested, intra-species level (Figure 2), showing that the relationship between estimates of clonal richness R and F_{is} transcends the species boundary and applies within species at the meadow scale. This phenomenon is reflected by a positive correlation between F_{is} and R among meadows for *P. oceanica*, *C. nodosa* and *Z. marina*

(Table 1), again supporting the predictive power of population genetics models explicitly taking partial clonality into account. Using a standard sampling strategy, many more clonal meadows (i.e., those exhibiting lower R values) tend to exhibit higher departures from HWE towards heterozygote excess (negative F_{is} values), again in line with theoretical predictions.

Considering the accumulated ecological and physiological knowledge of the rhizome growth and dynamics of these species, our comparison of the theoretical predictions, coming from a ‘simple’ Wright-Fisher-like model extended to explicitly include clonality, with the population genetics data gathered on four seagrass species shows remarkable congruence. This result highlights the need i) to extend population genetics theory to predict the dynamics of genetic diversity while accounting for various reproductive systems and in turn ii) to expand the production and interpretation of empirical data to enhance our understanding of the main drivers of demography and connectivity in natural populations as well as the possibility of achieving these goals.



Implications for understanding clonal versus sexual prevalence and its influence on the dynamics and evolution of natural populations

Species ranges are the result of multiple ecological and evolutionary drivers, among which genetic drift, selection and dispersal are essential processes strongly influenced by both environmental factors and demography (Gaggiotti, 2017). The accurate use of molecular markers and population genetics tools and models thus requires a good theoretical understanding of the ways in which evolutionary forces drive the temporal and spatial dynamics of genetic polymorphism, including the reproductive system, to in turn infer demographic dynamics and history from molecular data. Understanding the respective roles of clonal and sexual recruitment and dispersal in determining local demography and spatial connectivity is essential for forecasting their evolution in the context of global change and future range shifts. This knowledge is also a prerequisite for defining accurate management measures, and its quest has been a strong incentive underlying population genetic studies of seagrass during the past few decades (Alberto et al., 2005; Arnaud-Haond, Duarte, et al., 2007; Arnaud-Haond et al., 2012; Kendrick et al., 2012). Although the importance of clonal growth in the colonization, expansion and maintenance of meadows has long been acknowledged (Duarte, 1991b; Kendrick et al., 2012), recent studies proposed migration via the production and dispersal of seeds as a possible central driver of the dynamics and persistence of seagrasses (Kendrick et al., 2012; McMahon, K. et al., 2014). This hypothesis was rooted in the observation of moderate to elevated levels of genotypic and genetic diversity, together with limited genetic differentiation and isolation by distance, in a panel of species including the four targeted here together with the Australian *Posidonia australis*, the Pacific *Zostera pacifica* and the Atlantic *Thalassia testudinum* (Kendrick et al., 2012).

Despite being represented by a limited number of species (approximately 70), seagrasses are characterized by a diversity of life history traits (Hemminga & Duarte, 2000; McMahon, K. et al., 2014), as illustrated by the panel of four species examined here. The hypothesis proposed by Kendrick *et al.* (2012) may thus apply to some of them, particularly the short-lived species producing a large number of seeds (Phan et al., 2017), and even more so if one also accounts for the dispersal of vegetative fragments (McMahon, K. et al., 2014). However, the prevalence of asexuality is associated with a diminution of the influence of drift (Reichel et al., 2016), which may partly explain the elevated levels of genetic diversity observed in well-established meadows. Those high levels of alpha diversity in turn also intrinsically limit the maximum possible estimates of beta diversity (genetic differentiation as estimated through F_{st} , Charlesworth, 1998; Gregorius, 2010; Hedrick, W., 1999) and their saturation, which can be well described by the disruption of isolation by distance patterns over large scales (Kendrick et al., 2012). Part of the observations that led to the hypothesis of a central role of seed dispersal may thus reflect the prevalence of clonal reproduction, regardless of whether high connectivity exists. In fact, similar observations of high diversity and limited differentiation with no large-scale pattern of isolation by distance led to the exploration of mutation patterns across the distribution range of the long-lived species *P. oceanica* and *C. nodosa* at the scale of their distribution range (Arnaud-Haond et al., 2014). Disentangling the spatial distribution pattern of polymorphism revealed a stronger influence of the accumulation of somatic mutations through clonal propagation than of the pattern of dispersal of sexual propagules, implying a stronger influence of clonal than of sexual reproduction on the dynamics and evolution of meadows at large spatial (distribution range) and time (evolutionary) scales (Arnaud-Haond et al., 2014).

The results presented here thus support the co-existence of complex trade-offs between clonal and sexual reproduction and dispersal among seagrass species, which may be better understood by specifically taking into account the large diversity of life history traits they express (McMahon, K. et al., 2014). Disentangling these cases individually and accurately assessing the level of genotypic diversity as well as its influence on the resistance of natural populations (Hughes et al., 2008; Massa et al., 2013; Reusch et al., 2005), although challenging, are extremely important for designing sound management and restoration strategies in a fluctuating environment (McMahon, K. M. et al., 2017) and represent a major research axis to develop in the future. Marine connectivity research has been enriched in recent years by the improvement of predictive (oceanographic modelling, Robert & Sponaugle, 2009) and molecular (next-generation sequencing and high-density genome scan) tools (Riginos et al., 2016; Selkoe et al., 2016). The former can be readily enriched through the extensive knowledge gained by seagrass ecologists on the broad diversity of life history traits determining the timing and extent of the production and dispersal of sexual and clonal propagules (Duarte, 1991a; McMahon, K. et al., 2014). The latter will certainly benefit research on seagrass population genetics

by allowing a finer-grained snapshot of the distribution of polymorphism and the various origins of mutations at nested spatial scales to better disentangle the relative impact of different evolutionary forces on their emergence and spatial spread. Finally, the integration of oceanographic and molecular information in new integrative Bayesian frameworks of analysis is currently underway (Gaggiotti, 2017), showing a promising path for grasping the diversity and complexity of seagrass strategies in terms of demography and dispersal.

Conclusion

The data re-analysed here on four seagrass species support both i) the ecological hypothesis of higher clonality in longer-lived, lower-turnover species and ii) the mathematical hypothesis of greater deviations from HWE towards heterozygotes excess at higher levels of clonality. This re-analysis also supports iii) the statistical prediction that with the usual sampling density and grain size (although useful in comparative studies with rigorously standardized sampling strategies), the genotypic diversity index R will systematically grossly overestimate (by one to two (or more) orders of magnitude) the true clonal richness in natural populations. These results, in line with theoretical predictions, favour the use of the signature of heterozygote excess (a negative F_{is}), which is less prone to large sampling bias than genotypic richness (R), in assessing the extent of clonal *versus* sexual reproduction. By emphasizing this likely overlooked signature of clonal prevalence in the literature, the results also call for a revision of our perception of the balance between sexual and clonal reproduction in natural populations of partial asexuals such as seagrass meadows. Recent empirical and theoretical developments now provide directions towards improved estimates of these essential parameters to provide sound interpretation of population genetics data, elucidate the short-term drivers of the natural dynamics and resistance of meadows, forecast their resilience or shifts in their distribution range under global change, and define sound conservation strategies.

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Author Contributions

SAH and SS conceived the study, and SAH and DB wrote the manuscript. SAH and DB compiled

the data and performed the meta-analysis, data exploration and interpretation. All authors contributed to editing. SAH and SS were responsible for securing funding. All authors have read and approved the final manuscript.

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Supplementary material

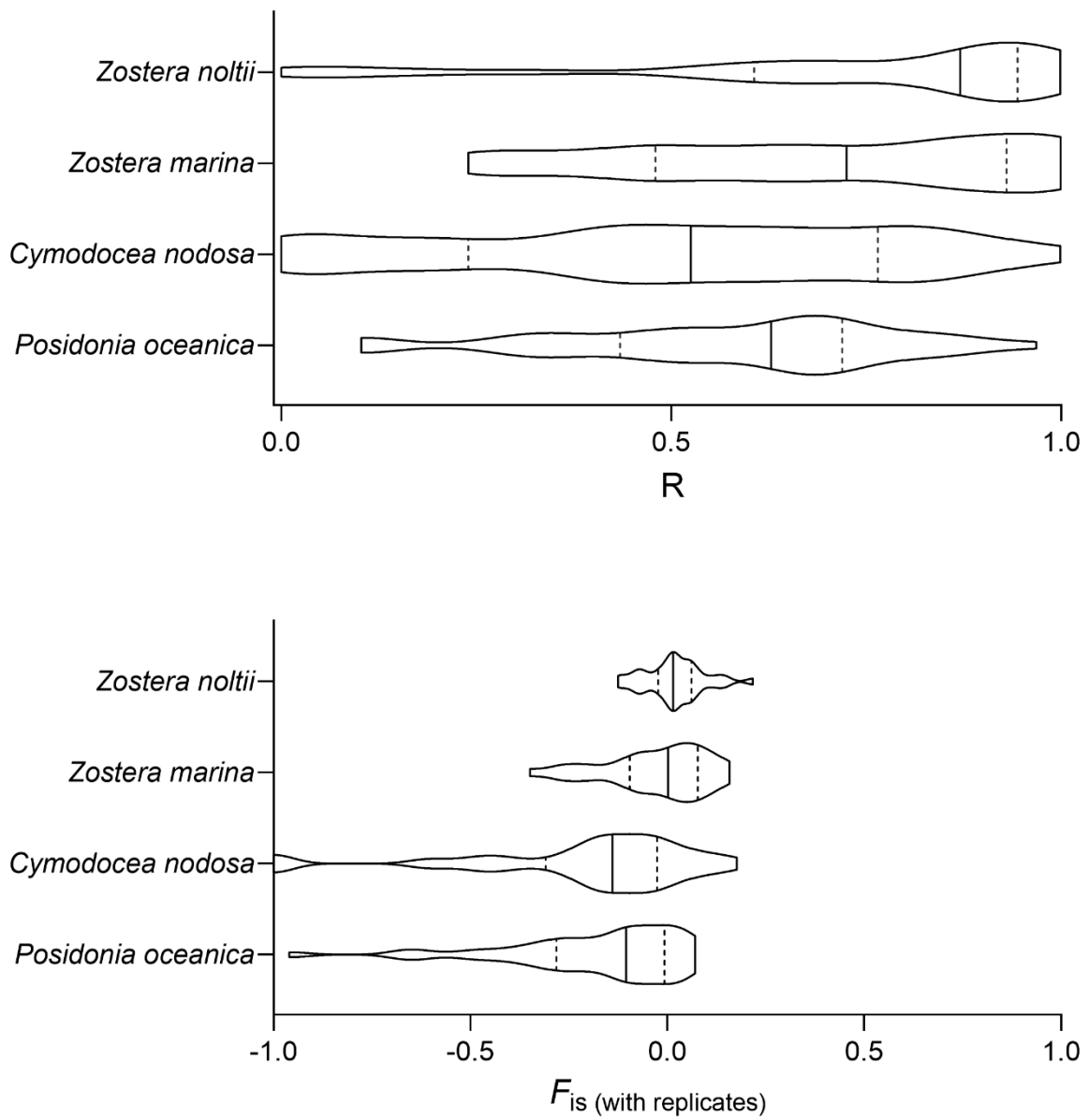


Figure S1: Boxplot showing the average departure from Hardy-Weinberg equilibrium (F_{is} , with replicates) and genotypic richness (R) values over all studied meadows for each of the four seagrass species: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Z. noltii*

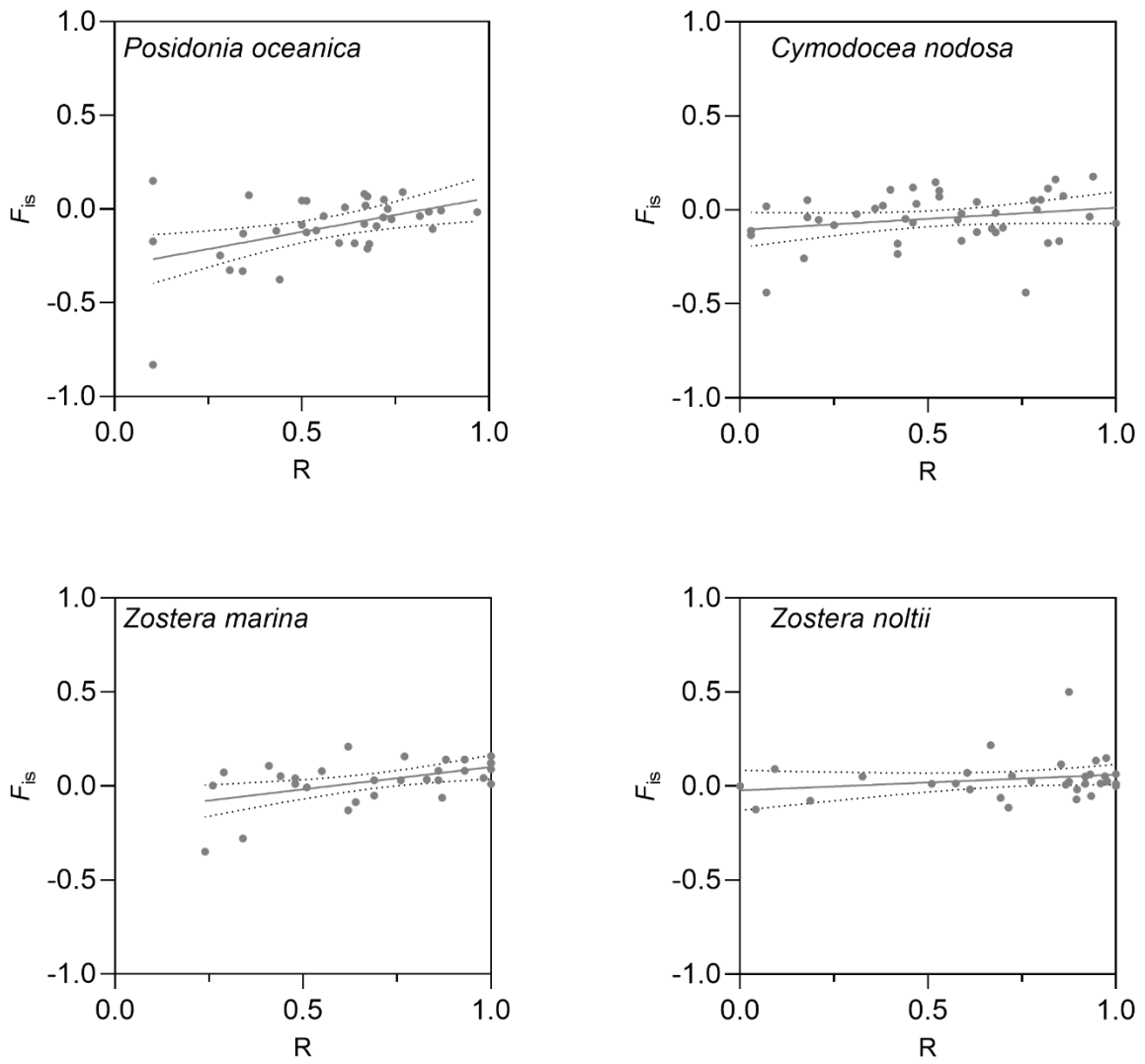


Figure S2: Relationships between the level of genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is} , at the ramet level, i.e., with replicates) at the meadow scale for each of the four seagrass species

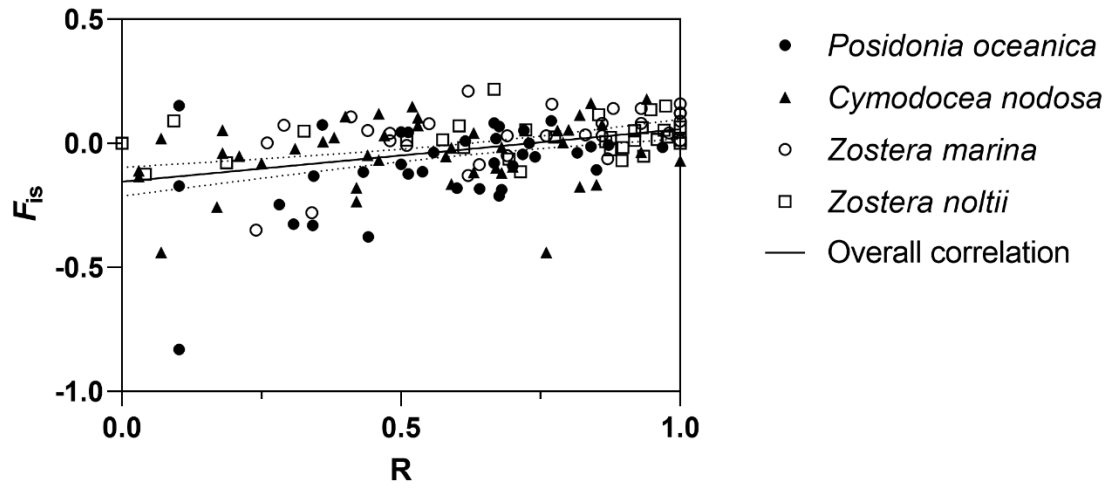


Figure S3: Overall relationships between the level of genotypic richness (R) and departure from Hardy-Weinberg equilibrium (F_{is} , at the ramet level, i.e., with replicates) at the meadow scale for each of the four seagrass species.