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Title page

Macroevolutionary insights in sedges (*Carex*: Cyperaceae): The effects of rapid chromosome number evolution on lineage diversification

Running title: Chromosome number evolution in *Carex* (Cyperaceae)

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Abstract and keywords

Changes in chromosome number as a result of fission and fusion in holocentric chromosomes have direct and immediate effects on genome structure and recombination rates. These in turn may influence ecology and evolutionary trajectories profoundly. Sedges of the genus *Carex* (Cyperaceae) comprise ca. 2000 species with holocentric chromosomes that evolve almost exclusively by fission and fusion. The genus exhibits a phenomenal range in chromosome number ($2n = 10-132$). In this study, we integrated the most comprehensive cytogenetic and phylogenetic data for sedges with associated climatic and morphological data to investigate the hypothesis that high recombination rates are selected for when evolutionary innovation is required, using chromosome number evolution as a proxy for recombination rate. We evaluated Ornstein–Uhlenbeck models to infer shifts in chromosome number equilibrium and selective regime. We also tested the relationship between chromosome number and diversification rates. Our analyses demonstrate significant correlations between morphology and climatic niche and chromosome number in *Carex*. Nevertheless, the amount of chromosomal variation that we are able to explain is very small. We recognized a large number of shifts in mean chromosome number, but a significantly lower number in climatic niche and morphology. We also detected a peak in diversification rates near intermediate recombination rates. In combination, these analyses point to the importance of chromosome evolution to the evolutionary history of *Carex*. Our work suggests that the effect of chromosome evolution on recombination rates, not just on reproductive isolation, may be central to the evolutionary history of sedges.

Key words: bioclimatic variables, BAYOU, Brownian motion, diversification, morphological characters, Ornstein–Uhlenbeck model, Phylogenetic Comparative Methods, QuaSSE, SLOUCH

Main text

1 Introduction

The genus *Carex* L. (Cyperaceae), with ca. 2000 accepted species, includes nearly 40% of total sedge diversity and is one of the three most diverse angiosperm genera (Roalson et al., submitted; WCSP, 2020). It is distributed worldwide, but especially rich in the temperate and cold regions of both hemispheres. This global distribution results from a series of dispersals and expansions from its cradle in southeastern Asia, where the genus originated about 37 Mya (Martín-Bravo et al., 2019), and a complex balance between in-situ diversification and migrations among regions (e.g., Hipp et al. 2006; Uzma et al. 2019). The last two decades have seen a flowering of *Carex* phylogenetic studies (Roalson et al., 2001; Waterway & Starr, 2007; Waterway et al., 2009; Escudero et al., 2012a; among other studies). As a result of a recent sampling push coordinated by the Global *Carex* Group (Jiménez-Mejías et al., 2016; Martín-Bravo et al., 2019), over 60% of the species of this megadiverse genus have DNA sequences, and a solid genomic backbone tree has solidified the broad-scale phylogeny of the genus (Villaverde et al., 2020). This has allowed for a more complete picture of evolutionary relationships at the species level and a robust framework for investigating the processes that shape the evolution of the genus.

The diversification bursts observed in *Carex* have been explained variously, with no clear unifying principle: some appear to be associated with morphological key innovations, others with ecological opportunity following establishment in new regions, still others with shifting dynamics in chromosome number evolution (Martín-Bravo et al., 2019 and references therein). The prospects for elucidating the partial effects of the various factors shaping diversification in a species-rich genus with such a patchwork of histories is daunting. For instance, self-compatibility in most *Carex* species facilitates

population establishment and expansion after dispersal events (interplaying with epizoochoric syndromes in some species; Villaverde et al., 2017a) and the formation of reproductive isolation (Whitkus 1988; Escudero et al. 2016). This aspect of its life history alone may shape speciation in some clades, especially in association with long-distance dispersal (Villaverde et al., 2015a, 2015b, 2017a, 2017b; Márquez-Corro et al., 2017). In others, colonization and adaptation to newly opened niches during the geographic expansion of the genus may have played important roles (Villaverde et al., 2017b; Benítez-Benítez et al., 2018, submitted). There are likely a multitude of explanations for global sedge diversity.

One of the most remarkable characteristics of the genus, shared by all its species and long suggested to be a diversification driver, is its peculiar chromosome architecture (Heilborn, 1932; Hipp, 2007; Hipp et al., 2010; Chung et al., 2012; Escudero et al., 2012b, 2014; Spalink et al., 2019; Márquez-Corro et al., 2019a). Sedges present a characteristic type of centromere, distributed along the chromosome (holocentric chromosomes) and fully functional during meiosis (holokinetic meiosis; see reviews in Hipp et al., 2013; Marques & Pedrosa-Harand, 2016). This characteristic is present in several lineages of plants and animals (Escudero et al., 2016; Márquez-Corro et al., 2018, 2019b). Because of the non-spatial separation of recombination and segregation function during meiosis, holocentric chromosomes are generally limited to one or two chiasmata during meiosis (Nokkala et al., 2004). Nevertheless, holocentricity allows fission and fusion to dominate karyotype evolution (Guerra, 2016); in monocentric groups, by contrast, chromosome evolution tends to be associated with genome duplication events. *Carex* has long been studied in terms of chromosome number, with reports ranging from $2n = 10$ to $2n = 132$ (*C. donnell-smithii* and *C. perplexa*, respectively; Heilborn, 1932; Tanaka, 1949; Davies, 1956; Naczi, 1999; Roalson, 2008;

Hipp et al., 2009). Chromosomal variation in *Carex* is second in angiosperm genera only to *Cyperus* ($2n = 10$ to $2n = 224$; Roalson, 2008), the second largest genus of Cyperaceae (ca. 950 spp; Larridon et al., 2013).

Fusion and fission of chromosomes during meiosis has led to high karyotype variation among species and among populations within species in *Carex* (e.g. Wahl, 1940; Tanaka, 1949; Naczi, 1999; Roalson, 2008). Because holokinetic chromosomes present kinetochore activity along the whole chromosome, chromosome fragments arising from fission and fusion events have the potential to segregate in Mendelian fashion (Escudero et al., 2014). Chromosome fission and fusion dominate to the exclusion of ploidy changes throughout *Carex* (Márquez-Corro et al., 2019a), with few but very notable exceptions. The most striking of these counterexamples is the subgenus *Siderosticta*, the sister lineage to the remainder of *Carex*, which has exceptionally low chromosome numbers and evolves by means of polyploidy (Tang & Xiang, 1989). The position of this subgenus as sister to the remainder of *Carex* suggests that the fusion / fission mode of chromosome evolution did not evolve with the other synapomorphies that define the genus, making the origin of *Carex* a stepwise process. Additionally, a small number of species groups (section *Racemosae* and the Humilis Clade; Lipnerová et al., 2013; species group names throughout follow Roalson et al., in revision) and species (*Carex dolichostachya*, *C. jackiana*, *C. roraimensis*; Hipp et al., 2006, 2009) within the other *Carex* subgenera also have been reported as polyploids. The transition to fusion and fission in *Carex*, associated with its high species diversity, suggests a possible role in the high diversification rate of the genus (Hipp, 2007; Hipp et al., 2010; Chung et al., 2012; Escudero et al., 2012b, 2014; Márquez-Corro et al., 2019a).

The reduced number of chiasmata per chromosome (typically one or two per chromosome) and the apparently negligible fitness costs of chromosome fusion and

fission make chromosome number a plausible proxy for recombination rates in the genus (Bell, 1982; Escudero et al., 2012a, 2018). Low within-chromosome recombination rates were demonstrated for *Carex* in the first published genetic linkage map for the genus (Escudero et al., 2018), in which recombination frequency was shown to be congruent with a single cross-over per homologous chromosome on average. Consequently, chromosome number is probably a reasonable proxy for recombination rate (Bell, 1982), and *Carex* may thus be an ideal study system for evaluating hypotheses regarding the effects of natural selection on recombination rates (Bell, 1982; Burt, 2000).

Bell (1982) hypothesized that in areas of densely packed niche space, already-established populations risk little with increased recombination rates. Plants can gamble on rare allelic combinations that may have extreme fitness, because high mean fitness in communities with high interspecific competition ameliorates reproductive risk. By contrast, low recombination rates should be favored when high reproductive potential is needed, and the risks of disadvantageous allelic combinations outweigh the potential advantages of recombination. Moreover, it also has been hypothesized that high recombination rates also would be adaptive in a scenario of quickly changing environments because the evolutionary potential of high recombination rates would allow evolutionary innovation to adapt to the new conditions, whereas low recombination rates may be selected when environmental conditions remain stable over time and evolutionary innovation would not be adaptive (e.g. Wang et al., 2019).

In this study, we test the hypothesis that high recombination rates are selected for in environments where evolutionary innovation is favored. We do so by fitting models that

test for a relationship between proxies of competitiveness and environmental stability on one hand, chromosome number as a proxy for recombination rate on the other. While chromosome number change may favour speciation by means of reproductive isolation (e.g. individuals with large numbers of rearrangements may not be interfertile; Chung et al., 2012), here we limit our questions to recombination rates rather than chromosome number per se. We gathered all available chromosome numbers for the genus *Carex*, covering around one third of the species in the genus and a broad range of its geographic and ecological diversity. Previous studies have sampled species more sparsely (5% species sampled in Escudero et al., 2012a; or floristic level in Spalink et al., 2019) or at a limited phylogenetic scale (e.g. within sects. *Cyperoideae* and *Spirostachyae*; Hipp, 2007; Escudero et al., 2010, respectively). We utilize chromosome counts from all the species that have been included in the most comprehensive phylogeny of the genus (Martín-Bravo et al., 2019) to investigate a number of questions about its evolutionary history. We investigate changes in chromosome number and selective environment using multi-optimum Ornstein-Uhlenbeck models to evaluate whether significant transitions among biomes entail replicable shifts in chromosome number. We then test whether recombination rate as estimated by chromosome number has an effect on diversification. Moreover, we evaluate whether different levels of environmental stability are correlated with variance in recombination rates or other ecologically-significant life history traits.

In combination, these analyses comprise the most comprehensive investigation to date of the effects of chromosome evolution on macroevolutionary success of the sedges.

2 Material and methods

2.1 Phylogeny and chromosome number data

The most comprehensive dated phylogeny of *Carex* was obtained from a previous study (Martín-Bravo et al., 2019), which includes 66% of the extant species. Diploid chromosome numbers were obtained from databases and recent cytogenetic studies (e.g. compilations by Roalson (2008) and Rice et al. (2015), the IAPT/IOPB periodical numbers and regional floras) for the species represented in the phylogeny of the genus (Fig. 1; Data S1 in Márquez-Corro, 2020), excluding prior to analysis chromosome counts that we considered unreliable (i.e., Löve & Löve's counts; Elven, 2020). Taxa for which there no chromosome data were pruned from the tree. The resulting phylogeny was resolved with the function 'multi2di' of the R package APE v5.4-1 (Paradis et al., 2018), and rendered ultrametric with the function 'nmls.tree' from the package PHANGORN v2.5.5 (Schliep, 2011). All analyses were conducted in R v3.6.3 (R Core Team, 2020).

Chromosome number means and squared standard errors were calculated for each species. However, due to the high among-species variability in how many counts were published, and as only 3% (22 tips) had 20 or more chromosome number reports, we use the average variance of all the species variance divided by the sample size (the weighted standard error of the mean, SEM^2_w). This procedure is recommended in Labra et al. (2009) and Hansen and Bartoszek (2012) for datasets with small sample sizes. Chromosome data were not transformed, as the residuals were normally distributed as tested using the 'powerTransform' function of the CAR package v3.0-10; Fox & Weisberg, 2019).

2.2 Tempo and mode of chromosome number evolution

In the current study, we follow previous research that treats *Carex* chromosome number as a continuous variable measured on an integer scale (e.g., Hipp et al., 2007; Escudero et al., 2010, 2012a; Chung et al., 2012; Carta et al., 2018). While this is an approximation (cf. Mayrose et al. 2010), it provides a gateway into a wide range of phylogenetic comparative models useful to the questions we are addressing.

To test for phylogenetic heritability / signal, we compared two models of continuous trait evolution using the Akaike Information Criterion (AIC) with the function ‘transformPhylo.ML’ in the R package MOTMOT v2.1.3 (Harmon & Freckleton, 2008; Data S2 in Márquez-Corro, 2020): i) the Brownian motion model (BM), in which chromosome evolution is modeled approximately as a random walk; and ii) Brownian motion with an additional parameter, Pagel’s lambda (BM + λ ; Pagel, 1999), which multiplies all off-diagonal elements of the phylogenetic variance-covariance matrix by a scalar. The latter has an effect on internal branch lengths relative to tips and estimates whether a character is more or less similar among species than expected from their phylogenetic relationships.

To test for clade-level shifts in chromosome number means, we fit alternative Ornstein-Uhlenbeck (OU) models (cf. Hansen, 1997) in the R package BAYOU v2.2.0 (Uyeda & Harmon, 2014). These models detect multi-optimum scenarios in trait evolution along the phylogeny using Bayesian reversible-jump Markov chain Monte Carlo (rjMCMC). Ten chains of 2.5 million iterations (burn-in = 0.3) were run. Prior values and parameters distributions were set based on previous knowledge of the genus (Data S3 in Márquez-Corro, 2020). For instance, the number of equilibria was set to follow a normal distribution with a mean near to the number of sections retrieved in the tree (ca. 107 sections), as many large-scale taxonomic groups in *Carex* appear to be associated

with shifts in chromosome number (Wahl, 1940; Hipp, 2007; Escudero et al., 2010).

The number of equilibria appear in Table 1.

2.3 Morphological and ecological predictors of chromosome number

To test for effects of selection on chromosome number, we used the ‘slouch.fit’ function in the SLOUCH package v2.1.4 (Kopperud et al., 2018) to fit a stochastic linear OU model for chromosome number evolution with morphological and climatic predictors.

Under this model, chromosome number is treated as evolving according to an OU model toward an optimum that is a linear function of the predictors. The predictors – morphological or climatic traits in our study – are modeled as evolving on the tree according to a BM process (Hansen et al., 2008). Under an OU model, the stationary variance (v_y) estimates the trait variance when the OU process has reached equilibrium, and the phylogenetic half-life ($t_{1/2}$) estimates the amount of time (in branch-length units) for a lineage to move halfway from its ancestral value to the OU equilibrium.

In *Carex*, chromosome number is highly labile and has been shown in at least some clades to evolve toward clade-specific optima (Hipp, 2007). To test the relationship of chromosome number to morphological traits we included a number of covariates that could explain the observed chromosome number variation. Variables that may shape aboveground competitive interactions (culm length and leaf width) and reproductive strategies (utricle length and length of lateral and terminal spikes) were selected from floras, taxonomic revisions and other published studies (Data S1 in Márquez-Corro, 2020). The midpoint of the ranges excluding outliers was used for analysis as a proxy for the character state mean, and 25% of that value as standard error (Escudero et al. 2012a). To model the evolution of environmental conditions that may select for

different chromosome numbers (recombination rates), we used bioclimatic variables from the WorldClim database (<https://www.worldclim.org/>). Occurrences of all the species were searched in the GBIF database (<https://www.gbif.org/>). Imprecise or duplicated coordinates (within the same 2.5 arcmin grid) were discarded. The data retained from the cleaning process were used to download the 19 bioclimatic variables of the WorldClim database. Mean and variance were calculated for each variable and taxon independently, and variance was used to estimate SEM2. To reduce error in SEM2 estimation for phylogenetic regression analyses, SEM2 for species with < 20 samples was estimated as SEM2w, dividing the variance averaged across species by the sample size for each species.

Multiple regressions were fitted using combinations of morphological and bioclimatic variables that reflect plausible explanations for chromosome evolution in the genus (Table 2). We expected higher recombination rates in highly stochastic environments or environments that promote high competitiveness among individuals (Bell, 1982; Burt, 2000; Escudero et al., 2012a; Wang et al., 2019). We modeled environmental stability for bioclimatic variables as less temperature variation across the year (lower BIO4 and BIO7). We modeled competitive conditions using morphological variables, where low competitiveness is associated with longer inflorescences units or smaller utricles, both associated with higher reproductive allocation; and larger leaves and height, associated with higher allocation to competition for space. As sample size varied widely, we reduced the dataset to the 564 taxa that had chromosome, morphological, and climatic data (Table 2, Data S4 in Márquez-Corro, 2020). AIC weights were calculated for all the models (function ‘*akaike.weights*’ from R package QPCR v1.4-1; Ritz & Spiess, 2008; Table 2, Data S4 in Márquez-Corro, 2020).

Phylogenetic transitions in morphological and bioclimatic niche as well as chromosome number were reconstructed using reversible-jump Markov chain Monte Carlo (rjMCMC) in the R package BAYOU v2.2.0 (Uyeda & Harmon, 2014). Ten chains of one million iterations (burn-in = 0.3) were run for the selected variables: culm length as vegetative character; lateral inflorescence length as reproductive character; and BIO1, BIO4, BIO7, BIO12 as proxies for climatic niche. Morphological traits were selected due to the wider range of variation among the studied variables. Bioclimatic variables were selected from those that most clearly distinguish species clustered in climatic space using the complete linkage method, which defines the distance between two clusters to be the highest distance between their individual components (Fig. S1 in Márquez-Corro, 2020; R Core Team, 2020).

2.4 Relationship between chromosome number and lineage diversification rates

The quantitative trait speciation-extinction model (QuaSSE) as implemented in the R package DIVERSITREE v0.9-14 (FitzJohn, 2012) was used to test for an effect of chromosome number on speciation and extinction rates. We evaluated all the possible model combinations of either constant, linear, sigmoid or unimodal relationship between recombination rates (chromosome number evolution) and speciation or extinction events. Global sampling fraction and standard error were set to 36% and SEM^2_w of each taxon, respectively (Data S5 in Márquez-Corro, 2020). We carried out an analysis with and without the subgenus *Siderosticta*, since it is a well-known early-diverging polyploid lineage, to check for any possible discrepancy caused by analyzing a predominantly dysploid clade (one dominated by fusion and fission) with its predominantly polyploid sister group included in analysis.

3 Results

3.1 Chromosome number, morphological and bioclimatic data

Chromosome number counts were found for 755 taxa (721 species) of the total of 1386 taxa (1312 species) included in the tree (Martín-Bravo et al., 2019). This represents 36% of *Carex* species (Fig. 1, Data S1 in Márquez-Corro, 2020). Data S1 (Márquez-Corro, 2020) contains the source information for the 3212 chromosome number reports used. Reports per taxon ranged from one to 131 counts (1st quartile = 1, median = 2, 3rd quartile = 5). Chromosome numbers were obtained for the six *Carex* subgenera (Villaverde et al., 2020) and most of the sections represented in the phylogeny (ca. 107 out of ca. 126; Figs. 1–2).

Carex exhibits broad, clade-level differences in chromosome numbers among subgenera that may have phylogenetic or ecological significance (Fig. 2). Subgenera *Carex* and *Vignea* present relatively broad chromosome number distributions, with a peak between $2n = 50–75$. Subgenus *Euthyceras* has a similar but somewhat flatter distribution. *Psyllophorae* presents three clusters of numbers that appear to have no particular relation to clade; section *Schoenoxiphium*, for example, includes species in each of the three chromosome number clusters. Finally, *Uncinia* presents a mode of $2n = 88$. The exceptions are a few New Zealand species and the South American taxa, which have higher chromosome numbers, and the remaining species of the subgenus, that exhibit mostly lower numbers.

Morphological data were available for more than 700 taxa for culm length (715 taxa), leaf width (722 taxa), and utricle length (734 taxa), with lateral and terminal inflorescence length available for 662 and 602 taxa respectively. Bioclimatic data were

obtained for 731 taxa, with occurrences ranging from one to 2328 data points (1st quartile = 28.5, median = 113, 3rd quartile = 330.5). We discarded any morphological or bioclimatic variable that was strongly correlated ($|r| < 0.70$) for the multi-predictor models.

3.2 Phylogenetic signal and clade-specific shifts

The BM+ λ model ($\sigma^2 = 5.23$, $\lambda = 0.75$ [95%CI: 0.67–0.81]) was significantly better supported than the Brownian motion model ($\Delta\text{AIC} = 168.09$, Data S2 in Márquez-Corro, 2020). The 95% CI for λ excludes 0.0 and 1.0, demonstrating that chromosome number has a significant phylogenetic component (Data S2 in Márquez-Corro, 2020), but that the phylogeny alone is insufficient to explain the patterns of chromosome evolution.

Reversible-jump MCMC analysis of OU models in BAYOU recovered a post-burn-in mean of 100 [HPD95%: 84–115] shifts in chromosome number, of which 21 were highly supported ($pp > 0.7$; Table 1, Data S3 in Márquez-Corro, 2020). The posterior rate of adaptation () was estimated at 3.4 [HPD95%: 0.7–8.1], which translates to a phylogenetic half-life of 0.2 Myr [HPD95%: 0.1–1.0]. The root mean was inferred as $2n = 38$ [HPD95%: 13–57]. However, the posterior distribution showed three peaks, with ca. 42 chromosomes as the most likely diploid number for the genus at the root, and the second and third with similar posterior probabilities between them, situated at approximately 16 and 56 chromosomes, respectively (Data S3 in Márquez-Corro, 2020).

3.3 Diversification-related pattern

QuaSSE models strongly support models in which diversification rates are related to chromosome number by a positive sigmoidal speciation relationship, either under the

OU process (AIC_w = 0.53) or the BM process (0.46). The remaining (state-independent) diversification models collectively share an AIC_w of 0.01 (Data S5 in Márquez-Corro, 2020). While these results might be subject to hidden states within chromosome number strata (Beaulieu and O’Meara 2016; O’Meara and Beaulieu 2016), the exceptionally high heterogeneity of chromosome numbers in our study makes it unlikely that additional unmeasured states within high-chromosome groups might explain this result. The relationship between chromosome number and speciation follows a positive sigmoidal distribution (Fig. 3), with 0.51 speciation events per million years (events/Myr) and an inflection at $2n = 58$, at which point speciation increases to 2.07 events/Myr. On the other hand, extinction was modeled similarly, with a rate of 0.31 extinction events/Myr below $2n = 60$, and an abrupt rate rise up to 1.83 events/Myr. This means that net diversification is low (0.20 species/Myr) for $2n < 58$, there is a burst in diversification of up to 1.76 species/Myr for $2n = 58–60$, and again low net diversification rates (0.24 species/Myr; Fig. 3) for $2n > 60$. This model was also the best supported in the analysis excluding the subgenus *Siderosticta* (Data S5 in Márquez-Corro, 2020).

3.4 Evaluation of the predictor variables

The sample size for the models used in SLOUCH varied between predictors, so a tree with all the variables was obtained (564 taxa). All of the tested models performed better than BM, whereas only three of them performed slightly better than the single-equilibrium OU null model at explaining chromosome number evolution (Table 2, Data S4 in Márquez-Corro, 2020). The single equilibrium (no-predictor) OU model indicated a very low deterministic effect on chromosome number evolution ($t_{1/2} = 13.50$ [9.05–23.92] Myr; Table 2, Data S4 in Márquez-Corro, 2020). This suggests a low rate of

evolution (α) to the equilibrium (θ), if it is the correct model. For single-predictor and multiple-predictor models, the half-life estimate varied similarly from 13.40 to 14.59 Myr. The range of the half-life values –within two units of log-likelihood– also varied similarly between 8.72 to 28.92 Myr (Table 2, Data S4 in Márquez-Corro, 2020). Thus, although instantaneous adaptation/no phylogenetic inertia ($t_{1/2} = 0, \alpha \rightarrow \infty$) and pure BM ($t_{1/2} = \infty, \alpha = 0$) are excluded from the confidence interval, our results are close to a BM model (e.g. single equilibrium OU model: $t_{1/2} = 13.5$ Myr, $\alpha = 0.05$ Myr⁻¹). Overall, there is little effect of predictors in all models. Moreover, little lag is shown between the evolutionary and optimal regressions using predictors for chromosome number because, despite the strong inertia of the variables, the root being old enough to allow the traits to evolve towards their optima (Table 2, Fig. 4). The three slightly supported models include the mean temperature of driest quarter (BIO9) plus culm or terminal inflorescence length, and the BIO9 alone as predictors of chromosome number (Fig. 4, Table 2). The model with BIO9 alone does not present a much higher AIC weight value vs. the OU no-predictor model (AICw = 0.44 vs. 0.40, respectively). Moreover, the two first models included the BIO9 variable, and did not exceed the AIC of the BIO9 model alone (AICw = 0.059 and 0.048 vs. 0.044 in BIO9). Thus, overall weak explanatory power was found (R^2 below 1%), considering either single-predictors or multi-predictors models (Table 2, Data S4 in Márquez-Corro, 2020). The multi-optima analyses showed high rates of evolution towards the equilibrium in chromosome number and low stochastic evolution ($\alpha = 3.41, \sigma^2 = 79.07$; Data S3 in Márquez-Corro, 2020). Morphological variables studied were estimated with the highest rates towards the optimum ($\alpha = 9.84$ and 41.91 for culm and lateral inflorescence length, respectively) and highest stochastic evolution as well ($\sigma^2 = 2693.43$ and 676.38 , respectively). In comparison with chromosome number, bioclimatic variables displayed mostly opposite

patterns, with most of them showing lower rates towards the optimum ($\alpha = 0.16, 0.63$ and 1.42 for BIO1, BIO4 and BIO12, respectively), with exception of BIO7 ($\alpha = 5.04$), and all displaying lower stochastic evolution ($\sigma^2 = 0.35, 64.39, 0.27$ and 1.44 , respectively; Data S3 in Márquez-Corro, 2020). Overall, despite the low explanatory ability detected for the predictors, their relationship with chromosome number is very similar to that obtained by Escudero et al. (2012a). This result is compelling, since we sampled about six times more taxa compared to that study.

3.5 Clade-level transitions among variables

Multi-optimum models (BAYOU) results showed more optima for morphological vs. bioclimatic variables (Table 1). On one hand, mean optima numbers of 48 [HPD95%: 37–58] and 98 [HPD95%: 84–114] were inferred for culm length and lateral inflorescence lengths, respectively. On the other hand, number of optima were modeled as 36 [HPD95%: 24–47], 37 [HPD95%: 25–48], 37 [HPD95%: 26–48] and 37 [HPD95%: 24–47] for BIO1, BIO4, BIO7 and BIO12, respectively.

At deep phylogenetic levels, clades corresponding to subgenera *Siderosticta* and *Uncinia* and the *Psyllophorae+Euthyceras* clade are reconstructed as having a single chromosome number equilibrium each (pp = 0.80, $\theta = 17$ chromosomes; pp = 0.75, $\theta = 82$ chromosomes; pp = 0.62, $\theta = 68$ chromosomes, respectively; Data S3 in Márquez-Corro, 2020), whereas subgenera *Vignea* and *Carex*, which are the two most diverse groups of the genus, have multiple chromosome number equilibria. However, *Vignea* seems to present a shift close to its crown node (pp = 0.75, $\theta = 59$ chromosomes), with few supported shifts further within the subgenus; the main ones include sections *Phleoideae* and *Physoglochin*, besides the above mentioned *Cyperoideae*. On the other

side, subgenus *Carex* is more variable, with multiple shifts occurring in different sections or lineages. For instance see Acrocystis-Liparocarpos-Humilis Clade, and the highly supported shift ($pp = 0.98$, $\theta = 58$ chromosomes) in a clade containing approximately half of the sampling of the subgenus and the vast majority of helophytic taxa (Mollicula, Gracillima, Stylosa, Scita, Squarrosa and Limosa Clades, sections *Scirpinae*, *Fecundae* and *Phacocystis*, among others). Moreover, further low to moderate supported shifts have been detected in the Hirta Clade ($pp = 0.54$, $\theta = 72$ chromosomes), sect. *Phacocystis* ($pp = 0.68$, $\theta = 76$ chromosomes), or *Spirostachyae* subsect. *Elatae* ($pp = 0.69$, $\theta = 72$ chromosomes). These are some examples that reveal the intricate scenario of chromosome number evolution, in conjunction with ecological conditions.

4 Discussion

Our work provides important new insights into the evolutionary history of chromosome diversity in *Carex*. First, chromosomes have evolved a much higher number of optima than morphological and bioclimatic variables (Table 1), suggesting a rapid rate of chromosome evolution in the clade relative to the evolution of climatic and functional niche, and relative to other stochastic factors. In association with this, morphological diversity and climatic niche appear to have shaped chromosome number evolution, but they explain only a small proportion of the total variance in chromosome number. The low predictive power of functional variation, perhaps a consequence of high diversity of lineage-specific life histories in our exceptionally high diversity study, contrasts with high predictive power at lower evolutionary scales (e.g., Escudero et al., 2013). The effects of chromosome evolution on ecological diversification may thus be more apparent within clades and regions than at broad phylogenetic scales. Finally, our work

suggests that the highest rates of lineage diversification in *Carex* are found at moderate chromosome numbers, with an eight-fold higher net diversification rate around 58–60 chromosomes than at the ends of the chromosome distribution (Fig. 3). Whether this might be an artifact of unmodeled heterogeneity in net diversification rates (cf. Beaulieu & O’Meara, 2016) or entanglement of chromosome number with the rate of chromosome evolution itself remains to be seen. In any case, our analyses demonstrate that chromosome variation in sedges is not a mere novelty, and does more than just shape reproductive isolation through reduced crossability. Chromosome evolution is an important macroevolutionary force in sedges, shaping the evolution of ecological and lineage diversity in its largest genus.

4.1 Chromosome number evolution in *Carex*

Karyotype in *Carex* has been shown to evolve through a continuous range, mainly by fusions and fissions (Márquez-Corro et al., 2019a) rather than through whole genome duplication. There are few exceptions to this rule in *Carex*, which is particularly striking given the high lineage diversity of the genus and the fact that the remainder of the family Cyperaceae is dominated by polyploid chromosome number evolution (Hipp et al., 2009). The most obvious exception in *Carex* is the polyploid subgenus *Siderosticta* (Figs. 1–2; Tang & Xiang, 1989), which is sister to the remaining subgenera (GCG, 2015; Jiménez-Mejías et al., 2016; Martín-Bravo et al., 2019). The shift in mean chromosome number we detect in *Siderosticta* vs. the remaining subgenera supports previous arguments that the evolution of the non-*Siderosticta* clade of *Carex* entailed a shift in the mode of chromosome evolution (Escudero et al., 2012b; Márquez-Corro et al., 2019a). Thus, while subgenus *Siderosticta* is clearly within *Carex* morphologically, from the standpoint of cytogenetic behavior, one could just as easily recognize it as a

distinct genus. It is a stepping-off point on the road into *Carex*, a transition from the more or less stable karyotypes and polyploidy that dominate in the remainder of Cyperaceae, reminding us of how important the mode of chromosome evolution has been in making this enormous genus what it is.

We detected a strong phylogenetic signal in chromosome number (Fig. 1, Data S2 in Márquez-Corro, 2020). We estimated ca. 42 chromosomes as the most likely ancestral diploid number (pp = 0.20), but chromosome numbers in *Carex* have varied so rapidly that this estimate should be considered a point estimate within a very broad confidence interval (less supported peaks around $2n = 16$ and $2n = 57$; Data S3 in Márquez-Corro, 2020). This result supports previous analysis using the ChromEvol model (Glick & Mayrose, 2014), in which a range including these numbers was obtained (Márquez-Corro et al., 2019a; ancestral $2n = 28$ with pp = 0.07 and pp values above 0.02 in a range that included numbers between $2n = 12$ –50]). Moreover, given the discussion above regarding the abrupt change in chromosome dynamics on the branch leading into *Carex* excluding subgenus *Siderosticta*, reconstructing the ancestral diploid number for the genus may not be practical. Our best estimate for the count at the oldest end of the stem leading into the genus might in fact be better arrived at using information from the taxa outside of *Carex*, while the estimate for the crown count of the genus might better be based on the ingroup taxa (as we have done). In any case, as many extant species present large variation in chromosome numbers (e.g., *C. laevigata* $2n = 69$ –84, *C. scoparia* $2n = 56$ –70), the ancestors of the main lineages of *Carex* might also show a wide range of chromosomes rather than just a single chromosome number or a narrow range. Nonetheless, since the dysploid (fusion / fission) variation in chromosome number is common within *Carex* species but not observed in subgenus *Siderosticta*, it would be conceivable to expect a less variable number for *Carex* ancestor.

When we considered OU models with multiple equilibria, we observed a more complex scenario, with a minimum of 84 equilibria shifts (Fig. 5, Table 3, Data S3 in Márquez-Corro, 2020). This complex evolutionary history of chromosome number evolution is supported by numerous previous studies. Hipp (2007) for instance found a shift in mean chromosome numbers within sect. *Cyperoideae* between the western and eastern North American clades that is also detected in this study. Moreover, our analysis also inferred an additional shift for sect. *Cyperoideae* itself (Fig. 5, Data S3 in Márquez-Corro, 2020). This fact, in combination with previous demonstrations of an increase in the rate of chromosome evolution (Chung et al. 2012) associated with an increase in diversification rate at the base of the clade (Martin-Bravo et al. 2019), echoes the transition in chromosome dynamics and diversification rates at the base of *Carex* itself (Escudero et al. 2012). Together, these provide evidence supporting the role of chromosome evolution in lineage diversification in the genus. Escudero et al. (2010) similarly studied chromosome number evolution of sect. *Spirostachyae* and found no shifts in equilibrium, whereas here we have been able to detect a shift within the section, corresponding to the subsect. *Elatae*. The history of sedge diversification appears to entail numerous shifts in chromosome number across a range of phylogenetic depths. Each of these shifts is a natural experiment in the evolution of recombination rates, each with the potential to yield insight into the ecological dynamics of *Carex* diversification.

4.2 Recombination rate optima for ecological strategies: Competitiveness and environmental stability ecological scenarios

The lack of supported shifts inferred for bioclimatic variables suggests that climatic transitions in the genus may proceed gradually or be limited by niche conservatism at

broad scales, in keeping with the observation that its species mostly inhabit cold or temperate areas (Reznicek, 1990). Nevertheless, previous studies have demonstrated niche shifts at shallow evolutionary scales (Benítez-Benítez et al., 2018, submitted; Villaverde et al., 2017b). In addition, niche shifts in two *Carex* sister species have been suggested to be related to changes in non-bioclimate preferences (soil pH, Benítez-Benítez et al., 2018). In combination with these inferred niche transitions at fine phylogenetic scales, our results suggest that the major lineages of *Carex* may not be characterized by dramatic and rapid climatic range expansions, but by radiations within and among relatively similar climatic conditions. These radiations may entail rapid shifts among different soil types or communities that differ in competitive interactions (Villaverde et al., 2017b; Martín-Bravo et al., 2019; Benítez-Benítez et al., submitted). But the fact that many clades are geographically widespread (Martín-Bravo et al., 2019) may point to a stronger importance of ecological diversification within major clades. Our work demonstrating the correlation of chromosome number with climatic regime and functional traits suggests that chromosome number and ecological diversification shape diversification at shallower evolutionary scales (e.g. Hipp, 2007; Escudero et al., 2010; Escudero et al., 2013), explaining the high regional and broad continental diversity of individual sedge clades. Morphological traits such as culm and lateral inflorescence lengths present a significantly greater number of shifts than bioclimatic variables (Table 1, Fig. 5). This fact and the geographic conservatism of many *Carex* clades (Dragon & Barrington, 2009; Hipp et al., 2006; Roalson et al., 2004) together suggest that morphological variation may facilitate habitat and life history transitions within geographic and climatic regions, shaping speciation.

The relationships among climate, morphology, and chromosome variation are not straightforward. Bioclimatic and morphological trait evolution show low correlation,

and both are poorly correlated with chromosome number evolution at deep evolutionary scales (Fig. 4, Table 2, Data S4 in Márquez-Corro, 2020). Nevertheless, climatic and morphological variation predict relatively small portions of the variability in chromosome number (less than 1%), the best supported models include several of the tested predictors. By contrast, a study of Cyperaceae assemblages of North America by Spalink et al. (2018) demonstrated that chromosome numbers in *Carex* species assemblages exhibit variation according to climate, with lower chromosome numbers in warmer, wetter, and less seasonal assemblages. However, this correlation between chromosome number and climatic niche became nonsignificant when tested at the species level (using PGLS) instead at the level of species assemblages. It may be that community biogeographic patterns allow us to detect more nuanced effects of selection than species centroids, which wash over much of the variation across species ranges. Nevertheless, phylogenetic ANOVA indicated that species with lower chromosome numbers grow in significantly drier and more shaded habitats (Spalink et al., 2018). These findings are congruent with the significant relationship also found by Bell (1982) between the similar habitat categories and chromosome number in North American and Britain *Carex* species. Escudero et al. (2012b) found that species with lower chromosome numbers tend to grow in areas with higher temperature seasonality and / or in dry habitats and tend to have smaller lateral inflorescences. These previous studies show mixed results in relation to the best ecological scenarios in which selection of potential for evolutionary innovation (high recombination rates) could be acting. By comparison, the only study at a shallow evolutionary scale (Escudero et al., 2013) infers that high recombination rates are selected for in communities with high interspecific competition. In the current study, the best models have as predictors the mean temperature of the driest month, culm length and terminal inflorescence length (Fig. 4,

Table 2). Higher chromosome numbers tend to be related with shorter culms and terminal inflorescence units and tend to grow in areas with higher temperature during the driest months. However, these best models are only marginally better than the single optimum OU model and have low model fit (as estimated using R^2), making it difficult to conclusively favor any of the ecological scenarios that relate recombination rates with potential for evolutionary innovation.

The history of chromosome evolution in sedges is likely as complex as the history of lineage diversification (Martin-Bravo et al., 2019). In different clades, recombination rates may be under divergent selection, or neutral, making it difficult or meaningless to infer a global relationship between chromosome number evolution and morphological traits or climate. Each of the clades in a genus the size of *Carex* tracks an individualistic history (cf. Roalson & Roberts, 2016), which complicates the finding of common overlying features for the genus as a whole. The two main ecological scenarios regarding our hypothesis of the evolution of recombination rate may be alternatively more or less important in different lineages and contexts and require further work.

Besides the detected effect of chromosome number in diversification, we have been able to compare whether shifts of the studied variables do occur in concert with previous reported diversification rate shifts (Martín-Bravo et al., 2019; Larridon et al., 2020). Bearing in mind the sampling differences among studies, we detected only a single lineage in which shifts in chromosome number equilibrium and diversification rate coincide, and also a shift in a morphological variable. This occurs in section *Cyperoideae*, for which we have retrieved strong and moderately supported shifts for chromosome number and lateral inflorescence, respectively. In addition, an inferred reduction in the diversification rate for the clade comprising subgenera *Psyllophorae* and *Euthyceras* (Larridon et al., 2020) matched a moderately supported chromosome

number equilibrium shift. For the remaining clades with inferred diversification rate shifts (Martín-Bravo et al., 2019), we were not able to find any supported shift in equilibria for chromosome number. However, some shifts were detected to have happened shortly after or before the diversification shift occurs. Such is the case of subgenus *Uncinia*, the sections *Clandestinae* and *Acrocystis* or the Hirta Clade. Thus, we found a diversification rate increase in numbers near 58–60 chromosomes, a range of chromosomes that are common in many different clades (Fig. 1), including the lineages in which diversification rate bursts were detected. The high diversification rates near the overall chromosome number range midpoint, in addition to the different morphological shifts and adaptations to possible different non-climatic niches points out to the assumption that recombination rates, probably followed by adaptive innovation, indeed constitute a force in this genus diversification. To what extent this occurs is yet to be clarified, as well as the effect of the chromosomes and genome regions involved in the fusion or fission events.

4.3 Final remarks and future works

Carex is remarkable both for its high diversification rates (Escudero et al., 2012b; Martín-Bravo et al., 2019) and its departure from the typical latitudinal species richness gradient, with a peak of diversity closer to the poles (Escudero et al., 2012b). The genus also exhibits high morphological variability (Egorova, 1900; Kükenthal, 1909; Jiménez-Mejías et al., 2016). In addition, an uncommon type of centromere and meiotic division is widespread if not ubiquitous in the genus (holocentric chromosomes, inverted meiosis, and pseudomonads instead of pollen tetrads; see Wahl, 1940; Brown & Lemmon, 2000; Halbritter et al., 2010 and references therein). Our work demonstrates that these attributes –lineage diversity patterns on one hand, chromosomal variation on

the other— jointly shape the ecological and morphological variety that characterize this enormous genus. In this study, we investigate the evolution of recombination rates at a macroevolutionary scale, using chromosome number as proxy. Although our work is based on a sampling of only one third of *Carex* species, our results are based on ca. 560–750 taxa, depending on the analysis. This is due to the high species richness that characterizes *Carex*, not only being among the top angiosperm genera, but also being the most species-rich of all holocentric plant lineages (Márquez-Corro et al., 2018). Overall, this mostly temperate genus has been studied broadly, whereas other plant and animal holocentric groups have been poorly studied at a macroevolutionary scale under this scope (e.g. Vershinina & Lukhtanov, 2017).

Here, we highlight the need for further work on chromosome number evolution in holocentric organisms. We know already that rapid evolution of chromosome number is not a common feature of holocentric lineages; so why do some holocentric lineages exhibit such rapid increases in chromosome evolution? Does chromosome variation shape diversification rates, either through the rate of chromosome evolution (shaping reproductive isolation) or through its effects on recombination rates? The discovery of common phylogenetic and selective patterns in holocentric chromosome evolution would greatly increase our understanding of the factors shaping biodiversity.

There is much work to do at shallower scale. This applies to *Carex* as well, for which only few sectional studies have been carried out to date (Hipp, 2007; Escudero et al., 2010). Further progress will require new data, from more taxonomically curated geographic occurrences to new ecological data that will enable deeper investigations of how chromosome number evolves at a shallower scale and its relationship with morphological and ecological history. This is especially complicated by and important due to the varying relationships between chromosome number and predictors across the

Carex phylogeny. Refining our understanding of macroevolutionary relationships between chromosomes and the evolution of the genus would vastly improve our understanding of the factors underlying the evolutionary success of this important temperate genus.

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References

- Beaulieu JM, O’Meara BC. 2016. Detecting hidden diversification shifts in models of trait-dependent speciation and extinction. *Systematic Biology* 65: 583–601.
- Bell G. 1982. *The masterpiece of nature: the evolution and genetics of sexuality*. Berkeley: University of California Press.
- Benítez-Benítez C, Escudero M, Rodríguez-Sánchez F, Martín-Bravo M, Jiménez-Mejías P. 2018. Pliocene–Pleistocene ecological niche evolution shapes the phylogeography of a Mediterranean plant group. *Molecular Ecology* 27. 1696–1713. <https://doi.org/10.1111/mec.14567>

- Benítez-Benítez C, Martín-Bravo S, BJORÅ CS, Gebauer S, Hipp AL, Hoffmann MH, Luceño M, Pedersen TM, Reznicek A, Roalson E, Volkova P, Yano O, Spalink D, Jiménez-Mejías P. Geographical vs. ecological diversification patterns in *Carex* sect. *Phacocystis* (Cyperaceae): patterns hidden behind a twisted taxonomy. *Journal of Systematics and Evolution* XX: XX–XX.
- Brown RC, Lemmon BE. 2000. The cytoskeleton and polarization during pollen development in *Carex blanda* (Cyperaceae). *American Journal of Botany* 87: 1–11. <https://doi.org/10.2307/2656679>
- Burt A. 2000. Perspective: sex, recombination, and the efficacy of selection – was Weismann right? *Evolution* 54: 337–351. <https://doi.org/10.1111/j.0014-3820.2000.tb00038.x>
- Carta A, Bedini G, Peruzzi L. 2018. Unscrambling phylogenetic effects and ecological determinants of chromosome number in major angiosperm clades. *Scientific reports* 8: 1–14. <https://doi.org/10.1038/s41598-018-32515-x>
- Chung K-S, Hipp AL, Roalson EH. 2012. Chromosome number evolves independently of genome size in a clade with nonlocalized centromeres (*Carex*: Cyperaceae). *Evolution* 66: 2708–2722. <https://doi.org/10.1111/j.1558-5646.2012.01624.x>
- Davies EW. 1956. Cytology, evolution and origin of the aneuploid series in the genus *Carex*. *Hereditas* 42: 349–365.
- Dragon JA, Barrington DS. 2009. Systematics of the *Carex aquatilis* and *C. lenticularis* lineages: Geographically and ecologically divergent sister clades of *Carex* section *Phacocystis* (Cyperaceae). *American Journal of Botany* 96: 1896–1906.

- Egorova TV. 1999. *The sedges (Carex L.) of Russia and adjacent states*. St Louis: Missouri Botanical Garden Press.
- Elven, R. 2020. Annotated Checklist of the Panarctic Flora (PAF) Vascular plants. Available from <http://panarcticflora.org/> [accessed 15 October 2020].
- Escudero M, Hipp AL, Luceño M. 2010. Karyotype stability and predictors of chromosome number variation in sedges: a study in *Carex* section *Spirostachyae* (Cyperaceae). *Molecular Phylogenetics and Evolution* 57: 353–363.
<https://doi.org/10.1016/j.ympev.2010.07.009>
- Escudero M, Hipp AL, Hansen TF, Voje KL, Luceño M. 2012a. Selection and inertia in the evolution of holocentric chromosomes in sedges (*Carex*, Cyperaceae). *New Phytologist* 195: 237–247. <https://doi.org/10.1111/j.1469-8137.2012.04137.x>
- Escudero M, Hipp AL, Waterway MJ, Valente LM. 2012b. Diversification rates and chromosome evolution in the most diverse angiosperm genus of the temperate zone (*Carex*, Cyperaceae). *Molecular Phylogenetics and Evolution* 63: 650–655.
<https://doi.org/10.1016/j.ympev.2012.02.005>
- Escudero M, Maguilla E, Luceño M. 2013. Selection by climatic regime and neutral evolutionary processes in holocentric chromosomes (*Carex* gr. *laevigata*: Cyperaceae): A microevolutionary approach. *Perspectives in Plant Ecology, Evolution and Systematics* 15: 118–129.
<https://doi.org/10.1016/j.ppees.2013.01.001>
- Escudero M, Martín-Bravo S, Mayrose I, Fernández-Mazuecos M, Fiz-Palacios O, Hipp AL, Pimentel M, Jiménez-Mejías P, Valcárcel V, Vargas P, Luceño M. 2014. Karyotypic changes through dysploidy persist longer over evolutionary time than

polyploid changes. *PLoS One* 9: e85266.

<https://doi.org/10.1371/journal.pone.0085266>

Escudero M, Hahn M, Brown BH, Lueders K, Hipp AL. 2016. Chromosomal rearrangements in holocentric organisms lead to reproductive isolation by hybrid dysfunction: The correlation between karyotype rearrangements and germination rates in sedges. *American Journal of Botany* 103: 1529–1536.

Escudero M, Márquez-Corro JI, Hipp AL. 2016. The phylogenetic origins and evolutionary history of holocentric chromosomes. *Systematic Botany* 41: 580–585. <https://doi.org/10.1600/036364416X692442>

Escudero M, Hahn M, Hipp AL. 2018. RAD-seq linkage mapping and patterns of segregation distortion in sedges: meiosis as a driver of karyotypic evolution in organisms with holocentric chromosomes. *Journal of Evolutionary Biology* 31: 833–843. <https://doi.org/10.1111/jeb.13267>

FitzJohn RG. 2012. Diversitree: Comparative Phylogenetic Analyses of Diversification in R. *Methods in Ecology and Evolution* 3: 1084–1092. <https://doi:10.1111/j.2041-210X.2012.00234.x>

Fox J, Weisberg S. 2019. *An R Companion to Applied Regression*, Third edition. Thousand Oaks CA: Sage.

Glick L, Mayrose I. 2014. ChromEvol: assessing the pattern of chromosome number evolution and the inference of polyploidy along a phylogeny. *Molecular Biology and Evolution* 31: 1914–1922. <https://doi.org/10.1093/molbev/msu122>.

- Global *Carex* Group. 2015. Making *Carex* monophyletic (Cyperaceae, tribe Cariceae): a new broader circumscription. *Botanical Journal of the Linnean Society* 179: 1–42.
<https://doi.org/10.1111/boj.12298>
- Guerra M. 2016. Agmatoploidy and symploidy: a critical review. *Genetics and Molecular Biology* 39: 492–496.
- Halbritter H, Weber M, Hesse M. 2010. Unique aperture stratification in *Carex* (Cyperaceae) pollen. *Grana* 49: 1–11. <https://doi.org/10.1080/00173130903566723>
- Hansen TF. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51: 1341–1351.
- Hansen TF, Bartoszek K. 2012. Interpreting the evolutionary regression: the interplay between observational and biological errors in phylogenetic comparative studies. *Systematic Biology* 61: 413–425. <https://doi.org/10.1093/sysbio/syr122>
- Harmon GH, Freckleton RP. 2008. MOTMOT: models of trait macroevolution on trees. *Methods in Ecology and Evolution* 3: 145–151. <https://doi.org/10.1111/j.2041-210X.2011.00132.x>
- Heilborn O. 1932. Aneuploidy and polyploidy in *Carex*. *Svensk Botanisk Tidskrift Bd.* 26: 137–145.
- Hipp AL, Reznicek AA, Rothrock PE, Weber JA. 2006. Phylogeny and classification of *Carex* section *Ovales* (Cyperaceae). *International Journal of Plant Sciences* 167: 1029–1048.
- Hipp AL. 2007. Nonuniform processes of chromosome evolution in sedges (*Carex*: Cyperaceae). *Evolution* 61: 2175–2194.

- Hipp AL, Rothrock PE, Roalson EH. 2009. The evolution of chromosome arrangements in *Carex* (Cyperaceae). *Botanical Review* 75: 96–109.
- Hipp AL, Rothrock PE, Whitkus R, Weber JA. 2010. Chromosomes tell half of the story: the correlation between karyotype rearrangements and genetic diversity in sedges, a group with holocentric chromosomes. *Molecular Ecology* 19: 3124–3138.
<https://doi.org/10.1111/j.1365-294X.2010.04741.x>
- Hipp AL, Escudero M, Chung K-S. 2013. Holocentric chromosomes. In: Maloy S and Hughes K (eds) *Brenner's Encyclopedia of Genetics*, pp 499–501. Elsevier: Amsterdam. <https://doi.org/10.1016/B978-0-12-374984-0.00723-3>
- Jiménez-Mejías P, Hahn M, Lueders K, Starr JR, Brown BH, Chouinard BN, Chung K-S, Escudero M, Ford BA, Ford KA, Gebauer S, Gehrke B, Hoffmann MH, Jin X-F, Jung J, Kim S, Luceño M, Maguilla E, Martín-Bravo S, Míguez M, Molina A, Naczi RFC, Pender JE, Reznicek AA, Villaverde T, Waterway MJ, Wilson KL, Yang J-C, Zhang S, Hipp AL, Roalson EH. 2016. Megaphylogenetic specimen-level approaches to the *Carex* (Cyperaceae) phylogeny using regions ITS, ETS, and *matK*: implications for classification. *Systematic Botany* 41: 500–518.
<https://doi.org/10.1600/036364416X692497>
- Kopperud BT, Pienaar J, Voje KL, Orzack SH, Hansen TF. 2019. SLOUCH: Stochastic Linear Ornstein-Uhlenbeck Comparative Hypotheses. R package version 2.1.2.
<https://CRAN.R-project.org/package=slouch>
- Kükenthal G. 1909. Cyperaceae-Caricoideae. In: Engler HGA ed. *Das Pflanzenreich*. Leipzig: W. Engelmann. 4: 1–247.

- Labra A, Pienaar J, Hansen TF. 2009. Evolution of thermal physiology in *Liolaemus* lizards: adaptation, phylogenetic inertia, and niche tracking. *The American Naturalist* 174: 204–220. <https://doi.org/10.1086/600088>
- Larridon I, Bauters K, Reynders M, Huygh W, Muasya AM, Simpson DA, Goetghebeur P. 2013. Towards a new classification of the giant paraphyletic genus *Cyperus* (Cyperaceae): phylogenetic relationships and generic delimitation in C4 *Cyperus*. *Botanical Journal of the Linnean Society* 172: 106–126.
- Larridon I, Spalink D, Jiménez-Mejías P, Márquez-Corro JI, Martín-Bravo S, Muasya AM, Escudero M. 2020. The evolutionary history of sedges (Cyperaceae) in Madagascar. *Journal of Biogeography*: XXX: XX–XX. <https://doi.org/10.1111/jbi.14048>
- Lipnerová I, Bureš P, Horová L, Šmarda P. 2013. Evolution of genome size in *Carex* (Cyperaceae) in relation to chromosome number and genomic base composition. *Annals of Botany* 111: 79–94. <https://doi.org/10.1093/aob/mcs239>
- Marques A, Pedrosa-Harand A. 2016. Holocentromere identity: from the typical mitotic linear structure to the great plasticity of meiotic holocentromeres. *Chromosoma* 125: 669–681. <https://doi.org/10.1007/s00412-016-0612-7>
- Márquez-Corro JI, Escudero M, Martín-Bravo S, Villaverde T, Luceño M. 2017. Long-distance dispersal explains the bipolar disjunction in *Carex macloviana*. *American Journal of Botany* 104: 663–673. <https://doi.org/10.3732/ajb.1700012>

- Márquez-Corro JI. 2020. Research compendium for “Macroevolutionary insights in sedges (*Carex*: Cyperaceae): The effects of rapid chromosome number evolution on lineage diversification”. Zenodo. <http://doi.org/10.5281/zenodo.4450475>
- Márquez-Corro JI, Escudero M, Luceño M. 2018. Do holocentric chromosomes represent an evolutionary advantage? A study of paired analyses of diversification rates of lineages with holocentric chromosomes and their monocentric closest relatives. *Chromosome Research* 26: 139–152. <https://doi.org/10.1007/s10577-017-9566-8>
- Márquez-Corro JI, Martín-Bravo S, Spalink D, Luceño M, Escudero M. 2019a. Inferring hypothesis-based transitions in clade-specific models of chromosome number evolution in sedges (Cyperaceae). *Molecular Phylogenetics and Evolution* 135: 203-209. <https://doi.org/10.1016/j.ympev.2019.03.006>
- Márquez-Corro JI, Martín-Bravo S, Pedrosa-Harand A, Hipp AL, Luceño M, Escudero M. 2019b. Karyotype evolution in holocentric organisms. In: *eLS*. John Wiley & Sons, Ltd: Chichester. <https://doi.org/10.1002/9780470015902.a0028758>
- Martín-Bravo S, Jiménez-Mejías P, Villaverde T, Escudero M, Hahn M, Spalink D, Roalson EH, Hipp AL, the Global *Carex* Group. 2019. A tale of worldwide success: behind the scenes of *Carex* (Cyperaceae) biogeography and diversification. *Journal of Systematics and Evolution* 57: 695–718. <https://doi.org/10.1111/jse.12549>
- Mayrose I, Barker MS, Otto SP. 2010. Probabilistic models of chromosome number evolution and the inference of polyploidy. *Systematic Biology* 59: 132–144.

- Naczi RFC. 1999. Chromosome numbers of some eastern North American species of *Carex* and *Eleocharis*. *Contributions from the University of Michigan Herbarium* 22: 105–119.
- Nokkala S, Kuznetsova VG, Maryanska-Nadachowska A, Nokkala C. Holocentric chromosomes in meiosis. I. Restriction of the number of chiasmata in bivalents. *Chromosome Research* 12: 733–739.
<https://doi.org/10.1023/B:CHRO.0000045797.74375.70>
- O’Meara BC, Beaulieu JM. 2016. Past, future, and present of state-dependent models of diversification. *American Journal of Botany* 103: 792–795.
- Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.
- Paradis E, Schliep K. 2018. APE 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35: 526–528.
- R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Reznicek AA. 1990. Evolution in sedges (*Carex*, Cyperaceae). *Canadian Journal of Botany* 68: 1409–1432.
- Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I. 2015. The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. *New Phytologist* 206: 19–26.
<https://doi.org/10.1111/nph.13191>
- Roalson EH, Columbus JT, Friar EA. 2001. Phylogenetic relationships in Cariceae (Cyperaceae) based on ITS (nrDNA) and trnT-L-F (cpDNA) region sequences:

assessment of subgeneric and sectional relationships in *Carex* with emphasis on section *Acrocystis*. *Systematic Botany* 26: 318–341.

Roalson EH, Friar EA. 2004. Phylogenetic relationships and biogeographic patterns in North American members of *Carex* section *Acrocystis* (Cyperaceae) using nrDNA ITS and ETS sequence data. *Plant Systematics and Evolution* 243: 175–187.

Roalson EH, Roberts WR. 2016. Distinct processes drive diversification in different clades of Gesneriaceae. *Systematic Biology* 65: 662–684.

Roalson EH. 2008. A synopsis of chromosome number variation in the Cyperaceae. *Botanical Review* 74: 209–393.

Roalson EH, Jiménez-Mejías P, Hipp AL, Benítez-Benítez C, Bruederle LP, Chung K-S, Escudero M, Ford BA, Ford K, Gebauer S, Gehrke B, Hahn M, Hayat MQ, Hoffmann MH, Jin X-F, Kim S, Larridon I, Lévillé-Bourret É, Lu Y-F, Luceño M, Maguilla E, Márquez-Corro JI, Martín-Bravo S, Naczi RFC, Reznicek AA, Spalink D, Starr JR, Uzma, Villaverde T, Waterway MJ, Wilson KL, Zhang S-R. Under review. A framework infrageneric classification of *Carex* (Cyperaceae) and its organizing principles. *Journal of Systematics and Evolution* XX: XX–XX.
<https://doi.org/10.1111/jse.12722>

Ritz C, Spiess AN. 2008. qPCR: an R package for sigmoidal model selection in quantitative real-time polymerase chain reaction analysis. *Bioinformatics* 24: 1549–1551. <https://doi.org/10.1093/bioinformatics/btn227>

Schliep KP. 2011. PHANGORN: phylogenetic analysis in R. *Bioinformatics* 27: 592–593. <https://doi.org/10.1111/2041-210X.12760>

Spalink D, Pender J, Escudero M, Hipp AL, Roalson EH, Starr JR, Waterway MJ, Bohs L, Sytsma KJ. 2019. The spatial structure of phylogenetic and functional diversity in the United States and Canada: An example using the sedge family (Cyperaceae).

- Journal of Systematics and Evolution* 56: 449–465.
<https://doi.org/10.1111/jse.12423>
- Tanaka N. 1949. Chromosome studies in the genus *Carex* with special reference to aneuploidy and polyploidy. *Cytologia* 15: 15–29.
- Tang Y-C, Xiang Q-Y. 1989. Cytological studies of *Carex siderosticta* Hance (Cyperaceae) and its importance in phytogeography. *Cathaya* 1: 49–60.
- Uyeda JC, Harmon LK. 2014. A novel Bayesian method for inferring and interpreting the dynamics of adaptive landscapes from phylogenetic comparative data. *Systematic Biology* 63: 902–918. <https://doi.org/10.1093/sysbio/syu057>
- Uzma, Jiménez-Mejías P, Amir R, Hayat MQ, Hipp AL. 2019. Timing and ecological priority shaped the diversification of sedges in the Himalayas. *PeerJ* 7: e6792.
- Vershina AO, Lukhtanov VA. 2017. Evolutionary mechanisms of runaway chromosome number change in *Agrodiaetus* butterflies. *Scientific Reports* 7: 8199. <https://doi.org/10.1038/s41598-017-08525-6>
- Villaverde T, Escudero M, Luceño M, Martín-Bravo S. 2015a. Long-distance dispersal during the middle-late Pleistocene explains the bipolar disjunction of *Carex maritima* (Cyperaceae). *Journal of Biogeography* 42: 1820–1831.
- Villaverde T, Escudero M, Martín-Bravo S, Bruederle LP, Luceño M, Starr JR. 2015b. Direct long-distance dispersal best explains the bipolar distribution of *Carex arctogena* (*Carex* sect. *Capituligerae*, Cyperaceae). *Journal of Biogeography* 42: 1514–1525.
- Villaverde T, Escudero M, Martín-Bravo S, Jiménez-Mejías P, Sanmartín I, Vargas P, Luceño M. 2017a. Bipolar distributions in vascular plants: a review. *American Journal of Botany* 104: 1680–1694. <https://doi.org/10.3732/ajb.1700159>.

- Villaverde T, González-Moreno P, Rodríguez-Sánchez F, Escudero M. 2017b. Niche shifts after long-distance dispersal events in bipolar sedges (*Carex*, Cyperaceae). *American Journal of Botany* 104: 1765–1774. <https://doi.org/10.3732/ajb.1700171>
- Villaverde T, Jiménez-Mejías P, Luceño M, Waterway MJ, Kim S, Lee B, Rincón-Barrado M, Hahn M, Maguilla E, Roalson EH, Hipp AL, the Global *Carex* Group. 2020. A new classification of *Carex* subgenera supported by a HybSeq backbone phylogeny. *Botanical Journal of the Linnean Society* 194: 141–163. <https://doi.org/10.1093/botlinnean/boaa042>
- WCSP. 2020. World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. Available from <http://wcsp.science.kew.org/> [accessed 14 December 2019].
- Wahl HA. 1940. Chromosome numbers and meiosis in the genus *Carex*. *American Journal of Botany* 27: 458–470.
- Wang S, Veller C, Sun F, Ruiz-Herrera A, Shang Y, Liu H, Zickler D, Chen Z, Kleckner N, Zhang L. 2019. Per-nucleus crossover covariation and implications for evolution. *Cell* 177: 326–338.e16. <https://doi.org/10.1016/j.cell.2019.02.021>
- Waterway MJ, Starr JR. 2007. Phylogenetics relationships in tribe Cariceae (Cyperaceae) based on nested analyses of four molecular data sets. *Aliso* 23:165–192.
- Waterway MJ, Hoshino T, Masaki T. 2009. Phylogeny, species richness, and ecological specialization in Cyperaceae tribe Cariceae. *Botanical Review* 75: 138–159.
- Whitkus R. 1988. Experimental hybridization among chromosome races of *Carex pachystachya* and the related species *Carex macloviana* and *Carex preslii* (Cyperaceae). *Systematic Botany* 13: 146–153.

Figures and tables

Fig. 1. Phylogenetic tree of the *Carex* taxa used in the analysis. Right side of the figure shows the diploid chromosome number for each taxon. Density distribution plots are shown above and along the right side of the plot, depicting the distribution of chromosome number in the genus and the sample size (number of chromosome number counts) per taxon, respectively. The grey band shows the range between 58 and 60 chromosomes.

Fig. 2. Chromosome number variation among *Carex* subgenera for the taxa studied in this work. Each count is included once per taxon. Minimum and maximum diploid values are displayed for the subgenera. Grey bars represent the cumulative chromosome number reports for the genus, and colored bars correspond to the observed numbers in each subgenus.

Fig. 3. Best-supported models of the relationship between chromosome number and speciation (blue), extinction (orange) and diversification (dashed line) rates in *Carex*, as inferred from QuaSSE analysis. Ornstein-Uhlenbeck (OU) and Brownian motion (BM) models are represented with dark and faint color lines, respectively. Diversification for the best-supported OU model is depicted in a dashed line.

Fig. 4. The left side depicts the log-likelihood space in function of phylogenetic signals ($t_{1/2}$) and stationary variances (v_y) in different models, whereas in the right side are shown the different optimal (orange line) and phylogenetic (black line) regressions of the chromosome number on each model with the reduced dataset with 564 taxa, in black dots. Grey dots represent taxa that were not used for the SLOUCH analysis. **A**, Phylogenetic effects in chromosome number based on a model with mean temperature of driest quarter (BIO9, °C). **B**, Phylogenetic effects in chromosome number based on a

model including culm length (cm). **C**, Phylogenetic effects in chromosome number based on a model with terminal inflorescence length (mm).

Fig. 5. Mean value and detected shifts for the different variables as inferred throughout the phylogeny: **A**, 2n chromosome number; **B**, culm length; **C**, lateral inflorescence length; **D**, temperature annual range (BIO7). Subgenera appear in capitals letters in their corresponding ancestral branch (Psy: *Psyllophorae*; Eut: *Euthyceras*). Inferred values for each variable are represented with coloured branches according to the colour legend. Circles indicate shifts in the equilibrium of the corresponding variable, with circle size proportional to the posterior probability inferred for that shift. Some clades for which moderately to highly supported shifts in the trait value equilibrium were detected are indicated with grey arches in the tips.

Table 1. Number of optima (θ) detected in bayou by ranges of posterior probability. Note that taxa and error estimation used in the analysis differ among some of the variables (see Materials and Methods section). Therefore, no comparison a priori can be made between chromosomal, morphological and bioclimatic variables.

	Mean	HPD95%	[0.10–0.30)	[0.30–0.50)	[0.50–0.70)	[0.70–1.00]
Chromosome number	101	85–116	83	30	15	21
Culm length	48	37–58	49	6	4	8
Lateral inflorescence length	98	84–114	124	32	17	32
Annual mean temperature (BIO1)	36	24–47	-	-	-	-

Temperature seasonality (BIO4)	37	25–48	-	-	-	-
Temperature annual range (BIO7)	37	26–48	1	1	-	-
Annual precipitation (BIO12)	37	24–47	-	-	-	-

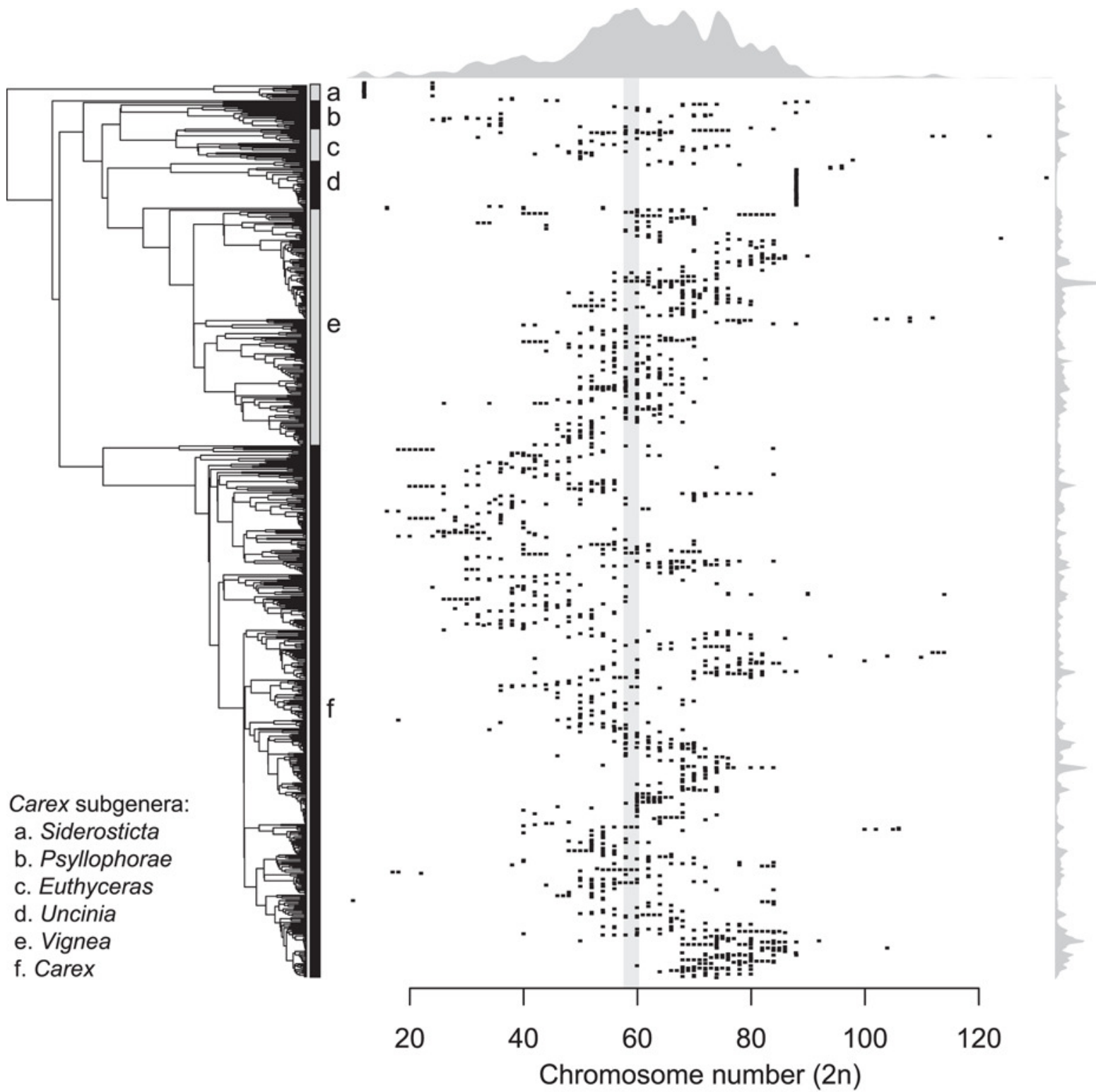
Table 2. Results for the linear modelling of continuous predictors. Models with AIC scores within two units of difference, ordered by values with exception of BM and single-equilibrium OU models, in the two firsts rows. Phylogenetic half-life ($t_{1/2}$, millions of years), stationary variance (v_y , squared chromosome number), intercept and standard error (diploid chromosome number value), slope and standard error (units specified below the table), and R-squared (R^2 in %) of the phylogenetic regression.

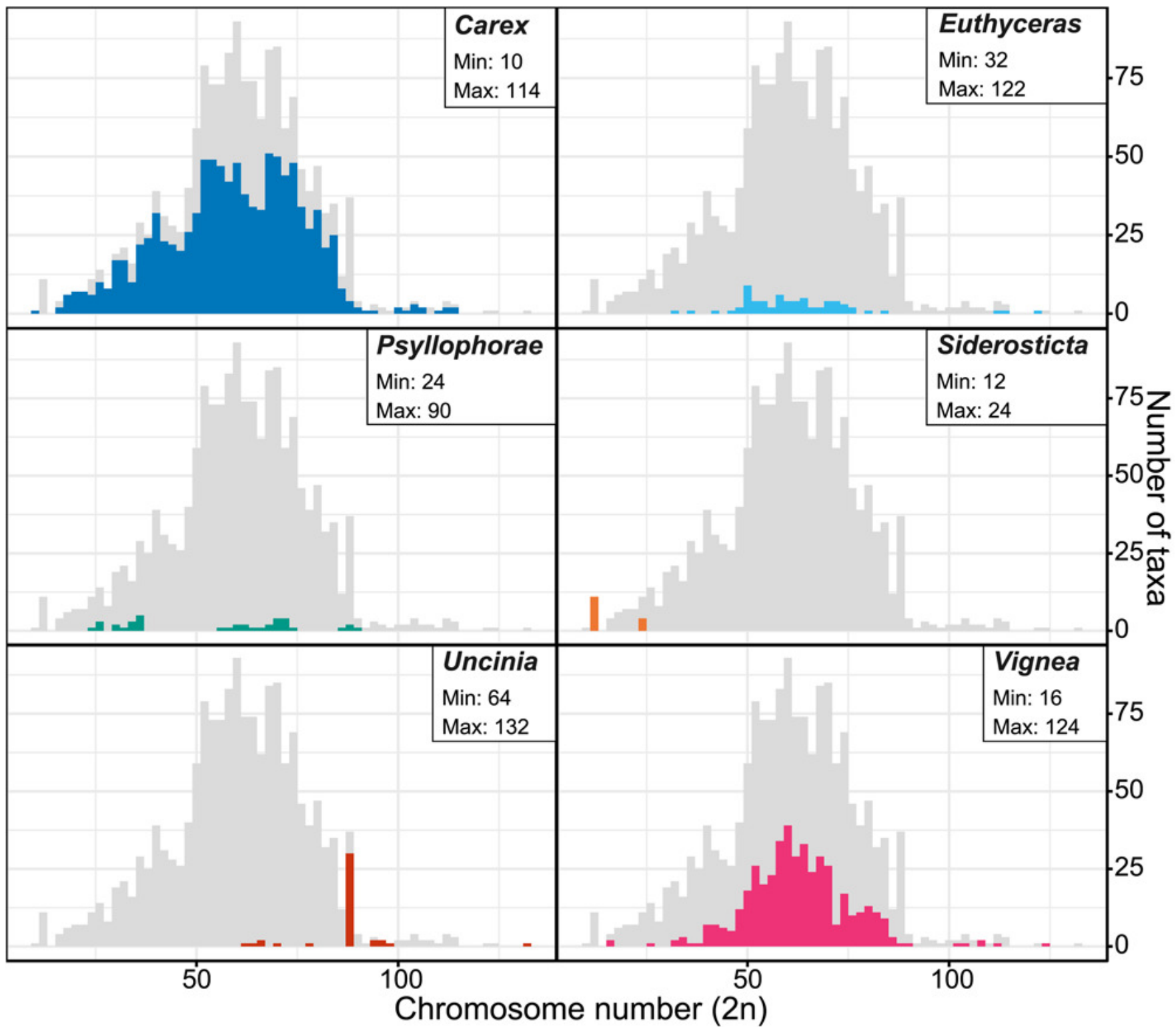
Model	AIC	$t_{1/2}$	v_y	Intercept (\pm SE)	Slope (\pm SE)	R^2 (%)
2n ~ 1 (BM)	4203.458	-	-	43.292 \pm 14.961	-	0.000
2n ~ 1 (OU)	4190.336	13.51 (9.05–23.92)	362.54	50.903 \pm 5.265	-	0.000
2n ~ culm length + mean temperature of driest quarter (BIO9)	4189.569	13.93 (9.05–25.27)	358.57	51.121 \pm 5.530	Culm: -0.061 \pm 0.039 [†] BIO9: 0.148 \pm 0.090 [§]	0.791
2n ~ terminal inflorescence length (TI length) + BIO9	4189.970	14.07 (9.05–26.08)	353.41	50.532 \pm 5.628	TI: -0.081 \pm 0.057 [†] BIO9: 0.144 \pm 0.090 [§]	0.709
2n ~ BIO9	4190.182	13.64 (9.05–24.26)	362.51	50.554 \pm 5.301	0.124 \pm 0.088 [§]	0.353
2n ~ culm length	4190.530	13.61 (9.05–24.05)	363.34	51.667 \pm 5.352	-0.051 \pm 0.038 [‡]	0.316
2n ~ culm length + mean temperature of	4190.644	13.86 (9.05–24.86)	359.20	51.487 \pm 5.506	Culm: -0.062 \pm 0.039 [†]	0.612

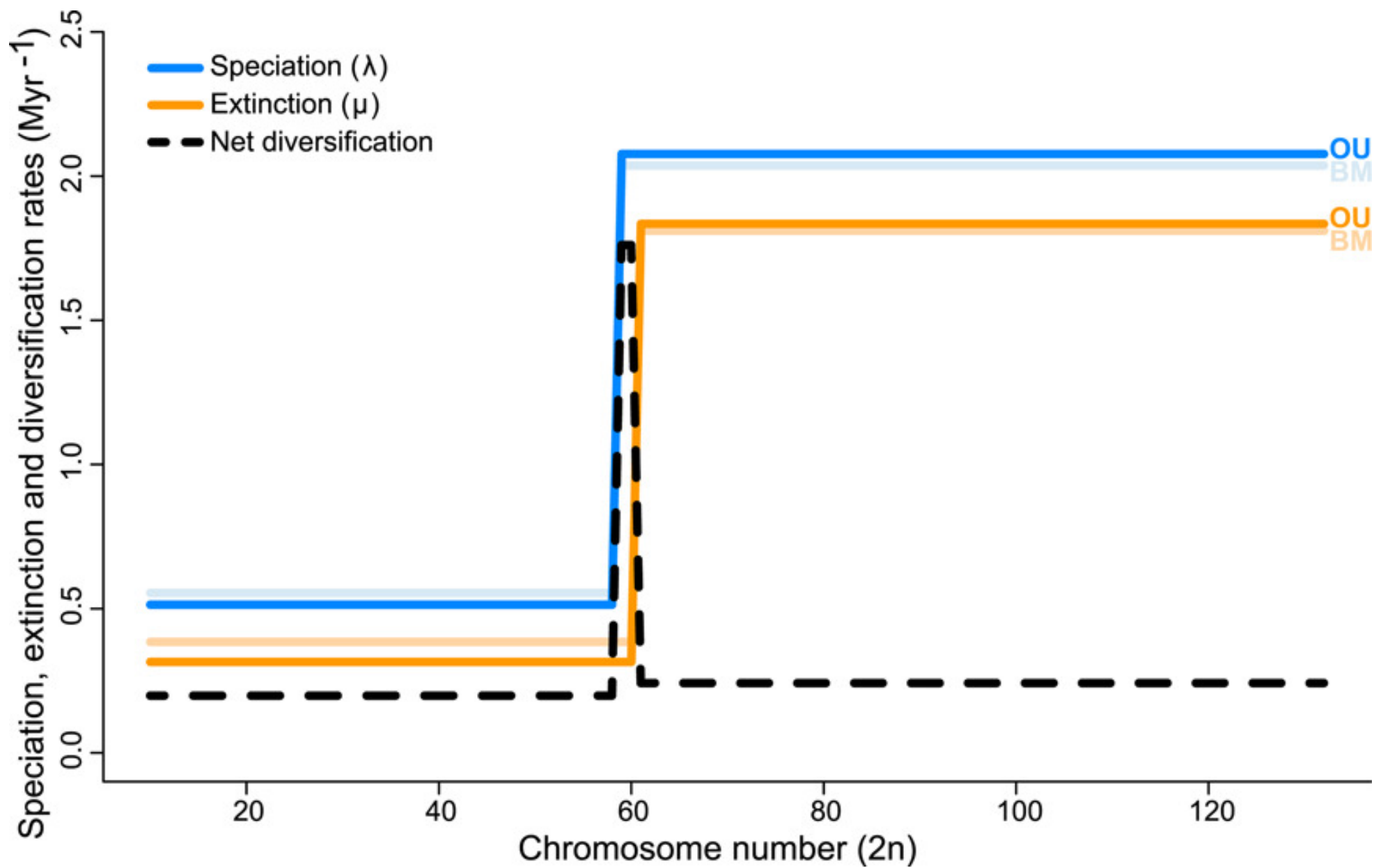
coldest quarter (BIO11)					BIO11: $0.141 \pm 0.108^{\S}$	
2n ~ BIO9 + precipitation of the warmest quarter (BIO18)	4190.706	13.65 (9.05–24.46)	359.57	51.945 ± 5.457	BIO9: $0.128 \pm 0.0988^{\S}$ BIO18: $-0.006 \pm 0.005^{\dagger}$	0.584
2n ~ TI length	4190.867	13.61 (9.05–24.05)	362.89	51.402 ± 5.324	$-0.068 \pm 0.056^{\dagger}$	0.260
2n ~ lateral inflorescence length	4190.910	13.41 (9.05–24.05)	359.04	51.311 ± 5.246	$-0.061 \pm 0.051^{\dagger}$	0.252
2n ~ culm length + BIO18	4191.010	14.11 (9.05–26.49)	347.10	52.042 ± 5.903	Culm: $-0.050 \pm 0.039^{\dagger}$ BIO18: $-0.006 \pm 0.005^{\dagger}$	0.498
2n ~ mean temperature of wettest quarter (BIO8)	4191.017	13.42 (8.72–24.26)	358.68	52.270 ± 5.360	$-0.167 \pm 0.150^{\S}$	0.221
2n ~ TI length + BIO18	4191.047	14.59 (9.05–28.92)	335.65	51.031 ± 6.196	TI: $-0.067 \pm 0.058^{\dagger}$ BIO18: $-0.006 \pm 0.006^{\dagger}$	0.431
2n ~ TI length + BIO11	4191.081	13.98 (9.05–25.68)	354.64	50.910 ± 5.586	TI: $-0.081 \pm 0.057^{\dagger}$ BIO11: $0.132 \pm 0.108^{\S}$	0.523
2n ~ BIO18	4191.086	13.47 (9.05–24.26)	360.18	52.289 ± 5.399	$-0.006 \pm 0.005^{\dagger}$	0.211
2n ~ BIO8 + BIO11	4191.095	13.51 (9.05–24.46)	356.73	52.581 ± 5.390	BIO8: $-0.219 \pm 0.155^{\S}$ BIO11: $0.141 \pm 0.108^{\S}$	0.521
2n ~ BIO8 + BIO9	4191.145	13.54 (9.05–24.46)	358.81	51.818 ± 5.397	BIO8: $-0.152 \pm 0.151^{\S}$ BIO9: $0.117 \pm 0.088^{\S}$	0.532
2n ~ BIO11 + BIO18	4191.155	13.56 (9.05–24.46)	358.84	52.663 ± 5.429	BIO11: $0.143 \pm 0.108^{\S}$ BIO18: $-0.008 \pm 0.006^{\dagger}$	0.517
2n ~ BIO11	4191.334	13.57 (9.05–24.26)	362.53	50.843 ± 5.282	$0.102 \pm 0.105^{\S}$	0.168
2n ~ maximum temperature of warmest month (BIO5)	4191.487	13.73 (9.05–24.26)	366.97	48.513 ± 5.915	$0.177 \pm 0.196^{\S}$	0.144
2n ~ minimum temperature of coldest month (BIO6)	4191.497	13.56 (9.05–24.26)	362.32	51.104 ± 5.283	$0.089 \pm 0.100^{\S}$	0.141

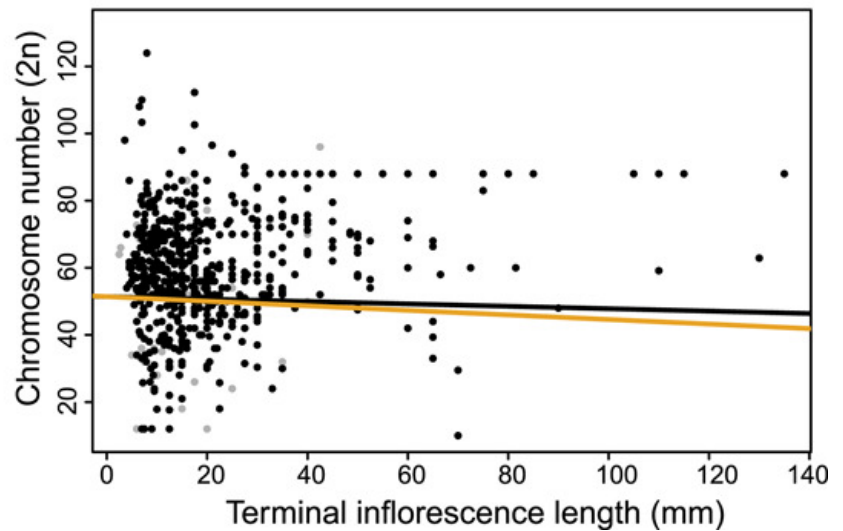
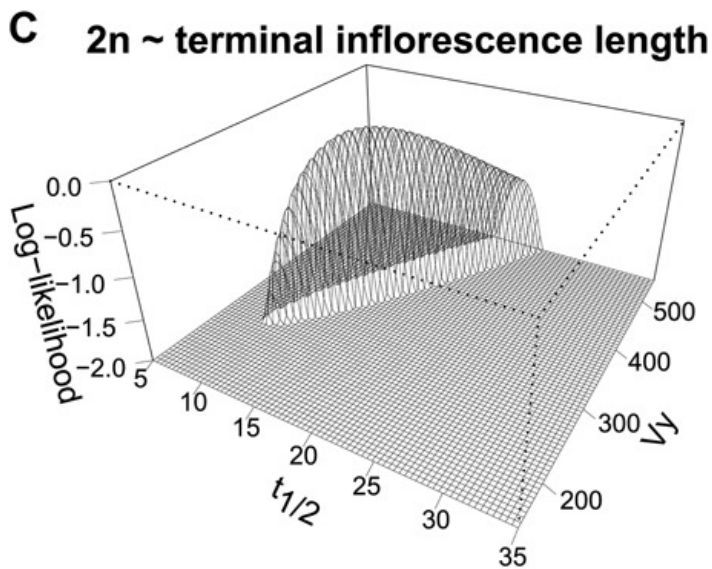
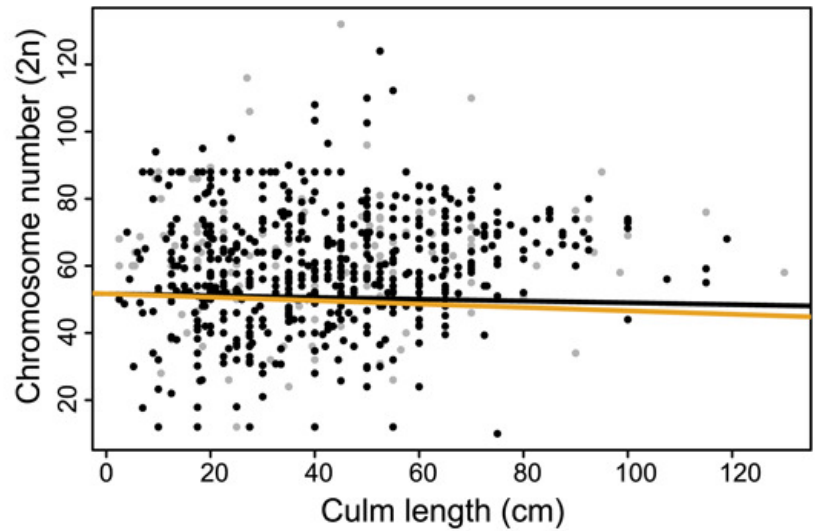
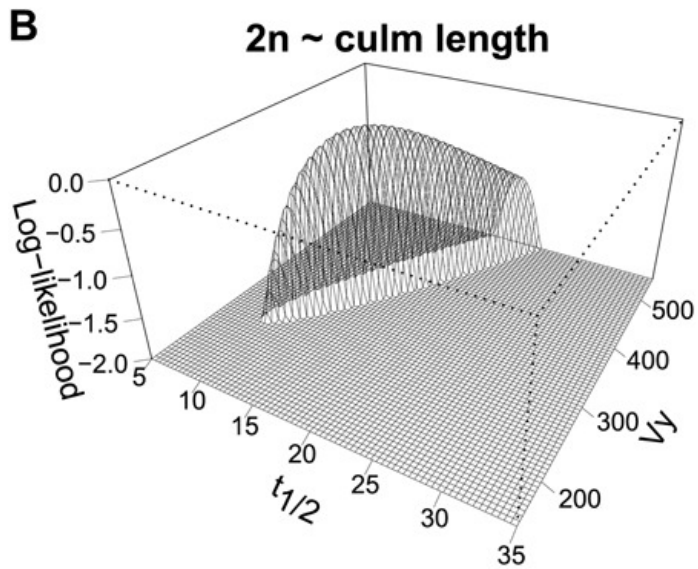
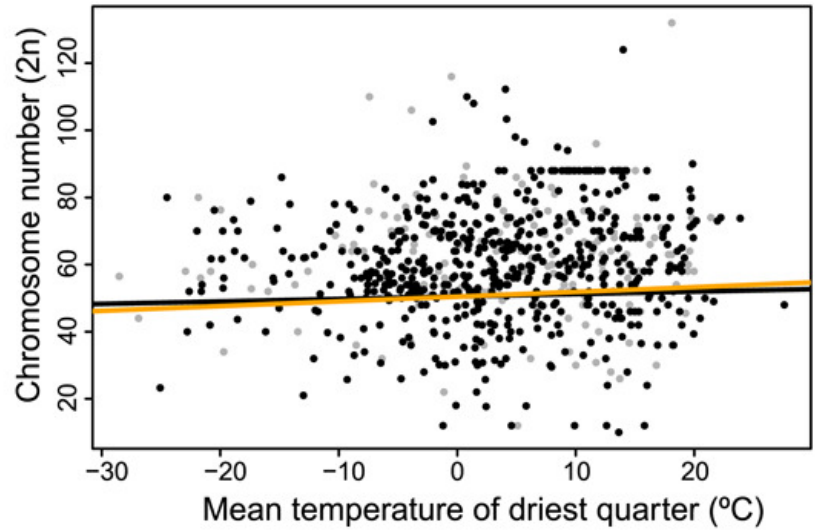
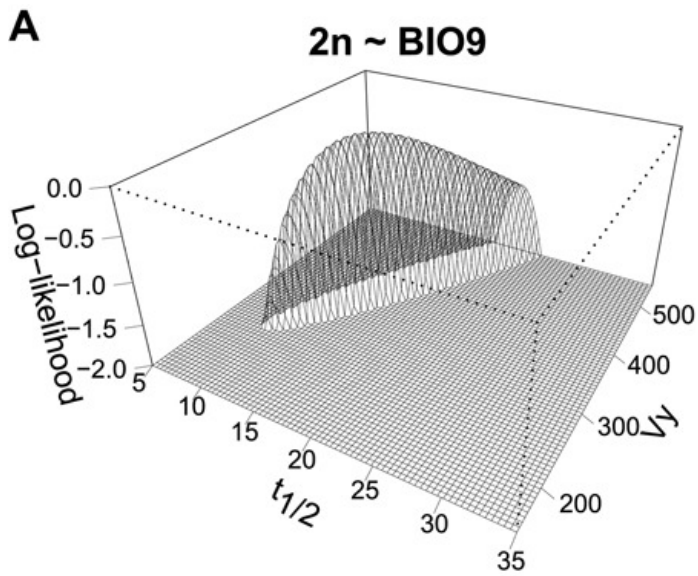
2n ~ mean diurnal range (BIO2)	4191.531	13.51 (9.05–23.92)	362.51	49.025 ± 5.684	0.383 ± 0.437 [§]	0.136
2n ~ annual mean temperature (BIO1)	4191.562	13.63 (9.05–24.26)	364.38	50.169 ± 5.363	0.132 ± 0.153 [§]	0.131

Slope in units of chromosome number per: [†]millimeter, [‡]centimeter, or [§]degrees Celsius.

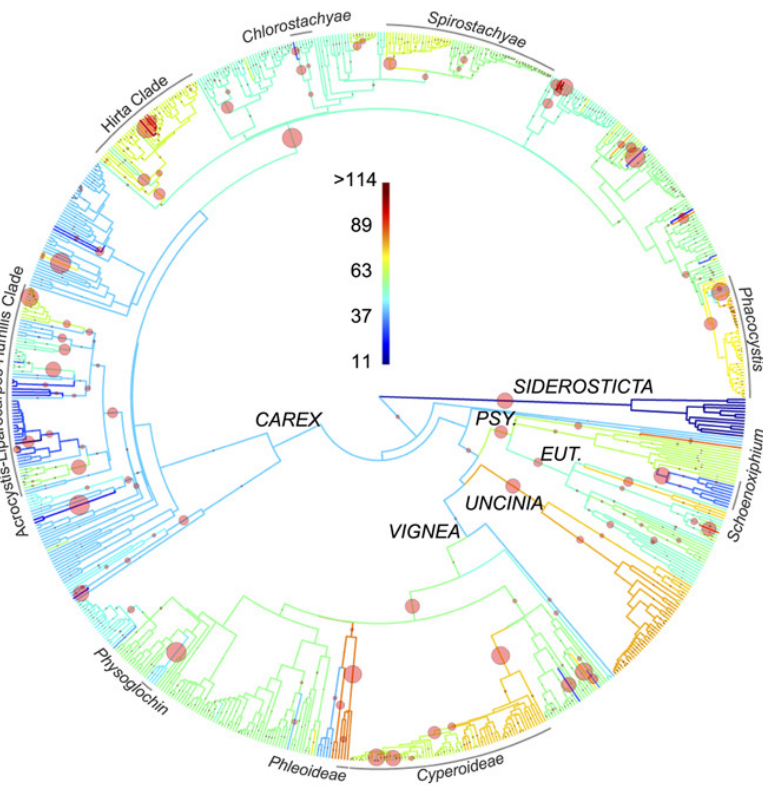




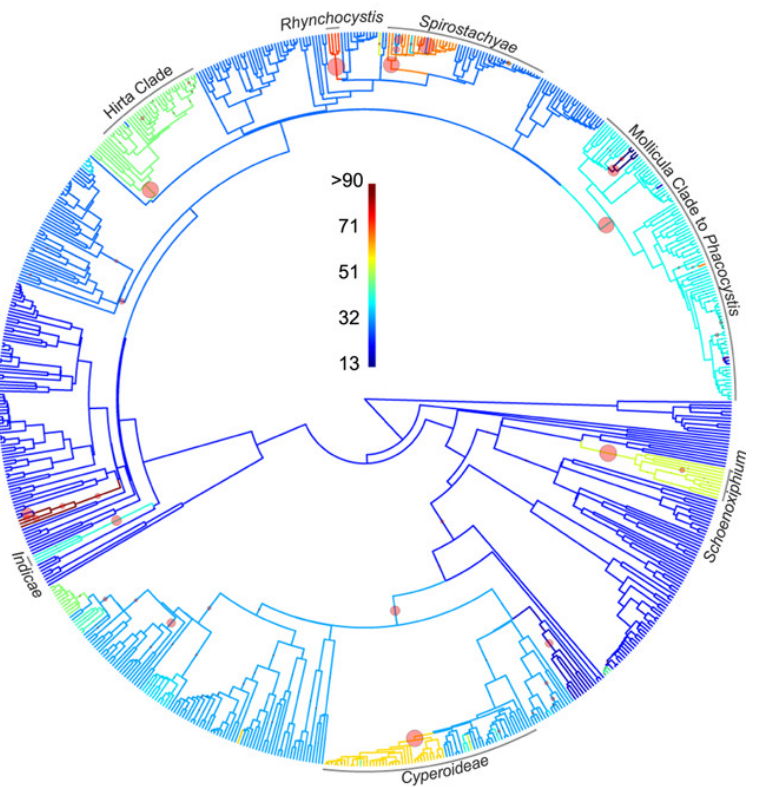




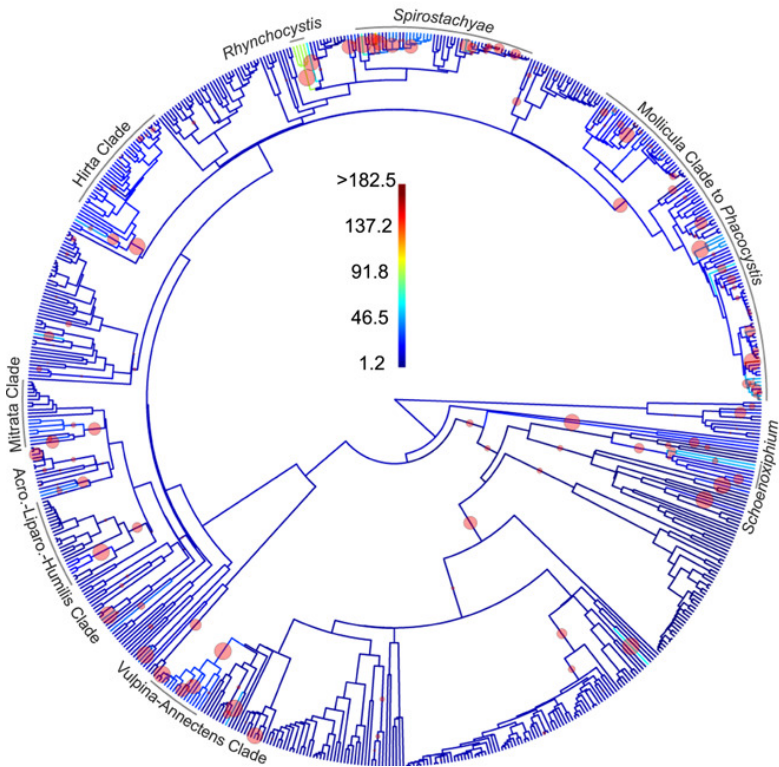
A Diploid chromosome number



B Culm length (cm)



C Lateral inflorescence length (mm)



D Temperature Annual Range (°C)

