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Research Article

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

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Abstract Changes in holocentric chromosome number due to fission and fusion 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. The genus exhibits a phenomenal range in the chromosome number ($2n = 10 - 132$) with almost not polyploidy. 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 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 toward 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: BAYOU, bioclimatic variables, Brownian motion, diversification, morphological characters, Ornstein–Uhlenbeck model, Phylogenetic Comparative Methods, QuaSSE, SLOUCH.

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., 2021; 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 after establishment in new regions, and still others with shifting dynamics in the 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 are 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 onset of reproductive isolation (Whitkus, 1988; Escudero et al., 2016a). 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, 2021). 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., 2019b). 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., 2016b; Márquez-Corro et al., 2018, 2019a). Due to the nonspatial 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 the 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 have led to a high karyotype variation among species and among populations within species in *Carex* (e.g., Wahl, 1940; Tanaka, 1949; Naczi, 1999; Roalson, 2008). As holokinetic chromosomes present kinetochore activity along the whole chromosome, chromosome fragments arising from fission and fusion events have the potential to segregate in a Mendelian fashion (Escudero et al., 2014). Chromosome fission and fusion dominate the exclusion of ploidy changes throughout *Carex* (Márquez-Corro et al., 2019b), with few but very notable exceptions. The most striking of these counter-examples 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., 2021) and species (*Carex dolichostachya*, *C. jackiana*, *C. roraimensis*; Hipp et al., 2006, 2009) within 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., 2019b).

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 crossover per homologous chromosome on average. Consequently, the chromosome number is probably a reasonable proxy for the 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 have little risk 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 a 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 a relationship between proxies of competitiveness and environmental stability on the one hand and chromosome number as a proxy for recombination rate on the other hand. Although chromosome number change may favor 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 regional 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 to date (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 the 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 the macroevolutionary success of 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 before analysis chromosome counts that we considered unreliable (i.e., Löve & Löve's counts; Elven, 2020). Taxa for which there is 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 & Schliep, 2018) and rendered ultrametric with the function "nnls.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 the number of counts that were published, and as only 3% (22 tips) had 20 or more chromosome number reports, we use the average variance

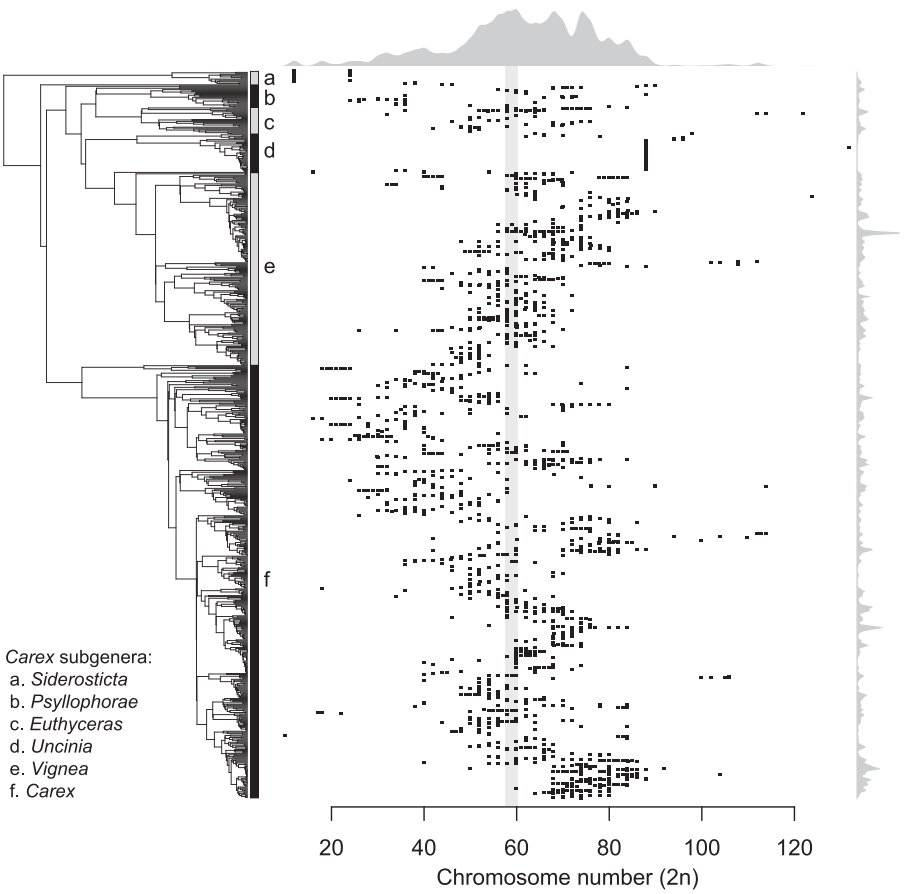

Fig. 1. The phylogenetic tree of the *Carex* taxa used in the analysis. The right side of the figure shows the diploid chromosome number for each taxon. Density distribution plots are shown above and along the right side of the plot, depicting the distribution of chromosome number in the genus and the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

of all the species variance divided by the sample size (weighted standard error of the mean, SEM^2_w). This procedure is recommended in Labra et al. (2009) and Hansen & Bartoszek (2012) for data sets 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, 2007; Escudero et al., 2010, 2012a; Chung et al., 2012; Carta et al., 2018). Although 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 the 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 (BM) model, in which chromosome evolution is modeled approximately as a random walk; and (ii) BM 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 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 on the basis of 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 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 the chromosome number (Wahl, 1940; Hipp, 2007; Escudero et al., 2010). The number of detected optima appears in Table 1.

2.3 Morphological and ecological predictors of chromosome number

To test effects of selection on the chromosome number, we used the "slouch.fit" function in the SLOUCH package v2.1.4 (Kopperud et al., 2019) to fit a stochastic linear OU model for chromosome number evolution with morphological and climatic predictors. Under this model, the 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*, the 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 with 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 be selected 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 SEM^2. To reduce error in SEM^2 estimation for phylogenetic regression analyses, SEM^2 for species with

Table 1 The number of optima (θ) detected in bayou by ranges of posterior probability

	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	–	–	–	–

It should be noted that taxa and error estimation used in the analysis differ among some of the variables (see Section 2). Therefore, no comparison a priori can be made between chromosomal, morphological, and bioclimatic variables.

<20 samples was estimated as SEM^2w, 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;

Table 2 Results for the linear modeling of continuous predictors. Models with AIC scores within two units of difference appear in bold, 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^2 value (R^2 in %) of the phylogenetic regression

Model	AIC	$t_{1/2}$	v_y	Intercept (±SE)	Slope (±SE)	R^2 (%)
2n ~ 1 (BM)	4203.458	–	–	43.292 ± 14.961	–	0.000
2n ~ 1 (OU)	4190.336	13.51 (9.05–23.92)	362.54	50.903 ± 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 ± 5.530	Culm: −0.061 ± 0.039‡ BIO9: 0.148 ± 0.090§	0.791
2n ~ terminal inflorescence length (TI length) + BIO9	**4189.970**	14.07 (9.05–26.08)	353.41	50.532 ± 5.628	TI: −0.081 ± 0.057† BIO9: 0.144 ± 0.090§	0.709
2n ~ BIO9	**4190.182**	13.64 (9.05–24.26)	362.51	50.554 ± 5.301	0.124 ± 0.088§	0.353
2n ~ culm length	**4190.530**	13.61 (9.05–24.05)	363.34	51.667 ± 5.352	−0.051 ± 0.038‡	0.316
2n ~ culm length + mean temperature of coldest quarter (BIO11)	4190.644	13.86 (9.05–24.86)	359.20	51.487 ± 5.506	Culm: −0.062 ± 0.039‡ BIO11: 0.141 ± 0.108§	0.612
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 ± 0.0988§ BIO18: −0.006 ± 0.005	0.584
2n ~ TI length	4190.867	13.61 (9.05–24.05)	362.89	51.402 ± 5.324	−0.068 ± 0.056†	0.260
2n ~ lateral inflorescence length	4190.910	13.41 (9.05–24.05)	359.04	51.311 ± 5.246	−0.061 ± 0.051†	0.252
2n ~ culm length + BIO18	4191.010	14.11 (9.05–26.49)	347.10	52.042 ± 5.903	Culm: −0.050 ± 0.039‡ BIO18: −0.006 ± 0.005†	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 ± 0.150§	0.221
2n ~ TI length + BIO18	4191.047	14.59 (9.05–28.92)	335.65	51.031 ± 6.196	TI: −0.067 ± 0.058† BIO18: −0.006 ± 0.006†	0.431
2n ~ TI length + BIO11	4191.081	13.98 (9.05–25.68)	354.64	50.910 ± 5.586	TI: −0.081 ± 0.057† BIO11: 0.132 ± 0.108§	0.523
2n ~ BIO18	4191.086	13.47 (9.05–24.26)	360.18	52.289 ± 5.399	−0.006 ± 0.005†	0.211
2n ~ BIO8 + BIO11	4191.095	13.51 (9.05–24.46)	356.73	52.581 ± 5.390	BIO8: −0.219 ± 0.155§ BIO11: 0.141 ± 0.108§	0.521
2n ~ BIO8 + BIO9	4191.145	13.54 (9.05–24.46)	358.81	51.818 ± 5.397	BIO8: −0.152 ± 0.151§ BIO9: 0.117 ± 0.088§	0.532
2n ~ BIO11 + BIO18	4191.155	13.56 (9.05–24.46)	358.84	52.663 ± 5.429	BIO11: 0.143 ± 0.108§ BIO18: −0.008 ± 0.006†	0.517
2n ~ BIO11	4191.334	13.57 (9.05–24.26)	362.53	50.843 ± 5.282	0.102 ± 0.105§	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 ± 0.196§	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 ± 0.100§	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, and §degree celsius.
AIC, Akaike information criterion; BM, Brownian motion; OU, Ornstein–Uhlenbeck.

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 data set 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, and 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 the 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*, as 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 the analysis.

3 Results

3.1 Chromosome number and 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) contain 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 at 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% confidence interval [CI]: 0.67–0.81]) was significantly better supported than the BM model (ΔAIC = 168.09, data S2 in Márquez-Corro, 2020). The 95% CI for λ excludes 0.0 and 1.0, demonstrating that the 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.

The reversible jump MCMC analysis of OU models in BAYOU recovered a post-burn-in mean of 100 [HPD95%: 84–115] shifts in the chromosome number, of which 21 were highly supported (pp > 0.7; table 1, data S3 in Márquez-Corro, 2020). The posterior rate of adaptation (α) 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 analyses strongly support models in which diversification rates are related to chromosome number by a positive sigmoidal speciation relationship, either under the OU process (AICw = 0.53) or the BM process (AICw = 0.46). The remaining (state-independent) diversification models collectively share an AICw of 0.01 (data S5 in Márquez-Corro, 2020). Although these results might be subject to hidden states within chromosome number strata (Beaulieu &

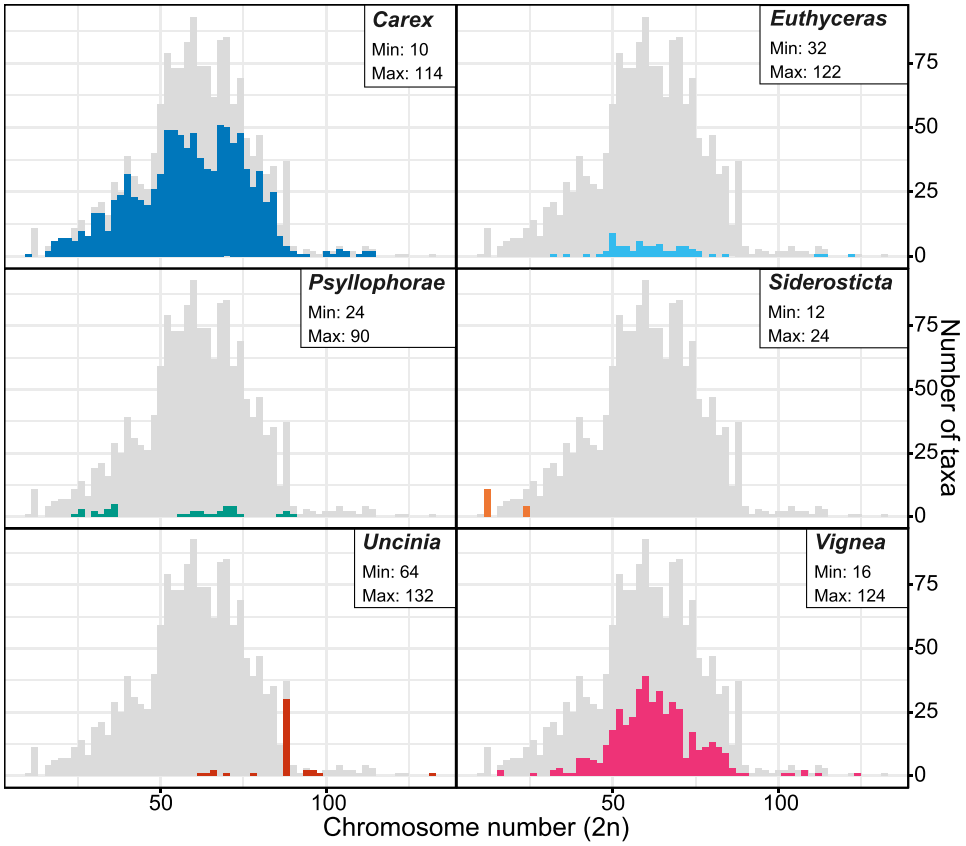

Fig. 2. Chromosome number variation among *Carex* subgenera for the taxa studied in this work. Each count is included once per taxon. Minimum and maximum diploid values are displayed for the subgenera. Gray bars represent the cumulative chromosome number reports for the genus and colored bars correspond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

O′Meara, 2016; O′Meara & 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 $2n = 58$, where speciation increases to 2.07 events/Myr. However, 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 was also the best-supported model 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 in 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 ($t_{1/2} = 0$, $\alpha \to \infty$) 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 a 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 is old enough to allow the traits to evolve toward 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 the chromosome number (Fig. 4; Table 2). The model with BIO9 alone does not present a much higher AIC weight value vs. the OU

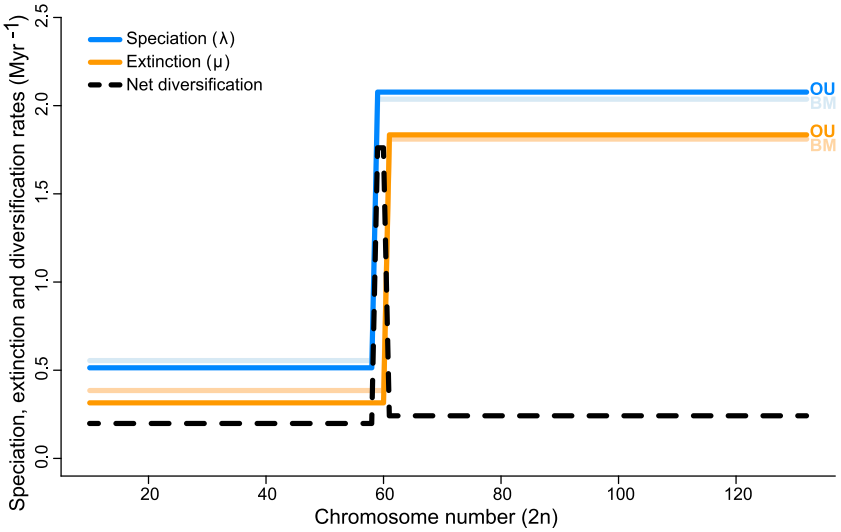

Fig. 3. Best-supported models of the relationship between chromosome number and speciation (blue), extinction (orange) and diversification (dashed line) rates in *Carex*, as inferred from the QuaSSE analysis. Ornstein–Uhlenbeck (OU) and Brownian motion (BM) models are represented with dark and faint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

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-predictor or multi-predictor models (table 2, data S4 in Márquez-Corro, 2020). The multi-optima analyses showed high rates of evolution toward the equilibrium in the 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 toward 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 the chromosome number, bioclimatic variables displayed mostly opposite patterns, with most of them showing lower rates toward 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, as we sampled about six times more taxa than 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 the 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*, *Physoglochin*, and *Cyperoideae*. On the other side, subgenus *Carex* is more variable, with multiple shifts occurring in different sections or lineages; for instance, 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

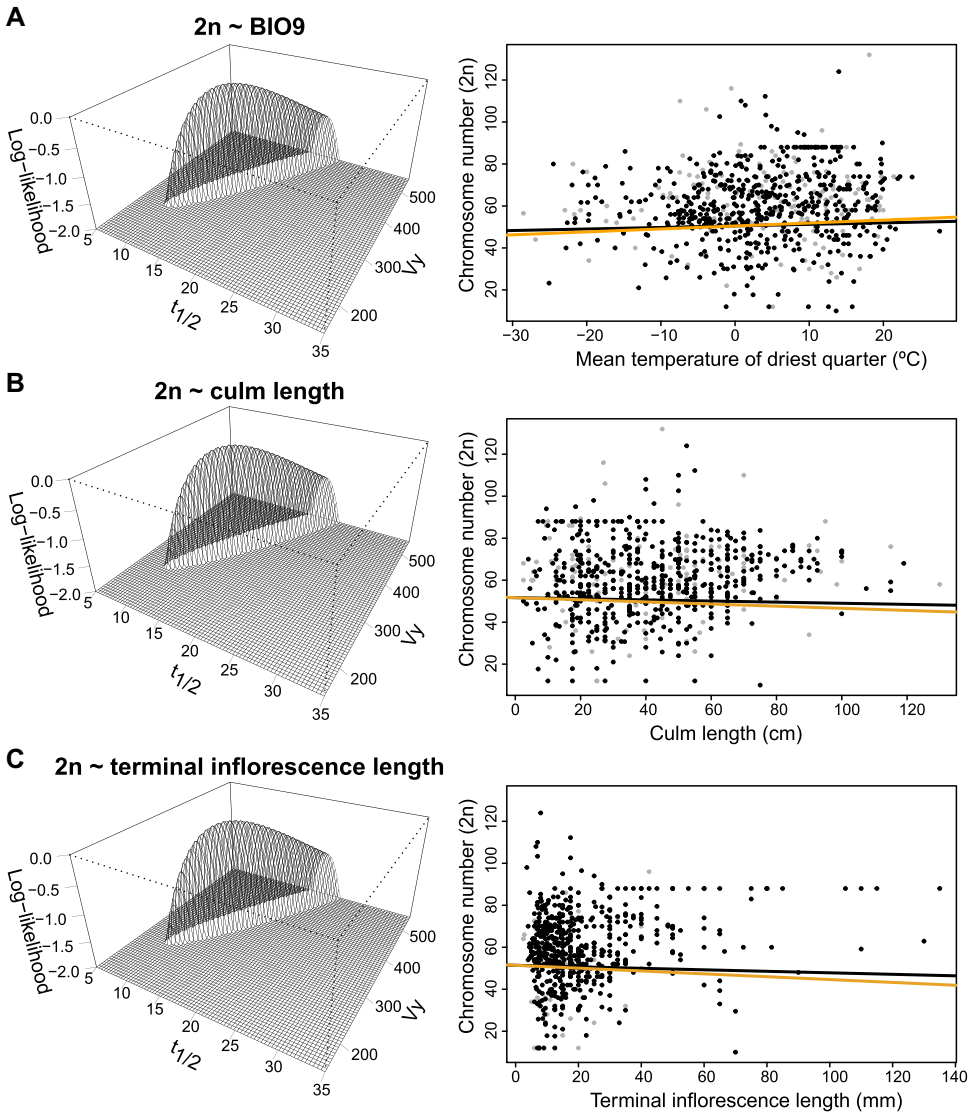

Fig. 4. The left side depicts the log-likelihood space in function of phylogenetic signals ($t_{1/2}$) and stationary variances (v_y) in different models, whereas the right side shows the different optimal (orange line) and phylogenetic (black line) regressions of the chromosome number on each model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

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 possibly 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 the chromosome number. The low predictive power of functional variation, perhaps a consequence of high diversity of lineage-specific life histories in our study organism, 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 the mean chromosome number detected 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., 2019b). Thus, although 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 the 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., 2019b; ancestral $2n = 28$ with $pp = 0.07$ and pp values above 0.02 in a range that included $2n = 12 - 50$). 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 using information from the taxa outside of *Carex*, whereas 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, as 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 (Martín-Bravo et al., 2019), echoes the transition in chromosome dynamics and diversification rates at the base of *Carex* itself (Escudero et al., 2012b). 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 the 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, 2021; Villaverde et al., 2017b). In addition, niche shifts in two *Carex* sister species have been suggested to be related to changes in non-bioclimatic 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., 2021). But the fact that many clades are geographically widespread (Martín-Bravo et al., 2019) may point toward 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 the chromosome number and ecological diversification shape diversification at shallower evolutionary scales (e.g., Hipp, 2007; Escudero et al., 2010, 2013), explaining the high regional and broad continental diversity of individual sedge clades. Morphological traits such as culm and lateral inflorescence lengths

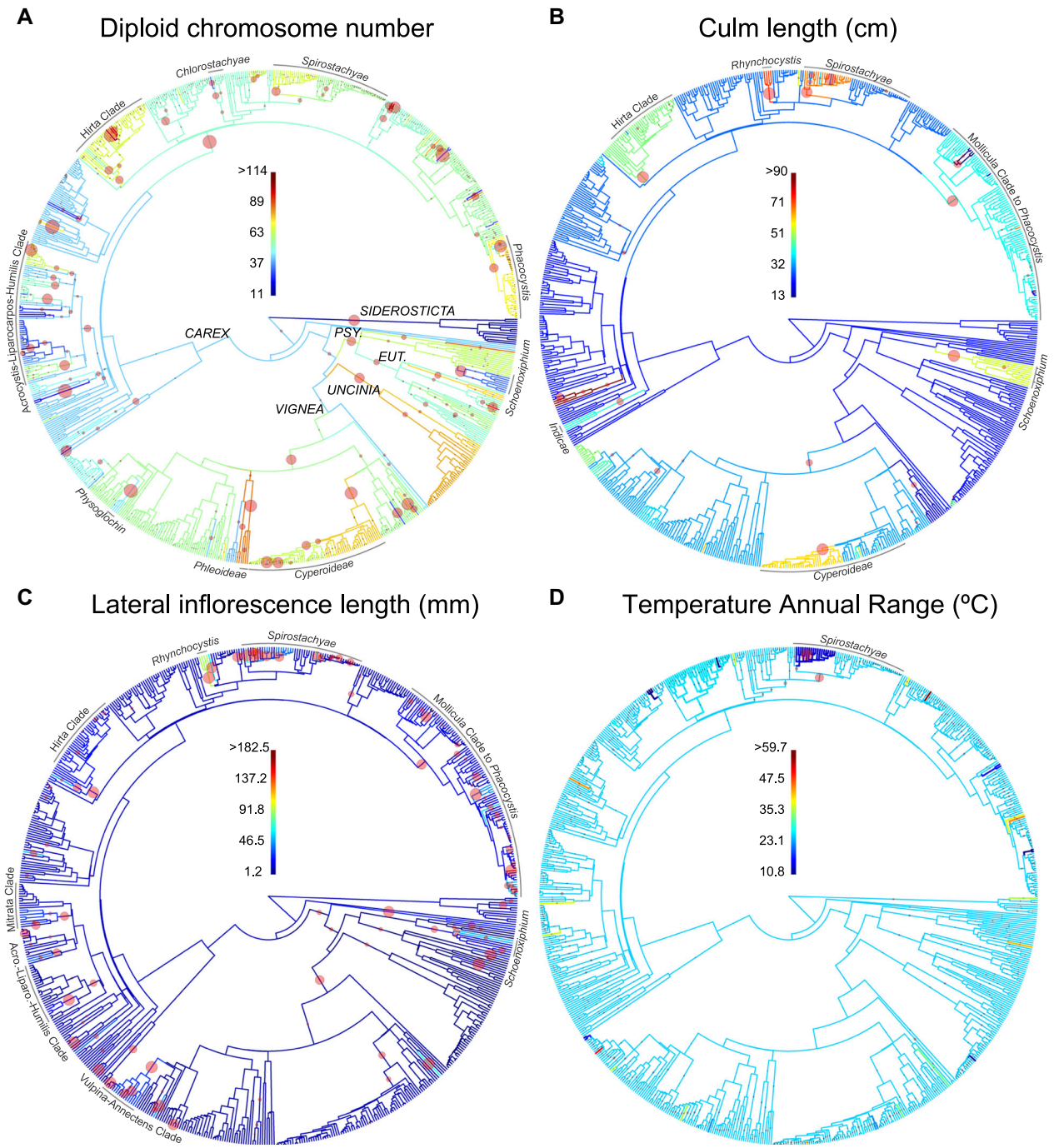

Fig. 5. Mean value and detected shifts for the different variables as inferred throughout the phylogeny: **A**, 2n chromosome number. **B**, culm length. **C**, lateral inflorescence length. **D**, temperature annual range (BIO7). Subgenera appear in capital letters in their corresponding ancestral branch (Psy: *Psyllophorae*; Eut: *Euthyceras*). Inferred values for each variable are represented with colored branches according to the color legend. Circles indicate shifts in the equilibrium of the corresponding variable, with circle size being 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 gray arches at the tips.

present a significantly greater number of shifts than bioclimatic variables (Table 1; Fig. 5). This fact and the geographic conservatism of many *Carex* clades (Roalson & Friar, 2004; Hipp et al., 2006; Dragon & Barrington, 2009) 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 a 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 the chromosome number (less than 1%), as 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 communities with a 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 a 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 (Martín-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 *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., 2021). Considering 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., 2021) 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 the chromosome number. However, some shifts were detected to have occurred shortly after or before the diversification shift. 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 nonclimatic niches points out the assumption that recombination rates, probably followed by adaptive innovation, indeed constitute a force in this genus diversification. However, to what extent this occurs, as well as the effect of the chromosomes and genome regions involved in the fusion or fission events, is yet to be clarified.

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 (Kükenthal, 1909; Egorova, 1999; 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 the one hand and chromosomal variation on the other hand—jointly shape the ecological and morphological variety that characterizes 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 already know that rapid evolution of the 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 a shallow 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, which will enable deeper investigations of how chromosome number evolves at a shallow scale and its relationship with morphological and ecological history. This is especially complicated 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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Conflict of Interest

The authors declare that there is no conflict of interest.

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