











Bursts of Rapid Diversification, Dispersals Out of Southern Africa, and Two Origins of Dioecy Punctuate the Evolution of *Asparagus*

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Abstract

The genus *Asparagus* arose ~9 to 15 million years ago (Ma), and transitions from hermaphroditism to dioecy (separate sexes) occurred ~3 to 4 Ma. Roughly 27% of extant *Asparagus* species are dioecious, while the remaining are bisexual with monoclinal flowers. As such, *Asparagus* is an ideal model taxon for studying the early stages of dioecy and sex chromosome evolution in plants. Until now, however, understanding of diversification and shifts from hermaphroditism to dioecy in *Asparagus* has been hampered by the lack of robust species tree estimates for the genus. In this study, a genus-wide phylogenomic analysis including 1,726 nuclear loci and comprehensive species sampling supports two independent origins of dioecy in *Asparagus*—first in a widely distributed Eurasian clade and then in a clade restricted to the Mediterranean Basin. Modeling of ancestral biogeography indicates that both dioecy origins were associated with range expansion out of southern Africa. Our findings also reveal several bursts of diversification across the phylogeny, including an initial radiation in southern Africa that gave rise to 12 major clades in the genus, and more recent radiations that have resulted in paraphyly and polyphyly among closely related species, as expected given active speciation processes. Lastly, we report that the geographic origin of domesticated garden asparagus (*Asparagus officinalis* L.) was likely in western Asia near the Mediterranean Sea. The presented phylogenomic framework for *Asparagus* is foundational for ongoing genomic investigations of diversification and functional trait evolution in the genus and contributes to its utility for understanding the origin and early evolution of dioecy and sex chromosomes.

Key words: Asparagaceae, biogeography, budding species, dioecious, phylogenomics.

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Significance

Asparagus is an important model system for studying dioecy (separate sexes) evolution in plants. *Asparagus* taxonomy has proved to be challenging, likely due to rapid species diversifications leading to highly variable species with complicated relationships that are impossible to resolve with limited DNA sequence data. Using phylogenomics and the largest species sampling to date, we show that all *Asparagus* lineages originated from an initial radiation in southern Africa and that separate range expansions out of southern Africa set the stage for two distinct origins of dioecy in *Asparagus*. Our findings provide a deeper understanding of species diversification and the role of long-distance dispersals in the evolution of dioecy. This study also illustrates the utility of phylogenomics for elucidating past and present speciation processes.

Introduction

The genus *Asparagus* Tourn. ex L. encompasses over 215 species and can be found in many different habitats spanning nearly all of Africa and Eurasia (POWO 2024). The most well-known species in the genus is garden asparagus (*Asparagus officinalis* L.), which is an important vegetable crop cultivated across the globe (Kanno and Yokoyama 2011) and a model system for studying dioecy and sex chromosome evolution in angiosperms (Harkess et al. 2015, 2016, 2017, 2020; Charlesworth 2019; Andreuzza 2020; Carey et al. 2021; Charlesworth and Harkess 2024). *Asparagus* belongs to the Asparagoideae subfamily of Asparagaceae, along with one other genus—*Hemiphylacus* S. Watson (Chase et al. 2009). The biodiversity hotspot for *Asparagus* is southern Africa but is distributed across the eastern hemisphere (Norup et al. 2015), whereas its sister genus, *Hemiphylacus*, is endemic to Mexico (Hernandez 1995). Approximately 73% of *Asparagus* species—and all five *Hemiphylacus* species—are bisexual with monoclinal flowers, while the rest of *Asparagus* are dioecious (Bentz et al. 2024). Previous studies have failed to confidently resolve species relationships across the *Asparagus* phylogeny despite many attempts using a few organellar and/or nuclear loci (Lee et al. 1997; Štajner et al. 2002; Fukuda et al. 2005; Wiegand 2006; Kubota et al. 2012; Norup et al. 2015). Ancestral rapid radiations likely account for the difficulty of phylogenetic inference in *Asparagus* due to incomplete lineage sorting (ILS) (Norup et al. 2015; Bentz et al. 2024). These phylogenetic challenges are compounded by the young origins of the genus (i.e. ~9 to 15 Ma) and a low substitution rate for the plastome (Bentz et al. 2024). Poor resolution of species relationships in *Asparagus* has stymied robust inference for the origin(s) of dioecy in the genus, with some analyses supporting a single origin (Fukuda et al. 2005; Kubota et al. 2012) and others opening the possibility for two separate origins (Norup et al. 2015; Bentz et al. 2024).

To explore evolutionary relationships and ancestral biogeography across the genus *Asparagus*, we employed a Hyb-Seq approach with Asparagaceae1726—a probe set

targeting 1,726 nuclear loci conserved in low copy numbers across Asparagaceae (Bentz and Leebens-Mack 2024)—and the most comprehensive species sampling of *Asparagus* to date. Hyb-Seq, or target sequence capture, is a cost-effective, reduced representation sequencing strategy that utilizes RNA probes designed to select and enrich for specific genes of interest (Weitemier et al. 2014). In this study, we (i) investigate how rapid radiations and range expansions out of southern Africa may have contributed to the origin and diversification of major *Asparagus* lineages; (ii) explore possible explanations for complicated speciation patterns and taxonomic uncertainties; (iii) test the hypothesized multiple origins of dioecy in *Asparagus* (Norup et al. 2015; Bentz et al. 2024); and (iv) infer the geographic origin of domesticated garden asparagus.

Materials and Methods

Sample Preparation and Sequencing

Samples were prepared for Hyb-Seq experiments with Asparagaceae1726 using the exact methods as described by Bentz and Leebens-Mack (2024). Total DNA was extracted from a silica-dried, flash-frozen, or herbarium voucher tissue, and Illumina DNA-sequencing libraries were prepared by using universal Y-yoke stub adapters and dual-indexed iTru primers (Glenn et al. 2019) aiming for an average fragment length of 350 to 550 bp. Genomic libraries were pooled for hybridization with Asparagaceae1726 probes, and then, captured target DNA fragments were enriched for 14 cycles of PCR (Bentz and Leebens-Mack 2024). Additional sample and data generation details are given in [supplementary appendix S1, Supplementary Material](#) online.

Species Sampling

We sampled as many species possible of *Asparagus*, attempting to include species spanning the entire geographic range of the genus, as well as one *Hemiphylacus*, and three outgroups from different Asparagaceae subfamilies (Table 1). Accessions for several undescribed taxa were included in our dataset (Table 1), to be described in a

Table 1 Species sampling across 12 major clades in *Asparagus* and outgroups

Clade	Samples	New species	Total species
<i>Asparagus</i> clades			
Africani	33	1	13 (+1 subsp.)
Asparagus	69	1	32
Exuviali	6	1	3
Macowanii	2	0	2
Myrsiphyllum	33	4	12
Nutlet	5	1	4
Racemose	90	17	49 (+1 var.)
Retrofracti	16	1	8 (+1 subsp.)
Scandens	5	0	2
Setaceus	19	1 (+1 subsp.)	9 (+1 subsp.)
Suaveolens	29	6 (+1 subsp.)	16 (+1 subsp.)
Sympodioidi	7	0	5
<i>Hemiphylacus</i>	1	0	1
Brodiaeoideae (outgroup)	1	0	1
Scilloideae (outgroup)	1	0	1
Lomandroideae (outgroup)	1	0	1
Total	318	33 (+2 subsp.)	162 (+4 subsp.)

Outgroup species include *Hemiphylacus*—the sister genus to *Asparagus* and sole other member of the Asparagaceae subfamily Asparagoideae—and samples from three different Asparagaceae subfamilies. New species circumscriptions are presented in a forthcoming monograph and revision of southern African *Asparagus* (J.E. Burrows and S.M. Burrows, in prep.).

forthcoming monograph and revision of the genus (J.E. Burrows and S.M. Burrows, in prep.), and are labeled with “MS”. All major clades of *Asparagus* were sampled in this study, including several DNA accessions from Norup et al. (2015). For DNA isolation, tissue was collected from wild populations, herbarium vouchers, or cultivated plants growing in greenhouses or botanical gardens. We aimed to sample multiple individuals from different populations of each species when possible. Ten accessions were from whole-genome shotgun sequencing (WGS) experiments repurposed for this study. Additional details regarding species and tissue sampling can be found in [supplementary appendix S1, Supplementary Material](#) online.

Target Ortholog and Paralog Assembly

Lingering adapter sequences were removed from reads, mismatched base pairs were corrected, and reads shorter than 21 bases were filtered out using fastp v.0.23.2 (Chen et al. 2018). The HybPiper v.2.1.6 pipeline (Johnson et al. 2016) was then used to (i) map the filtered reads to target nucleotide sequences with BWA-MEM (Li, 2013), (ii) assemble mapped reads into contiguous sequences with SPAdes (Bankevich et al. 2012), (iii) extend gene assemblies into flanking intronic regions, and (iv) test for paralogs with default HybPiper parameters. BLASTX was used for read alignments with HybPiper for some WGS

accessions ([supplementary appendix S1, Supplementary Material](#) online). The Asparagaceae1726 v.1.1 (<https://github.com/bentzpc/Asparagaceae1726>) target sequence file was used as reference for HybPiper processing, as performed by Bentz and Leebens-Mack (2024).

Phylogenomic Analysis

Multiple sequence alignments for each target locus were produced using MAFFT v.7.487 with the flag *-auto* (Katoh and Standley 2013). Poorly aligned sequences were trimmed using trimAl v.1.4.1 with the flag *-automated1* (Capella-Gutiérrez et al. 2009). IQ-TREE v.1.6.12 (Nguyen et al. 2015) was used for maximum likelihood (ML) gene tree inference with 1,000 ultrafast bootstraps (BS) and the *MFP* option which allows ModelFinder (Kalyaanamoorthy et al. 2017) to choose the best-fit substitution model based on a Bayesian information criterion. Species tree inference was derived from the full collection of unrooted ML gene trees by using wASTRAL-unweighted v1.16.3.4 (Zhang and Mirarab 2022) by optimizing the objective function of ASTRAL-III v.5.7.8 (Zhang et al. 2018), which employs a coalescent-based summary method that is statistically consistent under the multispecies coalescent model. Prior to the ASTRAL analysis, gene tree branches with <10% BS support were collapsed into polytomies to improve the accuracy of species tree inference (Zhang et al. 2018). The resulting ASTRAL tree was rooted with outgroup taxa (Table 1), and then, a polytomy test was performed with ASTRAL-III; species tree nodes were subsequently collapsed when a polytomy could not be rejected (*P*-value >0.05). Along with metrics reported by ASTRAL, gene tree quartet support (e.g. QD) was also assessed with Quartet Sampling v.1.3.1 (Pease et al. 2018) and RAXML-ng v.1.2.2 (Kozlov et al. 2019) as the likelihood evaluation engine, across all nodes. Quartet Sampling was performed with 1,000 replicates and a likelihood threshold of 2.0 (Pease et al. 2018). Coalescent-based, rather than concatenation, methods were used to analyze nuclear genes because concatenation implicitly assumes an absence of recombination and ILS (i.e. a single history is shared amongst all genes) and can result in statistical inconsistencies in multilocus datasets with high levels of gene tree discordance (Kubatko and Degnan 2007; Edwards et al. 2016), whereas coalescent approaches assume free recombination between genes, accounting for gene tree–species tree discordance due to ILS.

Ancestral Range Estimation

A second species tree reconstruction was performed with less terminal branches for ancestral range estimations by forcing closely related accessions together in quartet analyses of a subsequent ASTRAL-III analysis, which allows predefined lineages to coalesce into a single, terminal branch

(Zhang et al. 2018). Single-branch coalescence enables terminal branch length estimation, representing coalescent time units. Accessions were forced into a single lineage/branch only if they formed a well-supported clade (LPP ≥ 0.98 in the previously inferred ASTRAL tree) and exhibited overlapping geographic distributions (supplementary appendix S1, Supplementary Material online). For instance, lineages with a narrow geographic distribution nested in an otherwise broadly distributed clade/range were collapsed into a single branch. Based on the existing literature of *Asparagus* biogeography (Norup et al. 2015; Bentz et al. 2024; POWO 2024) and the biologically informative geographic areas defined by the Taxonomic Databases Working Group (Brummitt 2001), we defined 11 areas (states) for ancestral range estimation using stochastic mapping with BioGeoBEARS v.1.1.1 (Dupin et al. 2016; Matzke 2016). We analyzed and chose the best of three biogeographic models implemented with BioGeoBEARS: (i) the DEC model (Ree and Smith 2008), (ii) an ML implementation of the dispersal–vicariance analysis (Ronquist 1997) (termed DIVALIKE), and (iii) an ML implementation of BayArea (Landis et al. 2013) (termed BAYAREALIKE) (Ree and Smith 2008; Matzke 2014). We also tested whether allowing for founder events within clades (parameter j) increased the model fit, based on likelihood ratio tests for each nested model, considering a P -value of <0.05 as significant. The best-fit model was chosen based on an Akaike information criterion (AIC), for which we calculated sample size–corrected AIC (AICc) and then AIC weights (AICc_wt) to represent the relative likelihood of each model, choosing the model with the highest (best) AICc_wt score (Burnham and Anderson 2002). Each biogeographical test was unconstrained, allowing for equal probabilities among all possible area combinations for dispersal routes. Defined geographic areas included Southwestern + Central Europe; Eastern Europe + Caucasus; Northern Africa + Macaronesia; Northern Tropical Africa; Central Tropical Africa; Central Asia; Southern Africa + Madagascar; Western Asia to Mediterranean Basin; Arabian Peninsula; Eastern Asia to Malesia; and the Indian Subcontinent (supplementary fig. S8, Supplementary Material online). Prior to the analysis with BioGeoBEARS, a subset of noninformative branches were pruned from the input ASTRAL tree to make the analysis computationally tractable (supplementary appendix S1, Supplementary Material online).

Results

Species Sampling and Target Sequence Capture

In this study, we assembled the most comprehensive species sampling to date for phylogenomic investigation of *Asparagus*, including 314 accessions representing 158

Asparagus species (Table 1). Our sampling effort yielded phylogenomic support for 28 new species (+2 subsp.), the resurrection of nine previously described species, and elevation of three varieties to the species level (supplementary results S1, Supplementary Material online; supplementary appendix S1, Supplementary Material online). Formal taxonomic revision is outside the scope of the current study, but these new southern African *Asparagus* species, as well as other taxonomic updates, are described in a forthcoming publication (J.E. Burrows and S.M. Burrows, in prep.). After performing Hyb-Seq with the *Asparagaceae*1726 bait set (Bentz and Leebens-Mack 2024), we recovered $>1,300$ orthologous target loci in all samples (mean = 1,680) (supplementary appendix S2, Supplementary Material online). A small fraction of target loci (0.9% to 2.8%) exhibited gene copy number variation (i.e. paralogs) among taxa (supplementary results S1, Supplementary Material online; supplementary appendix S2, Supplementary Material online). However, analyses with and without paralogs resulted in congruent topologies and branch support, suggesting limited effect on phylogenomic inference in *Asparagus* (Bentz and Leebens-Mack 2024).

The *Asparagus* Phylogeny

Phylogenomic analyses yielded strong support (i.e. local posterior probability [LPP] = 1.0) for the earliest split in the *Asparagus* phylogeny, spawning one largely African branch leading to ten major clades and another spawning the *Asparagus* and *Exuvialis* sister clades (Fig. 1). Most species in the *Asparagus* clade are dioecious and are widespread across Eurasia and the Mediterranean Basin, whereas all other major clades are solely bisexual and largely restricted to Africa. Across the species tree, several paraphyletic species were revealed (supplementary fig. S1, Supplementary Material online; supplementary results S1, Supplementary Material online), some of which exhibited signatures of an ongoing budding speciation process (see Discussion). Multiple polyphyletic species were also observed in the *Asparagus* phylogeny, including a few highly polymorphic species known to be taxonomically troublesome (i.e. *Asparagus asparagoides* [L.] Druce, *Asparagus densiflorus* [Kunth] Jessop, and *Asparagus setaceus* [Kunth] Jessop) (supplementary results S1, Supplementary Material online).

Across the final species tree (supplementary fig. S1, Supplementary Material online), gene tree discordance (supplementary fig. S2, Supplementary Material online) was relatively low (ASTRAL normalized quartet score = 0.77), and quartets showed limited signs of deviation from assumptions of the multispecies coalescent, which accounts for ILS between speciation events (Pamilo and Nei 1988; Maddison 1997; Rannala and Yang 2003). For example, ancestral gene flow between diverging lineages can result in low quartet differential (QD) scores (47)

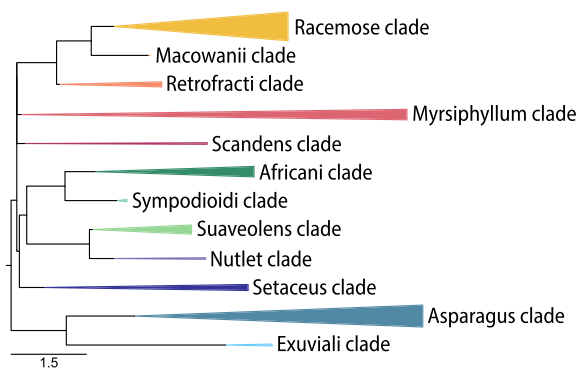


Fig. 1. Relationships among 12 major clades representing nearly the full extent of species diversity and geographic range of the genus *Asparagus*. All clades are solely composed of bisexual species exhibiting monodinous flowers, except for the *Asparagus* clade in which dioecy evolved twice independently. Summary tree based on larger phylogeny of 318 accessions (162 species) inferred from 1726 nuclear genes (Bentz and Leebens-Mack 2024) using wASTRAL-unweighted v1.16.3.4 (Zhang and Mirarab 2022) by optimizing the objective function of ASTRAL-III v.5.7.8 (Zhang et al. 2018). Triangular branch widths were scaled according to number of samples and initiation of tapering represents crown group radiations. Branch lengths/scale bar correspond to coalescent time units. Bifurcations were collapsed according to a polytomy test with ASTRAL-III (p -value >0.05). Local posterior probabilities (LPP) were >0.98 for all branches, except when including *A. fasciculatus* (sister species to the rest of Myrsiphyllum) in Myrsiphyllum (LPP = 0.64), otherwise LPP = 0.99 for the clade.

(QD = 0 when all discordant quartets at a node support one of the two alternative resolutions and QD = 1 when quartet frequencies for both alternatives are equal). The average QD across all nodes in the *Asparagus* phylogeny was 0.87 (supplementary fig. S2, Supplementary Material online). Furthermore, many of the nodes exhibiting QD <0.50 involved bifurcations between samples of the same species (supplementary fig. S2, Supplementary Material online). At the same time, the *Asparagus* phylogeny included 27 nodes for which we were unable to reject a polytomy (i.e. nodes with more than two daughter lineages) (supplementary fig. S3, Supplementary Material online). In a fully bifurcating tree, branches around these nodes exhibited low LPP and short branch lengths (supplementary fig. S4, Supplementary Material online) and similar gene tree quartet support for both alternative resolutions, rather than skewed support favoring one alternative topology over the other (supplementary figs. S2 and S5, Supplementary Material online).

A polytomy could not be rejected (P -value = 0.07; supplementary fig. S3, Supplementary Material online) among the Scandens, Myrsiphyllum, and Racemose–Macowanii–Retrofracti clades due to low support (LPP = 0.83) and a short branch length subtending a Myrsiphyllum–Scandens clade (supplementary fig. S4, Supplementary Material online), which also exhibited similar

quartet support for alternative resolutions (QD = 0.94) (supplementary figs. S2 and S5, Supplementary Material online). Additionally, a polytomy could not be rejected (P -value = 0.27) along the African *Asparagus* backbone—or branch subtending a (i) Africani–Sympodioidi–Suaveolens–Nutlet–Setaceus clade; (ii) Myrsiphyllum–Scandens clade; and (iii) Racemose–Macowanii–Retrofracti clade—due to low bifurcating support between the Myrsiphyllum–Scandens and Racemose–Macowanii–Retrofracti clades (LPP = 0.82; QD = 0.98) (supplementary figs. S2 to S5, Supplementary Material online). Collapsing of the two branches described above resulted in a polytomy with four leaves forming the backbone of the main African, bisexual clade in the genus (Fig. 1; supplementary fig. S1, Supplementary Material online). Aside from Myrsiphyllum, all other major clades were strongly supported (LPP ≥ 0.99) (supplementary fig. S1, Supplementary Material online). The inclusion of *Asparagus fasciculatus* Thunb. (a lineage sister to the rest of Myrsiphyllum) in the Myrsiphyllum clade was weakly supported (LPP = 0.64), but monophyly of the remainder of Myrsiphyllum was strongly supported (LPP = 0.99) (supplementary fig. S1, Supplementary Material online).

The *Asparagus* clade was strongly supported and showed virtually no skew in alternative quartet support (QD = 0.94) (supplementary fig. S2, Supplementary Material online). Within the *Asparagus* clade lie three smaller, well-supported (LPP = 1.0) clades, that is, a clade of four bisexual species that are distributed across central–southern Africa (Flagellaris clade: Fig. 2) and two entirely dioecious clades, namely a broadly distributed Eurasian clade and a less speciose and more narrowly distributed Mediterranean Basin clade (Fig. 2). All three of those nested clades were supported by most gene tree quartets, with limited to no skew between alternative resolutions (supplementary figs. S2 and S5, Supplementary Material online). Together, the Mediterranean Basin dioecy clade and Flagellaris bisexual clade formed a clade sister to the Eurasian dioecy clade (LPP = 1.0; QD = 0.98) (Fig. 2, supplementary fig. S2, Supplementary Material online). Lastly, the Exuviali clade, composed of three species restricted to southern Africa, was highly supported (LPP = 1.0; QD = 1.0) and placed sister to the *Asparagus* clade (Fig. 2).

Ancestral Biogeography Estimation

The best-fit biogeographic model for this dataset was dispersal–extinction–cladogenesis (DEC) (supplementary table S1, Supplementary Material online), and allowing for founder events within clades (parameter j) significantly improved the model fit relative to the DEC model (P -value = 0.026) (supplementary table S2, Supplementary Material online). Ancestral range inference under the DEC + J model implicates southern Africa as the most probable center of origin for all 12 major clades in *Asparagus* (supplementary fig. S6a,

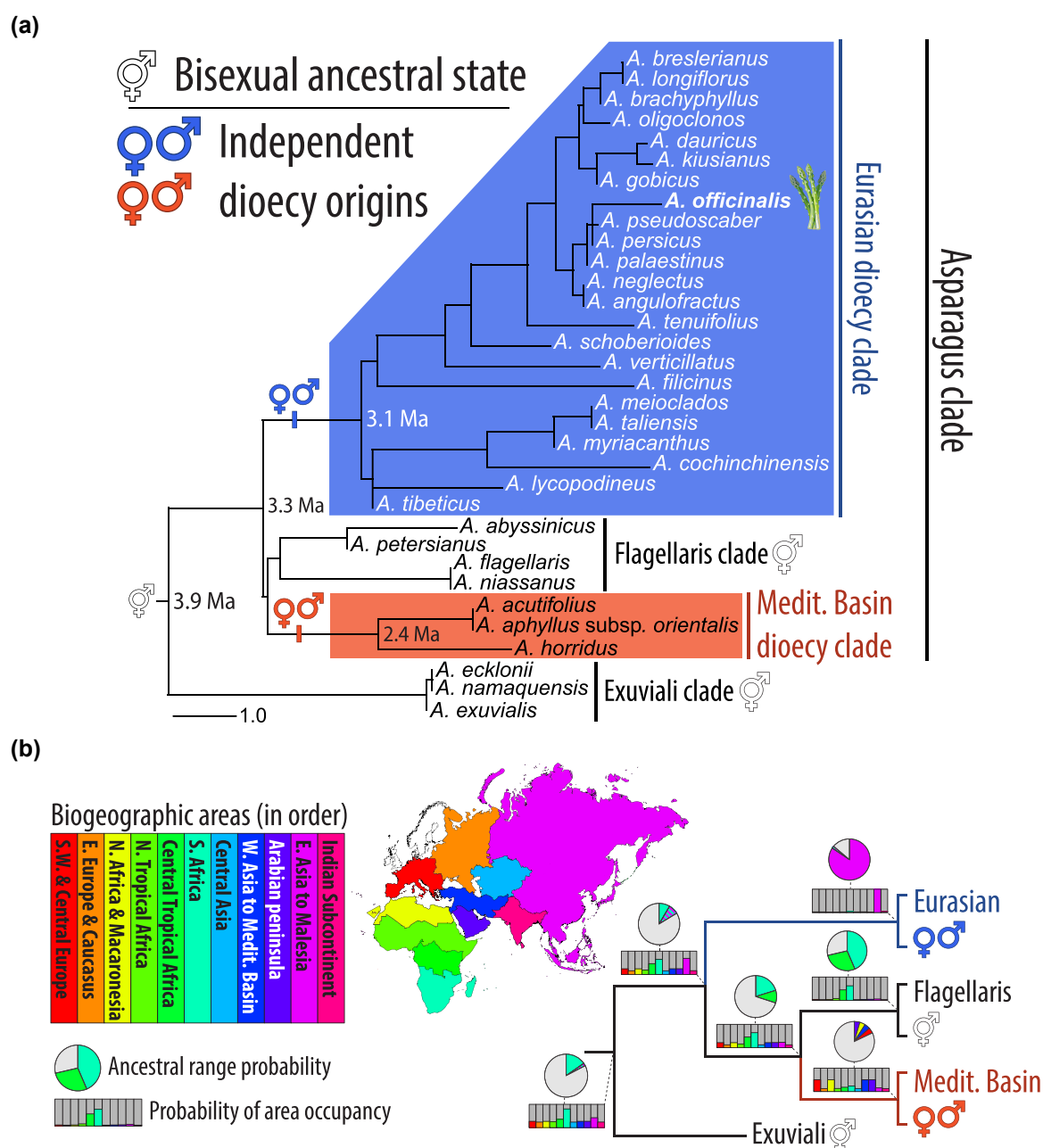


Fig. 2. a) Phylogeny of the Asparagus clade in the genus *Asparagus* showing support for two separate dioecious clades: i) a widespread Eurasian clade (blue) and ii) geographically restricted Mediterranean Basin clade (red). b) Ancestral range estimations suggest that the stem ancestor of the Medit. Basin dioecy clade dispersed out of southern or central Africa independently of the Eurasian dioecy clade, bolstering support for independent origins of dioecy in each of the dioecious clades (red and blue). a) Local posterior probabilities equalled 1.0 for all branches; bifurcations were collapsed according to a polytomy test with ASTRAL-III v.5.7.8 (Zhang et al. 2018). When present, multiple samples for a species were combined into a single terminal branch. Divergence time estimates (Ma = million years ago) are plotted at concordant nodes from Bentz et al. (2024) and branch lengths/scale bar represent coalescent time units (Zhang et al. 2018). b) Cladogram shows summarized results from BioGeoBEARS v.1.1.1 (Dupin et al. 2016; Matzke 2016) plotted at relevant nodes. Pies show the top two ancestral range probabilities in color (grey = all other possibilities) for internal nodes, except at the crown node of the Medit. Basin dioecy clade, which had four nearly equal top probabilities (all ~5%). Bar charts show the relative probability of geographic area occupancy (i.e. probability that a range included any of the 11 predefined areas). Area = discrete geographic region. Range = species distribution encompassing any combination of areas.

Supplementary Material online; supplementary appendix S3, Supplementary Material online). Our results indicated that at least eight independent range shifts, across the genus,

resulted in dispersal out of Africa (supplementary fig. S6a, Supplementary Material online; supplementary appendix S3, Supplementary Material online). One of these shifts out

Table 2 Ancestral range estimations for nodes implicated in the two origins of dioecy in *Asparagus* and the origin of garden asparagus (*A. officinalis*)

	<i>Asparagus</i> genus crown	<i>Asparagus</i> clade stem	<i>Asparagus</i> clade crown	Eurasian clade crown	Garden asparagus clade stem ^(a)	Mediterranean Basin clade stem	Mediterranean Basin clade crown	Flagellaris clade crown
A) Relative probability of occupancy per geographic area								
Areas								
South and Central Europe (D)	31%	27%	24%	3%	33%	21%	50%	4%
East Europe and Caucasus (E)	29%	24%	16%	3%	33%	12%	13%	3%
Northern Africa and Canary Isl. (F)	31%	27%	24%	2%	0%	21%	50%	4%
Northern Tropical Africa (J)	30%	25%	19%	2%	0%	16%	13%	8%
Central Tropical Africa (K)	36%	36%	46%	3%	0%	46%	18%	45%
Central Asia (L)	29%	24%	16%	3%	37%	12%	13%	3%
Southern Africa (M)	100%	80%	65%	4%	0%	64%	23%	62%
Western Asia to Mediterranean Basin (R)	31%	27%	24%	3%	100%	21%	50%	4%
Arabian Peninsula (T)	31%	27%	24%	2%	0%	22%	50%	4%
East Asia to Malesia (U)	39%	42%	69%	100%	46%	23%	15%	6%
Indian subcontinent (X)	29%	24%	16%	4%	37%	12%	13%	3%
B) Most probable ancestral ranges (in order of highest probability)								
#1	M	M	M	U	R	M	T	M
probability	9%	15%	8%	84%	24%	20%	5%	44%
#2	M + U	M + U	M + U	M + U	R + U	K	R	K
Probability	1%	2%	8%	2%	7%	10%	5%	28%
#3	K + M	K + M	K + U	U + X	D + ^(b)	M + U	D	K + M
Probability	1%	1%	4%	1%	6%	2%	5%	5%
#4	M + T	M + T	K	R + U	L + R	K + M	F	J
Probability	1%	1%	2%	1%	4%	1%	5%	1%

A) is the relative probability of occupancy for a given node in each of the 11 geographic areas (states) defined for biogeography analysis and illustrated as bar charts in Fig. 2. Considerable probabilities are bolded for each clade. B) is the most probable ancestral range (distribution of various area combinations) for a given node estimated using BioGeoBEARS v.1.1.1 (Dupin et al. 2016; Matzke 2016) and shown as pies in Fig. 2. ^aA well-supported branch with polytomy among *A. officinalis*, *A. persicus*, and *A. pseudoscaberr* (Fig. 2). ^bE + L + R + U + X.

of Africa occurred following the split between the Exuviali and the *Asparagus* clades, in the *Asparagus* clade crown group, which most likely originated in southern Africa (Fig. 2; Table 2). However, a disjunct range including southern Africa and eastern Asia was almost as probable for the crown group of the *Asparagus* clade (Fig. 2, supplementary fig. S6b, Supplementary Material online; Table 2), which is practically impossible considering the geographic distance between these regions. Such uncertainty at the crown of the *Asparagus* clade may be explained by sampling bias (i.e. eastern Asia is the biodiversity hotspot for the Eurasian clade and is more speciose than its sister clade) and can be further assessed at surrounding splits in the tree, which suggest that a transition from Africa to Asia occurred following cladogenesis of the Eurasian lineage (supplementary figs. S6 and S7a, Supplementary Material online; supplementary appendix S3, Supplementary Material online). Nonetheless, the ancestral range for the Exuviali–*Asparagus* crown group was inferred as southern Africa, whereas the crown range for the Eurasian dioecy clade was placed in eastern Asia with high confidence (Fig. 2; Table 2). Also in the *Asparagus* clade, a separate ancestral range shift was estimated at the stem

branch of the Mediterranean Basin dioecy clade, initiating out of either southern or central Africa and ending with colonization of the Arabian Peninsula or Mediterranean Basin by the crown group (Fig. 2, supplementary figs. S6a and S7b, Supplementary Material online; Table 2).

Discussion

Rapid Radiations and Dispersals out of Africa

Biogeographic analyses strongly support southern Africa as the ancestral center of origin for the genus *Asparagus* (Table 2), which agrees with previous findings (Norup et al. 2015). The short branches and polytomous backbone of the *Asparagus* phylogeny indicate that early and rapid diversification in southern Africa gave rise to all extant major clades in the genus (Fig. 1). Following its origin in southern Africa, eight independent range expansion events, in four major clades, led to colonization of Eurasia (supplementary fig. S6, Supplementary Material online). One of these expansions out of southern Africa initiated at the crown node of the *Asparagus* clade and led to a

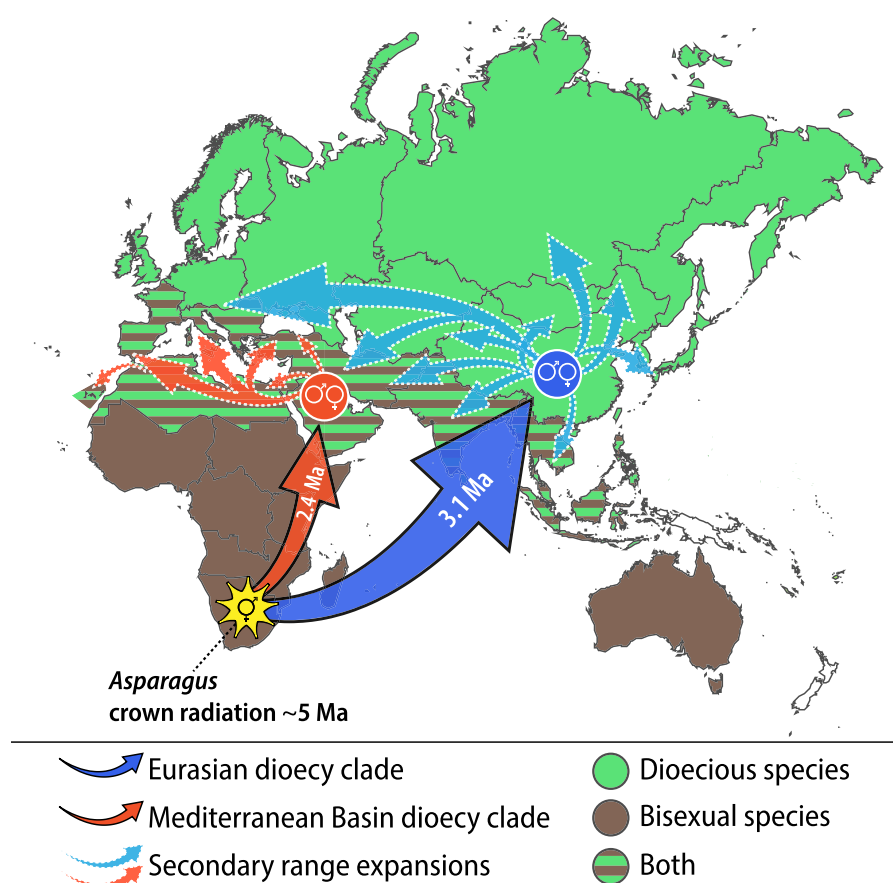


Fig. 3. Hypothesized long-distance dispersals out of southern Africa and into Eurasia by separate bisexual ancestral lineages (solid arrows), leading to independent founding events and origins of dioecy in two *Asparagus* clades (red and blue circles). Arrows do not represent specific dispersal routes, which remain uncertain, and are intended to simply point to destinations. The inferred founding events in Eurasia (circles) are based on the most likely ancestral ranges for each dioecious clade. It is nearly as probable that founders of the Mediterranean Basin dioecy clade first occurred elsewhere in the Medit. Basin, rather than the Arabian Peninsula (Table 2). Divergence time estimates in solid arrows correspond to mean crown group ages for each dioecy clade from Bentz et al. (2024) (Ma = million years ago). Dotted arrows represent subsequent range expansions following the hypothesized Eurasia founding events.

founding event in eastern Asia, where dioecy evolved in the most recent common ancestor (MRCA) of the Eurasian dioecy clade (Fig. 2; Table 2) and was followed by rapid diversification of dioecious lineages and secondary dispersals across the continent (Fig. 3). Our results suggest that the MRCA of the Mediterranean Basin dioecy clade dispersed out of southern Africa after that of the Eurasian dioecy clade, perhaps through the Arabian Peninsula (Figs. 2 and 3; Table 2).

Interestingly, our results suggest that ancestors of extant, non-African bisexual *Asparagus* lineages expanded northeastward out of southern Africa, through the Arabian Peninsula, into Eurasia and that dispersal to Macaronesia occurred independently three times (supplementary results S1, Supplementary Material online). Altogether, our biogeographic analysis suggests that range expansions out of southern Africa occurred independently in various *Asparagus* lineages following an initial radiation

that formed all extant major clades (Fig. 1) and alongside clade-specific secondary radiations. Across the *Asparagus* phylogeny, we found virtually no evidence of secondary evolutionary histories (e.g. ancient hybridizations, lineage-specific rate heterogeneity, or heterogeneous base compositions) as indicated by a skew in quartet support for a single alternative resolution, or a low QD (Pease et al. 2018). This includes the four-way polytomy representing the backbone of the main ten African clades in the genus (Fig. 1), which implicates only ILS as the cause of gene tree discordance for those nodes.

Two Independent Origins of Dioecy in *Asparagus*

The inferred *Asparagus* phylogeny resulted in strong support for a bifurcation between the Eurasian dioecy clade and two sister clades: a dioecious Mediterranean Basin clade and a bisexual clade primarily composed of African

species (Flagellaris clade) (Fig. 2). Support for two dioecious clades in *Asparagus* was previously found based on whole plastome sequences, although a polytomy among those and the Flagellaris clade showed higher support compared with bifurcations (Bentz et al. 2024). In contrast, our analysis of 1,726 nuclear loci and many more species enabled rejection of a polytomy among those three clades (Fig. 2). Norup et al. (2015) also reported two dioecious lineages within the *Asparagus* clade, but with very poor support. Parsimony mapping of trait shifts across the *Asparagus* phylogeny may imply equal support for a single origin of dioecy—on the stem branch of the *Asparagus* clade—followed by a loss in the bisexual Flagellaris clade, or two independent origins in the Eurasian and Mediterranean Basin dioecy clades (Fig. 2). However, when considering extant and ancestral species ranges in the genus, as well as the sister relationship between the Mediterranean Basin dioecy clade and bisexual Flagellaris clade (Fig. 2), the inference of two separate dioecy origins is strengthened. The biogeographic analysis suggests that if dioecy evolved only once in *Asparagus*, then this would have occurred in sub-Saharan Africa (see *Asparagus* clade crown in Fig. 2b). However, the practically complete absence of dioecious *Asparagus* in Africa (outside of the Mediterranean Basin) suggests that a sub-Saharan dioecy origin is unlikely.

Ancestral range estimations implicated eight independent range shifts out of southern Africa, two of which are associated with long-distance dispersal events and the evolution of dioecy in the *Asparagus* clade. Transitions from hermaphroditism to dioecy, following long-distance dispersals and associated with colonization bottlenecks, support the idea that dioecy may evolve in response to selective pressure to avoid inbreeding and deleterious mutational loads in founding populations (Charlesworth 1999). A founding population that formed after a long-distance dispersal event may be at increased risk to experience inbreeding depression due to a higher propensity to self, which is thought to be a required condition for long-distance dispersal and colonization of new habitats (Baker 1955). To that end, long-distance dispersals and founder population bottlenecks are hypothesized to have contributed to the evolution of dioecy in many angiosperms endemic to oceanic islands (Carlquist 1966; Pannell et al. 2015). In *A. officinalis*, some genotypically male (XY) plants bear a combination of strictly male flowers and bisexual flowers (i.e. andromonoecy) that readily self-pollinate and set fruit with no obvious barriers present, and the resulting progeny typically exhibit traits thought to correlate with inbreeding depression (e.g. loss of germinability and vigor) (Franken 1970; Galli et al. 1993). Similarly, andromonoecy and selfing in bisexual flowers have been observed in several other dioecious *Asparagus* (e.g. *Asparagus horridus* L., *Asparagus cochinchinensis* [Lour.] Merr., and *Asparagus taliensis* F.T. Wang & Tang ex S.C. Chen). Such high selfing

rates in andromonoecious individuals of *A. officinalis* and others may be reminiscent of a bisexual ancestral state with a predisposition to self (Galli et al. 1993). Therefore, if the ancestrally bisexual founder populations, of extant dioecious lineages of *Asparagus*, exhibited high selfing rates, then inbreeding depression could have promoted selection for outcrossing and the evolution of dioecy in both dioecious clades.

Genomic comparisons between *A. officinalis* (Eurasian dioecy clade) and *A. horridus* (Mediterranean Basin dioecy clade) also support independent origins of dioecy in these clades, as each exhibits novel sex chromosomes that separately evolved from different autosomes (Bentz 2024; Bentz et al. in prep.). Furthermore, no homologous genes exist between the *A. officinalis* and *A. horridus* Y-linked sex-determining regions (Bentz 2024), where the master sex-determining genes reside (e.g. *SOFF* and *aspTDF1* in *A. officinalis*) (Harkess et al. 2017). Expression assays of the male promoter gene, *aspTDF1*, also support the absence of a Y-linked *aspTDF1* in *A. horridus* and another member of the Mediterranean Basin dioecy clade (*Asparagus acutifolius* L.) as evident by its equal expression in both sexes of each species (Murase et al. 2017). The presence of completely different sex chromosomes and disparate genetic pathways for sex determination, between the Eurasian and Mediterranean Basin dioecy clades, along with the biogeographic and phylogenomic support presented here (Fig. 2; Table 2), suggests strongly that dioecy arose twice independently in *Asparagus* and in association with separate range expansions from southern Africa to Eurasia (Figs. 2 and 3).

Active Speciation in *Asparagus*

The *Asparagus* phylogeny includes some paraphyletic and polyphyletic species, typically involving narrowly distributed species nested within geographically widespread and highly polymorphic species (supplementary results S1, Supplementary Material online). We identified several examples of paraphyletic species (e.g. Fig. 4) that may be explained as a consequence of species with restricted ranges or ecological niches forming from within a more geographically widespread and phylogenetically diverse (now paraphyletic) extant species. This process of speciation, through genetic divergence of a population from an extant parent species, in association with reproductive isolation, is commonly referred to as “budding speciation” (Mayr 1954). Whether driven by geographic isolation or niche specialization (Grossenbacher et al. 2014), budding speciation giving rise to phylogenetically distinct species nested within extant paraphyletic species may be common in plants (Rieseberg and Brouillet 1994). Three clear-cut examples of possible budding speciation in *Asparagus* were identified in the Racemose, Suaveolens, and Exuviali clades (Fig. 4).

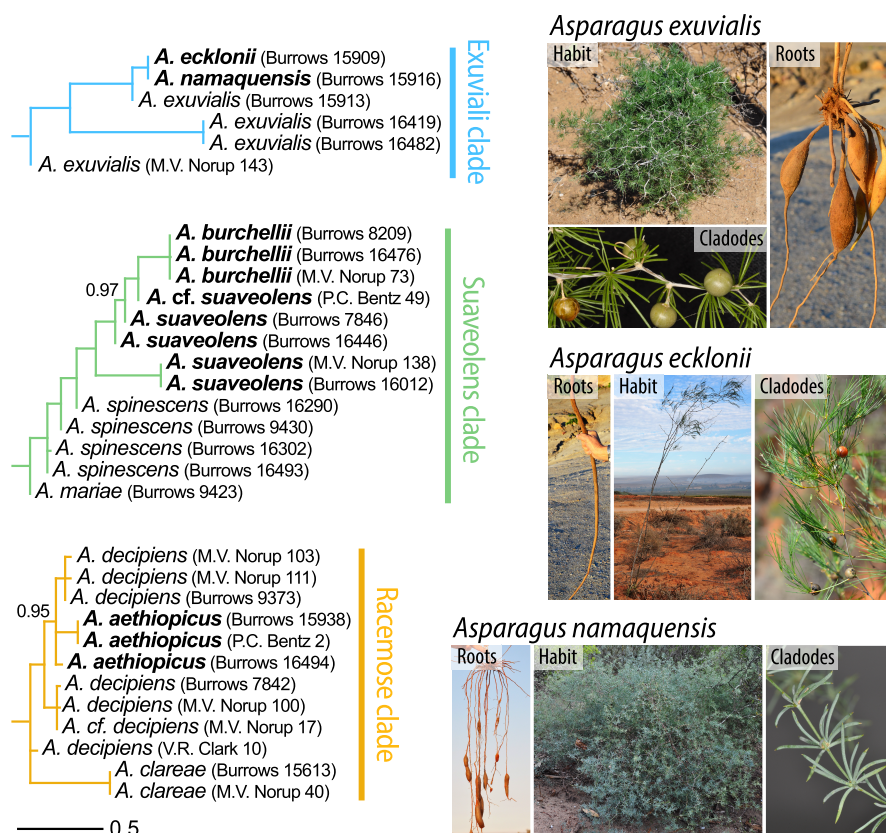


Fig. 4. Three subtrees illustrating paraphyly caused by budding speciation from within extant ancestral species of *Asparagus*. Species images show contrasting phenotypes of two budded species (*Asparagus ecklonii* Baker and *Asparagus namaquensis* MS) that evolved from an ancestral population of *Asparagus exuvialis* Burch. in the Exuviali clade. Branch lengths/scale bar correspond to coalescent time units (Zhang et al. 2018). Local posterior probabilities shown when <0.98. Bolded tip labels refer to putative budded species. Species images are excerpts from a forthcoming monograph and taxonomic revision of *Asparagus* taxa in southern Africa (J.E. Burrows and S.M. Burrows, in prep.).

Interestingly, there was no strong evidence of interspecific gene flow or any departure from the multispecies coalescent model (Pamilo and Nei 1988; Maddison 1997; Rannala and Yang 2003) at any of the nodes (all QD > 0.70) shown in Fig. 4, suggesting establishment or initiation of reproductive barriers between ancestral and budded species.

In the Racemose clade, short and largely polytomous branches among paraphyletic accessions of *Asparagus aethiopicus* L. and *Asparagus decipiens* (Baker) MS suggest that *A. aethiopicus* budded from the extant ancestral species *A. decipiens* (Fig. 4). The ranges of these two species are largely disjunct, implicating geography as a reproductive barrier: *A. aethiopicus* is mainly confined to the coastal belt of the Western Cape of South Africa, whereas *A. decipiens* occurs throughout much of the drier regions of the Eastern Cape and eastern reaches of the Western Cape of South Africa (J.E. Burrows and S.M. Burrows, in prep.). Although hybridization potential between these two species is unknown, the patterns listed above are nonetheless suggestive of an ongoing speciation process.

In the Suaveolens clade, multiple species gradients (i.e. branching events) among accessions of *Asparagus spinescens* Steud. ex Schult. and Schult.f., *Asparagus suaveolens* Burch., and *Asparagus burchellii* Baker (Fig. 4) support the notion that speciation is not some discrete event that always results in the emergence of bifurcating lineages and extinction of the MRCA, but rather speciation occurs on a continuum and multiple budded species can evolve simultaneously from various ancestral populations (e.g. sympatric formation of multiple host races and species in herbivorous insects) (Drès and Mallet 2002). Interestingly, *A. spinescens* is more geographically restricted compared with the two budded species, but it remains possible that the budding of *A. suaveolens* occurred in a small, narrowly distributed population of *A. spinescens*. On the other hand, *A. suaveolens* exhibits one of the widest distributions of African *Asparagus* species and is more widespread than the budded *A. burchellii* (Obermeyer et al. 1992).

In the Exuviali clade, *Asparagus exuvialis* Burch.—a species that extends widely across South Africa, Namibia, Botswana,

southern Zimbabwe, and southern Mozambique (Obermeyer et al. 1992)—is paraphyletic with two budded species (*Asparagus ecklonii* Baker and *Asparagus namaquensis* MS) that arose from an ancestral *A. exuvialis* (Fig. 4). *A. ecklonii* and *A. namaquensis* are broadly sympatric and narrowly distributed in the Northern Cape and Western Cape of South Africa, each preferring distinct dominant soil types: in the north of its range *A. namaquensis* prefers red Aeolian sands, whereas *A. ecklonii* prefers deep sandy soils in shrublands often dominated by *Restio* (J.E. Burrows and S.M. Burrows, in prep.). Although the extant ranges of *A. exuvialis*, *A. ecklonii*, and *A. namaquensis* overlap in the Western Cape region, each species exhibits strikingly different growth habits among several other distinct phenotypes (Fig. 4), suggesting realization of different ecological niches in the budded species.

Two extremely polymorphic species in the Myrsiphyllum clade—*Asparagus ovatus* T.M. Salter and *A. asparagoides*—are both highly polyphyletic in a fashion that is not associated with geography or intraspecific phenotypic variation (supplementary fig. S1, Supplementary Material online). The polyphyly among these Myrsiphyllum lineages may be indicative of a larger taxonomic problem involving the lumping of several cryptic species. Alternatively, *A. asparagoides* and *A. ovatus* may represent “ochlopecies” that formed through a process of repeated isolations and refusions among divergent populations driven by climatic shifts during the Pleistocene (Prance 1982; Bentz et al. 2024), or by rapid range expansion and colonization of new habitats without an allopatric phase, leading to a complex of variable intraspecific phenotypes/genotypes (Cronk 1998). Interestingly, two accessions of *A. asparagoides* (R. Boon 162 and 163) were sampled from different populations invasive to Australia and placed in disjunct clades within Myrsiphyllum; suggesting at least two separate introductions to Australia—each likely involving progenitors from the Western Cape of South Africa. Polyphyly was also observed among other taxonomically troublesome species including *A. setaceus* and *A. densiflorus* (supplementary results S1, Supplementary Material online), further illustrating the need for continued taxonomic and population-level work to better delimit species within the genus and elucidate the speciation process.

Major Clades of *Asparagus*

Previously, six major clades were hypothesized to encapsulate all *Asparagus* species diversity (Norup et al. 2015), including the following labeled clades: Racemose, Africani-Capenses, *Asparagus*, Lignosus, Setaceus, and Myrsiphyllum clades. Those six major clades were largely reinforced by morphological synapomorphies and biogeography (Norup et al. 2015) and plastome sequences (Bentz et al. 2024). The species tree analysis in this study also strongly supported those six previously defined clades (Norup et al.

2015; Bentz et al. 2024). However, increased species sampling in our analyses enabled splitting of the former Africani-Capenses clade into four distinct clades and allowed us to test taxonomic hypotheses generated over 100 years ago (supplementary results S1, Supplementary Material online). For instance, subgeneric taxon groups proposed by Obermeyer et al. (1992) were generally more congruent with the 12 major clades in Fig. 1 compared with those hypothesized by Baker (1896) and Jessop (1966) (supplementary table S3, Supplementary Material online). Our analyses also revealed congruence between the former Capenses and Lignosus clades from Norup et al. (2015) and the taxonomic series’ *Suaveolens* and *Retrofracti*, respectively, of Obermeyer et al. (1992); therefore, we refer to those clades as such in this study (Table 1; Fig. 1). The substantial species sampling in our analyses enabled delineation of an additional four major clades in *Asparagus* (i.e. Macowanii, Nutlet, Scandens, and Sympodioidi) which were defined based on phenotypic synapomorphies and strong support for monophyly (supplementary results S1, Supplementary Material online).

We found strong support for a sister relationship between the *Asparagus*–*Exuvialis* clade and the rest of the genus (Fig. 1), whereas previous studies either weakly (Norup et al. 2015) or strongly (Bentz et al. 2024) suggested that the Setaceus clade is sister to the rest of *Asparagus* and that the *Asparagus*–*Exuvialis* clade is more closely related to the Racemose clade (Norup et al. 2015; Bentz et al. 2024). Our analysis strongly supports a sister relationship between the Setaceus clade and an Africani–Sympodioidi–*Suaveolens*–Nutlet clade, which together form a larger clade that is equally related to three other African (bisexual) clades (see backbone polytomy with four leaves in Fig. 1). Bifurcations among the other major clades in *Asparagus* mostly resulted in poor support in previous studies, aside from moderate support for close relations between *Suaveolens* and Africani (Norup et al. 2015; Bentz et al. 2024).

Myrsiphyllum was the only major clade in our analyses with LPP < 1.0, which is in line with previous findings (Norup et al. 2015) in that inclusion of *A. fasciculatus* was weakly supported. It is possible that *A. fasciculatus* is not in fact part of the Myrsiphyllum clade, but rather a distinct lineage arising from a polytomous node subtending (i) the core Myrsiphyllum clade, (ii) *A. fasciculatus*, and (iii) the Scandens clade; however, that hypothesis was rejected in a polytomy test. Further, *A. fasciculatus* exhibits diagnostic traits typical of Myrsiphyllum, including connivent filaments and perianth segments (Malcomber and Demissew 1993). The ancestral flower form for the Myrsiphyllum clade likely included those diagnostic floral traits, which are ubiquitous across the clade, except in the species *Asparagus ramosissimus* Baker which exhibits spreading perianth segments and filaments (supplementary results S1, Supplementary Material

online). *Asparagus scandens* (Thunb.) Oberm. of the Scandens clade is another taxonomically troublesome species, and due to multiple shared traits (e.g. flattened, leaf-like cladodes), *A. scandens* is often lumped into *Myrsiphyllum* (formerly genus *Myrsiphyllum* Willd.) (Malcomber and Demissew 1993). However, unlike its sister lineage, *Asparagus mollis* (Oberm.) Fellingham and N.L. Mey., *A. scandens* has spreading filaments and perianth segments (Malcomber and Demissew 1993). Due to shared morphology and weak support in phylogenomic analyses (LPP = 0.83), it is possible that the Scandens clade is sister to *Myrsiphyllum*, but our results failed to show statistical support for that hypothesis (supplementary fig. S3, Supplementary Material online). According to our analyses, the most probable relationship between the *Myrsiphyllum* and Scandens clades was a polytomy including two additional major clades (Fig. 1).

Origin of Cultivated Garden Asparagus and Implications for Breeding

Garden asparagus (*A. officinalis*) was cultivated ~2,000 to 2,500 years ago (Moreno-Pinel et al. 2021) and has since been widely naturalized around the globe (POWO 2024), resulting in dubious reports of its “natural” range (Kay et al. 2001) which remains unknown. However, studies have shown that contemporary garden asparagus cultivars significantly differ genetically from putative wild populations in Turkey and Iran (Geoffriau et al. 1991; Sarabi et al. 2010), suggesting a center of origin in western Asia. To test the geographic origin of *A. officinalis*, we used the extant ranges of its closest relatives to estimate the ancestral, predomestication range of the domesticated species. *Asparagus persicus* Baker, *Asparagus pseudoscaber* Grecescu, and *A. officinalis* form a well-supported polytomy (garden asparagus clade: Table 2) sister to *Asparagus palaestinus* Baker (Fig. 2). The estimated ancestral range of the garden asparagus clade was the western Asia/Mediterranean Basin region (100% probability: Table 2), which is where the closest extant relatives of *A. officinalis* occur and is generally in line with previous hypotheses that also implicate the Caucasus region as a possible center of origin (Ellison 1986). A greater understanding of interspecies genetic relatedness is crucial to focused breeding efforts aimed to improve the economically important *A. officinalis*, due to its relatively low genetic diversity in breeding programs (Štajner et al. 2002) and absence of valuable agronomic traits that are more common in wild *Asparagus* (e.g. disease resistance and salt, drought, and acid soil tolerance) (Venezia et al. 1993). The robust species tree inference from this study will contribute to future breeding efforts, for either vegetables (e.g. *A. officinalis*) or herbal medicinal purposes (e.g. *Asparagus racemosus* Willd. and *A. cochinchinensis*) (Kanno and Yokoyama 2011), by lending insight into divergence of candidate material for interspecific crosses.

Supplementary Material

Supplementary material is available at *Genome Biology and Evolution* online.

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Author Contributions

P.C.B. conducted the experiments, analyzed the data, and wrote the manuscript. P.C.B., J.E.B., S.M.B., E.M., Z.M., and J.L.-M. conceived the study. P.C.B., J.E.B., S.M.B., Z.M., and J.L.-M. developed the experimental designs. Z.L., J.-B.Y., M.P., O.S., and G.P. contributed to the early design and analysis. All authors contributed to data collection/generation and manuscript edits.

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Conflict of Interest

No competing interests were reported for any of the authors.

Data Availability

All relevant result files and original scripts from this study are available at <https://zenodo.org/doi/10.5281/zenodo.10804898>. Sequencing reads from this study were deposited in the NCBI Sequence Read Archive under the BioProject numbers PRJNA1088837 and PRJNA1088858.

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