

Does size matter? The integrated roles of light, adaptive sex expression, and hybridization in a widespread *Arisaema* (Araceae) species from western Japan

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Abstract

Background and aims – Reproductive plasticity and hybridization are known to play key roles in local adaptation and range expansion in plants, yet the environmental conditions under which canonical size-dependent models of sex expression break down remain poorly understood. *Arisaema serratum* (Araceae), a widespread species in Japan, has long been characterized as exhibiting size-dependent sex determination, whereby individuals transition from producing staminate to pistillate inflorescences upon reaching a size threshold. Field observations from western Japan further suggest that sex expression in *A. serratum* may be decoupled from plant size, raising the possibility that environmental cues such as light availability override intrinsic size thresholds in some populations. Because such ecological flexibility can increase spatial and temporal overlap among sympatric congeners, we additionally evaluate whether relaxed sex determination may coincide with increased introgression within this species complex.

Materials and methods – We conducted field-based demographic surveys across five populations of *Arisaema* on Shikoku Island, Japan, quantifying plant size, sex expression, and light intensity to evaluate environmental and morphological predictors of reproductive phase. To assess hybridization, we generated genome-wide SNP data through genotyping-by-sequencing, constructed a maximum likelihood phylogeny, and performed f-branch analyses to detect patterns of introgression among sympatric species.

Key results – Our data indicate that size in fact may not be the key factor in sex determination, with light availability emerging as a stronger predictor of sex expression than size alone for populations on Shikoku Island. Preliminary genomic analyses support evidence of gene flow between *A. serratum* and sympatric species, indicating ongoing hybridization and introgression.

Conclusions – Our findings indicate that the shift away from a strict allometric model of sex determination on Shikoku Island to a photosensitive model allowing for diphasic sexual expression likely enhances reproductive flexibility and leads to an increase in seed production in open-canopy and disturbed environments. Together, these findings provide ecological and demographic insight contributing to the broad distribution, sex determination, and morphological diversity of *A. serratum*.

Keywords

Araceae, colonization, diphasic sexual expression, niche expansion, plasticity, Shikoku Island

INTRODUCTION

Species of *Arisaema* Mart. are perennial, tuberous geophytes that occur primarily as forest understory herbs, persisting across years via a subterranean corm but

relying largely on sexual reproduction rather than clonal spread (Bierzychudek 1982; Murata 2011; Murata et al. 2018). Although typically associated with shaded forest interiors, many taxa, including *A. serratum* (Thunb.) Schott, readily establish in canopy gaps, forest edges,

and managed plantation forests where light availability is spatially heterogeneous (Kinoshita 1987; Masaki et al. 2017; Matsumoto et al. 2018). Pollination is mediated mainly by small dipterans attracted and temporarily trapped within the spathe, a system that can facilitate heterospecific pollen transfer when congeners flower synchronously, while seeds are dispersed locally by gravity and small animals into newly opened microsites (Atkinson 1898; Bierzychudek 1982; Murata and Ohno 1989; Kudo et al. 2008).

The development of sexual expression in plants, here defined as the production of male or female flowers by an individual, is often constrained by intrinsic factors such as plant size and resource availability (Policansky 1981). In sexually diphasic species, these constraints can influence or trigger transitions between reproductive phases. In *Arisaema* (Araceae), sex expression has traditionally been considered size-dependent, with smaller individuals producing staminate (male) flowers and larger individuals producing “pistillate” or ovulate (female) flowers, due to the higher energetic demands associated with female function (Policansky 1981; Bierzychudek 1982). This pattern is supported by early morphological and demographic studies in North American congeners such as *A. triphyllum* (L.) Schott and *A. dracontium* (L.) Schott, where sex changes are linked to plant size (Atkinson 1898; Camp 1932; Doust and Cavers 1982; Ewing and Klein 1982; Clay 1993), nutrient status (Schaffner 1922), and age (Gow 1913; Camp 1932). Similar trends have been documented in several Japanese *Arisaema* species. For example, individuals of *A. urashima* H.Hara are reported to transition from male to female flowering with increasing plant size (Takasu 1987). The same size-dependent model has been applied to *A. japonicum* Blume (now considered a synonym of *A. serratum*), with early studies noting a general male-to-female transition in flowering accompanied by increases in plant age and plant size under controlled settings (Maekawa 1924, 1927). However, these studies were conducted under homogeneous greenhouse conditions, limiting their ability to assess the influence of natural environmental gradients.

More recent field-based work by Kinoshita (1987) provides explicit support for a size–sex relationship in *A. serratum* populations from Nagano Prefecture in north-central Japan. This study documented that larger plants were more likely to produce female flowers, consistent with the canonical size-dependent model. Yet, this pattern may not be universal. Preliminary field observations of *A. serratum* populations on Shikoku Island in western Japan suggested an absence or weakening of this correlation, with female-flowering individuals frequently observed across a broad range of plant sizes, including individuals below traditionally inferred size thresholds. These observations prompted a re-evaluation of the relative roles of intrinsic (size-based) and extrinsic (environmentally induced) cues in determining sex expression in this variable species.

The size-dependent model of sexual expression has been widely documented across plant families and is thought to optimize reproductive success under stable ecological conditions (Charnov and Bull 1977). However, in disturbed environments, where rapid colonization is favoured, strict size-dependent sex expression may be maladaptive (Dorken and Barrett 2004). In these situations, environmental parameters might select for, or result in the selection of, greater plasticity in reproductive strategies (Barot et al. 2005) including the timing of transition between the production of male vs female flowers.

Disturbance regimes, particularly those affecting canopy structure and light availability, can substantially alter reproductive dynamics in forest understory plants by modifying patterns of resource allocation and growth (Protchazka et al. 2024). In species capable of facultative or environmentally sensitive sex expression, external cues such as light intensity may override or interact with intrinsic, size-dependent factors to determine reproductive phase transitions (Obeso 2002). Light availability is especially critical in shaping fitness within early successional or plantation forests, where fluctuating microsite conditions favour rapid growth and reproductive responsiveness (Lichstein et al. 2021). In such contexts, sexually diphasic plants that relax their dependence on intrinsic size thresholds and respond directly to environmental conditions may gain a selective advantage, particularly in shaded or periodically disturbed landscapes common throughout western Japan.

Across many plant lineages, ecological expansion into novel or transitional habitats can also increase opportunities for interspecific hybridization. Species capable of colonizing light-variable, post-disturbance environments often come into secondary contact with congeners occupying adjacent native forests, creating conditions conducive to gene flow and introgression (Ellstrand and Schierenbeck 2000; Rieseberg et al. 2007). When such contact zones coincide with individuals exhibiting flexible or asynchronous sex expression, the likelihood of heterospecific pollen transfer may increase, facilitating admixture and the emergence of hybrid lineages.

The interaction between environmentally responsive sex expression and ecological disturbance may therefore have broader evolutionary implications. Morphological observations alone cannot determine whether apparent intermediacy among sympatric *Arisaema* taxa reflects phenotypic plasticity, shared ancestral variation, or genetic introgression, limiting inferences based solely on form. Because environmentally responsive sex expression may increase flowering synchrony and microsite overlap among congeners under variable light regimes, we evaluate introgression not as a predictor of sex expression itself, but as a potential evolutionary outcome of this reproductive flexibility in the plantation and secondary-forest landscapes of western Japan.

In this study, we test how environmental and morphological factors interact to shape reproductive phase transitions in *Arisaema serratum*, a widespread Japanese species commonly found in post-disturbance evergreen plantations and secondary forests (Matsumoto et al. 2018). Specifically, we evaluate whether light availability serves as a stronger predictor of sex expression than plant size—challenging the traditional size-dependent model—and whether this ecological flexibility may contribute to the species' success in dynamic, human-modified landscapes. To explore potential evolutionary consequences of this flexibility, we also analyse genome-wide SNP data as an exploratory framework for detecting possible gene flow between *A. serratum* and sympatric congeners. Together, these approaches aim to clarify how environmentally responsive reproductive strategies may facilitate both local adaptation and the maintenance of porous species boundaries in forest understory taxa.

MATERIAL AND METHODS

Sampling

Field sampling was conducted from 2–20 May 2024 across five populations on north-western Shikoku Island, Japan (Kugawa (n = 71), Ishizuchi (n = 24), Kumakogen (n = 95), Kamigawa (n = 84), and Ozu (n = 31)). These sites were selected to encompass the regional range of *Arisaema serratum* and closely related congeners occurring in managed plantation and secondary-forest habitats. All populations were situated within mixed evergreen conifer plantations dominated by *Cryptomeria japonica* (Thunb. ex L.f.) D.Don and *Chamaecyparis obtusa* (Siebold & Zucc.) Endl., landscapes shaped by post-World War II reforestation and periodic thinning. These management practices create heterogeneous light environments with canopy gaps and intermittent ground disturbance, conditions representative of much of western Japan's low- to mid-elevation forests.

At each site, all flowering *Arisaema* individuals encountered along 100–250 m transects were recorded and identified to species following Murata (2011) and Murata et al. (2018). Sampling captured the full range of vegetative and reproductive morphologies present within each population. The five study sites spanned elevations from approximately 250 m (Ozu) to 1050 m (Ishizuchi) and included both interior forest and edge microhabitats. All sites occurred within mixed evergreen conifer plantations dominated by *Cryptomeria japonica* and *Chamaecyparis obtusa*, with occasional secondary broadleaf elements such as *Castanopsis cuspidata* Schottky and *Acer sieboldianum* Miq. Across all localities, we measured and recorded a total of 305 flowering individuals (*A. serratum* (n = 114), *A. ehimense* J.Murata & J.Ohno (n = 56), *A. tosaense* Makino (n = 86), *A. ringens* (Thunb.) Schott. (n = 30), and *A. iyoanum* Makino (n = 19).

Morphological and light availability measurements

For each flowering individual, plant height (cm; measured from soil level to the apex of the tallest leaflet) and pseudostem base diameter (cm; measured at the midpoint of the pseudostem formed by petiole sheaths) were recorded as indicators of vegetative size from live plants. Within each species and site, we aimed to capture a wide range of morphological variation, and sampling included individuals spanning the full observed spectrum of plant height and pseudostem diameter present at the time of survey, given that an inflorescence was present for sexual classification. This approach minimized sampling bias by size class by ensuring that both smaller and larger sexually mature individuals were proportionally represented within each population.

To estimate microsite light availability, photosynthetically relevant light intensity (kilolux) was measured at each individual's position using a URCERI MT-912 digital illuminance meter. Measurements were taken on clear days between 10:00 and 14:00 local time to minimize temporal variation due to sun angle or cloud cover. The sensor was positioned 30 cm above the soil surface and oriented parallel to the plant lamina to approximate the ambient light environment experienced by each individual.

Individuals were categorized as staminate (male) or pistillate (female) based on the presence of respective floral morphs within the inflorescence. Only individuals bearing mature inflorescences were included in the analyses.

Statistical analyses

To evaluate the relative influence of size and light on sex expression, logistic regression models were implemented for each species using the `glm()` function in R v.4.2.1 (R Core Team 2021) with a binomial error distribution and logit link function:

sex ~ height + pseudostem diameter + light intensity

Sex was coded as a binary response variable (male = 0, female = 1). Model performance was assessed using Akaike's Information Criterion (AIC) and pseudo-R². Pairwise correlations among predictor variables (height × light, diameter × light) were tested using Pearson's *r* to ensure predictors were not collinear. Light availability was not strongly correlated with either plant height or pseudostem diameter (Pearson's *r* ≤ 0.11; *p* ≥ 0.28 across species), indicating low collinearity and supporting the inclusion of these predictors within the same model. All tests were evaluated at $\alpha = 0.05$. Models were specified a priori to include all biologically relevant predictors (plant height, pseudostem diameter, and light intensity), and inference focused on evaluating the relative contribution of each variable within a single multivariate framework rather than on stepwise model building or sequential model comparison.

Genomic data generation

To complement the ecological analyses, we generated exploratory genomic data to assess potential genetic relationships and gene flow among sympatric *Arisaema* species. Fresh leaf tissue samples were collected from individuals of *A. ternatipartitum* Makino (n = 1), *A. ringens* (n = 1), *A. ishizuchiense* Murata (n = 1), *A. sikokianum* Franch. & Sav. (n = 1), *A. iyoanum* (n = 1), *A. serratum* (n = 3), *A. ehimense* (n = 2), and *A. tosaense* (n = 1) and dried in silica. DNA was extracted using a CTAB-based method (Doyle and Doyle 1987), and fragments within the 5–10 kb range were selected using SPRIselect beads (Beckman Coulter 2012) through Solid Phase Reversible Immobilization (SPRI). Because of limited sample sizes, these genomic data are treated as exploratory rather than population-level estimates, intended to reveal qualitative patterns of relatedness and introgression rather than to infer demographic parameters. Likewise, we did not perform PCA or model-based genetic structure analyses, which require broader population-level sampling to yield stable and interpretable results.

Genotyping-by-sequencing (GBS) libraries were generated with the ApeKI restriction enzyme. Library preparation and sequencing were conducted at the University of Wisconsin Biotechnology Center DNA Sequencing Facility on an Illumina NovaSeq 6000 platform, producing 150 bp paired-end reads. Raw sequencing data were demultiplexed and analysed using Stacks (Catchen et al. 2013). All generated sequence data are publicly available on Zenodo (Scholten and Specht 2026).

Sequence data from all individuals were pre-processed using fastp (Chen 2023) before being aligned to the draft genome of *A. tosaense* (Scholten and Specht 2025) with the Burrows-Wheeler Aligner (BWA v.0.07.18) (Li and Durbin 2009). The alignment output in SAM format was converted to BAM, sorted, and indexed using SAMtools v.1.21 (Danecek et al. 2021).

Variant calling and genotyping were performed using GATK v.4.2.0 (McKenna et al. 2010). Genotypes were called in diploid mode, and both homozygous and heterozygous genotypes were retained in the resulting VCF. Because sequencing depth varied among loci, particularly across taxa represented by few individuals, variants were filtered to retain sites with a minimum genotype depth (DP) of 3 and minimum genotype quality of 50%. This threshold was selected to balance retention of informative heterozygous sites with locus recovery in a low-coverage GBS dataset, and to maximize the number of loci available for allele-sharing analyses.

The resulting VCF file was filtered to retain variants with minimum genotype quality scores of 50% and a sequencing depth of at least 3 using VCFR (Knaus and Grunwald 2017) and SNPfiltR (DeRaad 2022). While higher depth thresholds can increase confidence in

heterozygous genotype calls, exploratory analyses with stricter filtering (DP \geq 5) substantially reduced locus retention without qualitatively altering patterns of allele sharing; therefore, the more inclusive threshold was retained for downstream analyses.

Phylogenetic structure of genetic diversity

Phylogenetic structuring was used to quantify admixture and assess the influences of hybridization and gene flow between *Arisaema serratum* and sympatric species. The VCF file generated from genomic sequencing was converted into phylip format for maximum likelihood phylogenetic inference using vcf2phylip v.2.8 (Ortiz 2019). The resulting phylip alignment contained 165,325 SNPs after filtering across 11 taxa, 64.37% of which are invariant sites. A maximum likelihood (ML) phylogeny was generated for this dataset with RAxML-NG (Kozlov et al. 2019) using a GTR+G model to account for both substitution rate heterogeneity across sites and unequal base frequencies, 50 starting trees to ensure broad exploration of tree space and avoid suboptimal local maxima, and 200 bootstrap replicates to provide robust support values for inferred relationships and assess the stability of the phylogenetic topology (Pattengale et al. 2010). *Pinellia tripartita* Schott (Araceae) was included as an outgroup taxon for genomic library construction and downstream phylogenetic analyses; leaf material was collected concurrently with *Arisaema* samples on Shikoku Island and prepared using identical silica-drying and DNA extraction protocols, and the species was selected based on its well-supported placement outside *Arisaema* within Araceae (Murata et al. 2018). Ascertainment bias correction was not applied, as invariant sites were retained in the alignment and such correction is deemed unnecessary when the data include both variant and invariant positions (Kozlov et al. 2019).

To assess patterns of introgression and excess allele sharing among taxa, we used Dsuite (Malinsky et al. 2021), which implements Patterson's D-statistics and related f-statistics directly from VCF files. Using the maximum likelihood phylogeny as a guide topology, we first calculated D-statistics for all possible taxon quartets using the Dtrios module, which tests for asymmetries in allele sharing inconsistent with the inferred species tree. These statistics were then summarized using the f-branch (fb) statistic, which estimates the proportion of the genome exhibiting excess allele sharing between pairs of lineages and assigns this signal to specific internal branches of the phylogeny. This approach allows introgression to be visualized across multiple species simultaneously while accounting for shared ancestry and phylogenetic structure. Because of the small number of individuals per taxon, these analyses are exploratory and interpreted as indicative of potential admixture rather than definitive evidence of ongoing hybridization.

RESULTS

Sex expression in relation to plant size and light availability

Logistic regression analyses comparing plant sex (male vs female) and two size variables (plant height and pseudostem diameter) for each species demonstrates that both plant height (Fig. 1, column 1) and pseudostem diameter (Fig. 1, column 2) are significant predictors of sex expression in four of the five sampled species, with *A. serratum* being the only exception (Fig. 1A). In *Arisaema ehimense*, *A. tosaense*, *A. ringens*, and *A. iyoanum*, plant size is shown to be a strong predictor of sex expression (Fig. 1B–E), with shorter individuals bearing a thin pseudostem producing male flowers (left/top of graphs) and taller individuals exhibiting wider a pseudostem more likely to produce female flowers (bottom/right of graphs). The logistic regression between the individuals shows a steep curve ($P < 0.05$), indicating a significant correlation between sex (male vs female) and plant size (columns 1, 2).

In *A. serratum*, by contrast, there is no detected significant correlation between sex expression and plant size (Fig. 1A, columns 1, 2). Instead, logistic regression analyses for this species indicate that light intensity rather than plant size is the primary determinant of sex expression (Fig. 1A, column 3). The logistic regression reveals that in *A. serratum*, individuals exposed to higher light intensity are significantly more likely to express female reproductive structures ($P < 0.05$), whereas no significant correlation is found between sex and plant height (column 1) or pseudostem diameter (column 2). Correlation tests indicated that light availability was not strongly correlated with plant height ($r = 0.11$, $p = 0.28$) or pseudostem diameter ($r = 0.09$, $p = 0.34$), confirming that the observed relationship between light and sex was not simply an indirect effect of larger plants occupying brighter sites.

Phylogenetic structure of genetic diversity

The maximum likelihood (ML) phylogenetic tree constructed from genome-wide SNP data allows us to assess overall relationships among sampled individuals while also detecting levels of admixture that represent hybridization and/or ongoing gene flow. In our reconstruction, *A. serratum* does not form a clade, instead appearing as a grade with respect to other sampled species (Fig. 2B; Suppl. material 1). Bootstrap support values are high ($> 85\%$) for deeper nodes in the tree but lower ($< 50\%$) for relationships among closely related species, particularly among *A. serratum*, *A. ehimense*, and *A. tosaense*. The analysis also indicates that *A. ehimense* is polyphyletic, with its two defined morphotypes (red-appendix and green-appendix) appearing separately in the phylogeny. The red form is recovered as sister to *A. tosaense* while the green form is sister to the large clade

formed by the *A. serratum* grade plus the *A. tosaense*–*A. ehimense* red form sister pair. Neither of these relationships for *A. ehimense* is well supported (bootstrap < 55), indicating either incomplete lineage sorting or ongoing hybridization among the populations represented by these individuals.

Exploratory f-branch (fb) analyses were conducted to identify putative instances of gene flow among sampled individuals and to assess whether admixture patterns correspond with the observed phylogenetic relationships. The strength of admixture between pairs of taxa is visualized using shaded boxes (Fig. 2C), with darker hues indicating higher levels of excess allele sharing between species pairs. The dotted lines correspond to inferred ancestral branches, helping to contextualize observed admixture patterns within the context of the broader phylogeny. Our results reveal elevated allele sharing (fb > 0.025) between *A. serratum* and *A. ternatipartitum*, *A. ringens*, *A. ishizuchiense*, *A. sikokianum*, *A. iyoanum*, *A. ehimense*, and *A. tosaense*, suggesting a history of introgression within this regional complex (Fig. 2C). This suggests that hybridization is likely not limited to a single species pair but instead may be widespread across the sampled taxa, with *A. serratum* acting as a central contributor to gene flow. Although individual *A. serratum* samples showed qualitative differences in allele-sharing signals, all sampled populations occurred in similar plantation or secondary-forest habitats and exhibited broadly comparable sex-expression patterns, with no obvious population-level differences detected. Notably, *A. tosaense* also exhibits strong signals of excess allele sharing with multiple species, further suggesting that gene flow may be a recurrent process within this lineage and that there is widespread historical and/or ongoing introgression among taxa on Shikoku Island. Both morphotypes of *A. ehimense* show strong evidence of admixture with *A. serratum* (Fig. 2C), despite their polyphyletic position in the phylogeny.

DISCUSSION

Our results demonstrate that *Arisaema serratum* exhibits a relaxed dependence on plant size for determining sex expression, with light availability emerging as a more consistent predictor of reproductive phase among western Japanese populations. This contrasts sharply with the canonical size–sex relationship documented in other *Arisaema* species, including the four congeners analysed here, and challenges the long-held assumption that sex expression in this genus is strictly allometric (Atkinson 1898; Camp 1932; Doust and Cavers 1982; Ewing and Klein 1982; Takasu 1987; Clay 1993). In *A. serratum*, the probability of producing pistillate inflorescences increases under brighter microsite conditions, regardless of individual size, implying that light availability may act as an environmental cue overriding the intrinsic thresholds governing sex expression (Obeso 2002).

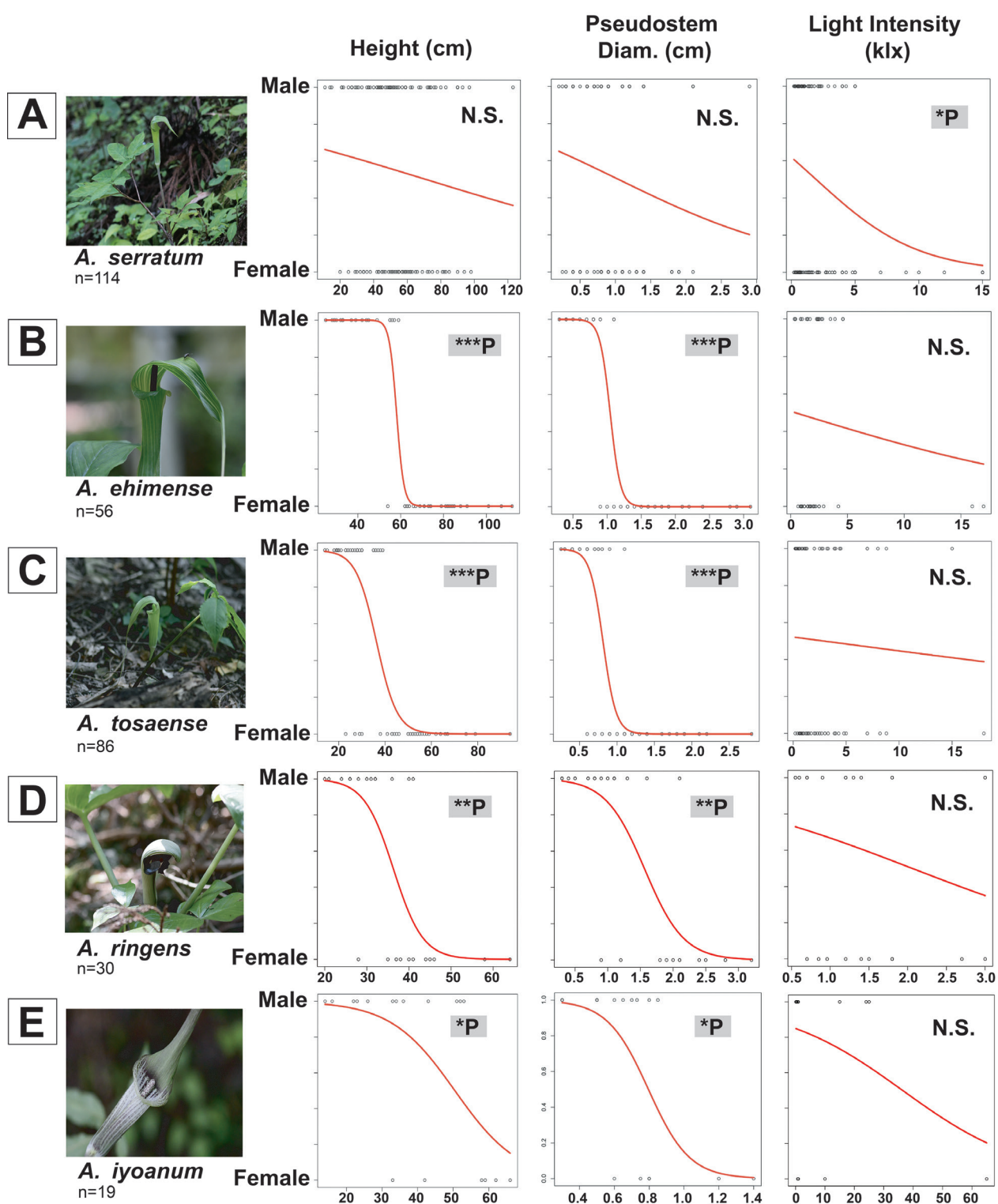


Figure 1. Pairwise logistic regression between plant sex and size/light intensity variables. N.S., *P, **P, and ***P indicate statistical significance at the levels of $P > 0.5$, $P < 0.05$, $P < 0.005$, and $P > 0.0005$, respectively. **A.** *Arisaema serratum*. **B.** *A. ehimense*. **C.** *A. tosaense*. **D.** *A. ringens*. **E.** *A. iyoanum*. Open circles in each plot indicate individuals, either Male (top) or Female (bottom), recorded at each height in cm (column 1), pseudostem diameter in cm (column 2), or light intensity in klx (column 3). The red line indicates the logistic regression curve with the pitch indicating the strength of differentiation between measurements pertaining to individuals producing either male or female inflorescences.

While similar light–sex relationships have been observed in *A. triphyllum* populations in high-light habitats of Ontario (Doust and Cavers 1982), those systems showed a strong correlation between light and plant size, making the causal driver ambiguous. In contrast, our analyses revealed no significant correlation between light intensity and either plant height or pseudostem diameter in *A. serratum*, suggesting that growth traits are not the proximate mediators of the light effect. This decoupling may reflect a shift toward direct physiological sensitivity to light in determining reproductive investment, a response that could confer adaptive advantages in patchy or periodically illuminated forest floors. For an understory geophyte such as *A. serratum*, this plasticity allows individuals to opportunistically reproduce when canopy openings increase resource availability, rather than waiting to achieve a fixed vegetative size threshold.

Our findings contradict early foundational work by Maekawa (1924, 1927), who reported that individuals of *A. japonicum* (now synonymized under *A. serratum*

(Scholten et al. 2025)) generally transitioned from male to female with increasing size and age. However, Maekawa's studies were conducted under homogeneous greenhouse conditions and did not assess the influence of light on reproductive expression. In fact, Maekawa himself acknowledged that energy assimilation and nutrient status likely played a role in sex transitions, hinting at complexity beyond mere size thresholds.

The departure from strict size dependence in *A. serratum* also provides an explanation for regional variation in reproductive strategies across Japan. Kinoshita (1987) found a clear size–sex correlation in *A. serratum* populations from Nagano Prefecture, where deciduous broadleaf forests dominate. In contrast, the evergreen conifer plantations and secondary mixed forests of Shikoku Island—characterized by reduced understory light and irregular canopy gaps (Ovington 1983)—present a markedly different selective environment. In such low-light systems, a reproductive strategy responsive to transient illumination may increase both individual

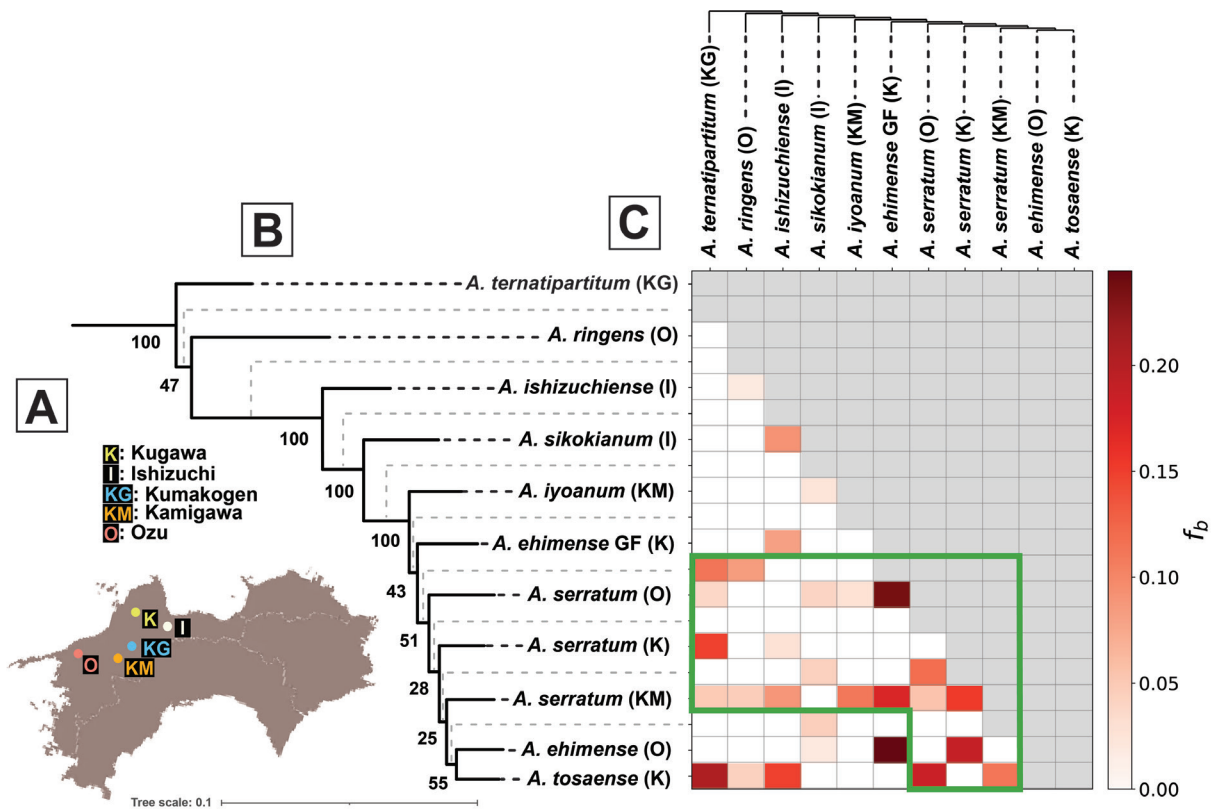


Figure 2. Assessment of admixture in *Arisaema* species on Shikoku Island, Japan. **A.** Sample locations on map of Shikoku Island. **B.** Maximum likelihood phylogeny of *Arisaema* individuals. Support values represented by bootstrap values. Branch lengths are proportional to the tree scale. Outgroup (not shown): *Pinellia tripartita*. Localities of collections indicated following the species name in parentheses with letters corresponding to abbreviations indicated on the map. “GF” after *A. ehimense* refers to the green appendix morphotype. **C.** Results from the *f*-branch analysis of excessive allele sharing. Grey dotted lines indicate internal (ancestral) branches of the phylogeny to which introgression signals are assigned in the *f*-branch framework. These ancestral branches are shown only to aid interpretation of the *f*-branch analysis and do not represent additional taxa or alternative phylogenetic relationships beyond those depicted in panel B. Shaded cells represent *f_b* values, with darker red indicating a higher proportion of the genome exhibiting excess allele sharing between a focal lineage and a given internal branch. Cells outlined in green highlight allele sharing involving *A. serratum*.

fitness and population persistence. These findings suggest that forest composition and management history can exert a strong influence over the evolution of reproductive plasticity in perennial understory plants.

The ecological consequences of such flexibility extend beyond individual fitness. Light-mediated sex expression likely facilitates colonization of plantation landscapes, where disturbance and canopy thinning create transient windows of high light. Increased reproductive output in these microsites can elevate local population density and the probability of contact among species. The co-occurrence of *A. serratum* with congeners such as *A. ehimense*, *A. tosaense*, and *A. ringens* within the same plantation mosaics provides numerous opportunities for heterospecific pollen transfer and hybridization. Our genomic analyses—although exploratory due to limited sampling—revealed extensive allele sharing among *A. serratum* and sympatric species, consistent with recurrent introgression. These results align with previous reports of hybrid individuals and morphologically intermediate populations throughout western Japan (Murata and Ohno 1989; Murata and Ohashi 2009) and indicate that anthropogenic habitats may serve as arenas for both ecological convergence and genetic exchange.

This pattern of admixture supports a causal sequence in which light-driven reproductive plasticity promotes colonization of plantation forests, thereby increasing opportunities for hybridization. Specifically, our demographic analyses show that sex expression in *A. serratum* is decoupled from plant size and instead strongly associated with light availability (Fig. 1A), a pattern not observed in sympatric congeners, indicating that ecological flexibility is a species-specific trait rather than a consequence of hybridization. In parallel, phylogenetic and f-branch analyses reveal widespread allele sharing between *A. serratum* and multiple sympatric taxa (Fig. 2B–C), consistent with recurrent gene flow occurring in the same disturbed, light-variable habitats where relaxed sex expression is observed. Together, these results favour an interpretation in which ecological flexibility enables range expansion and increased interspecific contact, after which gene flow among sympatric taxa reinforces variation and potentially facilitates adaptation, rather than hybridization acting as the primary driver of sex-expression plasticity. The frequent occurrence of morphologically intermediate individuals in plantation understories further supports this feedback, in which environmental heterogeneity, reproductive plasticity, and introgression operate jointly to maintain demographic and genetic diversity within the *A. serratum* complex (Fig. 3). While our data do not allow us to test whether introgression quantitatively modifies sex-expression strategies, the concurrence of relaxed size dependence and widespread allele sharing suggests that reproductive plasticity may facilitate opportunities for gene flow rather than result from it.

An intriguing question emerging from these findings is why sympatric species such as *A. ehimense*, *A. tosaense*, and *A. ringens* retain strict size-dependent sex

determination despite occupying similar habitats. One possibility is that their divergent phenology or differing energy reserves limit responsiveness to transient light changes, constraining the expression of plasticity. Alternatively, underlying hormonal or gene-regulatory mechanisms may differ among taxa, with *A. serratum* having a greater physiological sensitivity to photic cues. Future work should experimentally test these hypotheses through controlled shading and light-manipulation experiments, coupled with hormone assays to determine whether endogenous regulators such as gibberellins or cytokinins mediate the observed responses. Comparative transcriptomic or methylation studies could also identify candidate pathways underlying light responsiveness and reveal whether these mechanisms are shared or species-specific.

The genomic results presented here, though limited in resolution, underscore the evolutionary fluidity within this species complex. The parphyly of *A. serratum* relative to *A. ehimense* and *A. tosaense*, combined with widespread allele sharing, points to weak reproductive barriers and frequent historical gene flow. While the small number of individuals analysed prevents robust population-genetic inference, the congruence of morphological and molecular patterns strongly suggests that introgression is ongoing. Broader sampling across the geographic range of *A. serratum*—including both plantation and native-forest populations—will be essential to test whether hybridization rates are indeed higher in managed habitats and to disentangle incomplete lineage sorting from contemporary gene exchange. Such work could also clarify whether adaptive alleles associated with light sensitivity have experienced introgression across species boundaries, potentially linking hybridization to the evolution of such plastic sexual expression.

The unexpected polyphyly of *A. ehimense* in the phylogeny is particularly significant, as this study represents the first phylogenetic resolution of *Arisaema* species on Shikoku Island. The original description of *A. ehimense* by Murata and Ohno (1989) identifies two distinct morphotypes, one with a red appendix and another with a green appendix. Our analysis indicates that individuals with a green appendix do not form a monophyletic group with the more widely recognized red-appendix form. This suggests that the green-appendix morphotype, while superficially matching the morphological criteria of *A. ehimense*, may represent a hybrid between *A. serratum* and another species outside the *A. serratum*–*A. tosaense* hybrid complex. The f-branch analysis (Fig. 2C) supports this hypothesis, indicating one-way allele sharing with *A. serratum* and no other sampled species, suggesting that the second parental species was not included in our analysis. Further genetic sampling across Japan, targeting more individuals and taxa not included in this study, could help provide insight into the taxonomy of this species.

The increasing number of documented natural hybrids between *A. serratum* and its congeners (Murata and

Ohno 1989; Murata and Ohashi 2009) further supports the idea that hybridization and introgression significantly influence the genetic structure of *Arisaema* populations

on Shikoku Island. Collectively, our findings suggest that the demographic success of *A. serratum* across western Japan arises from the interaction of environmental

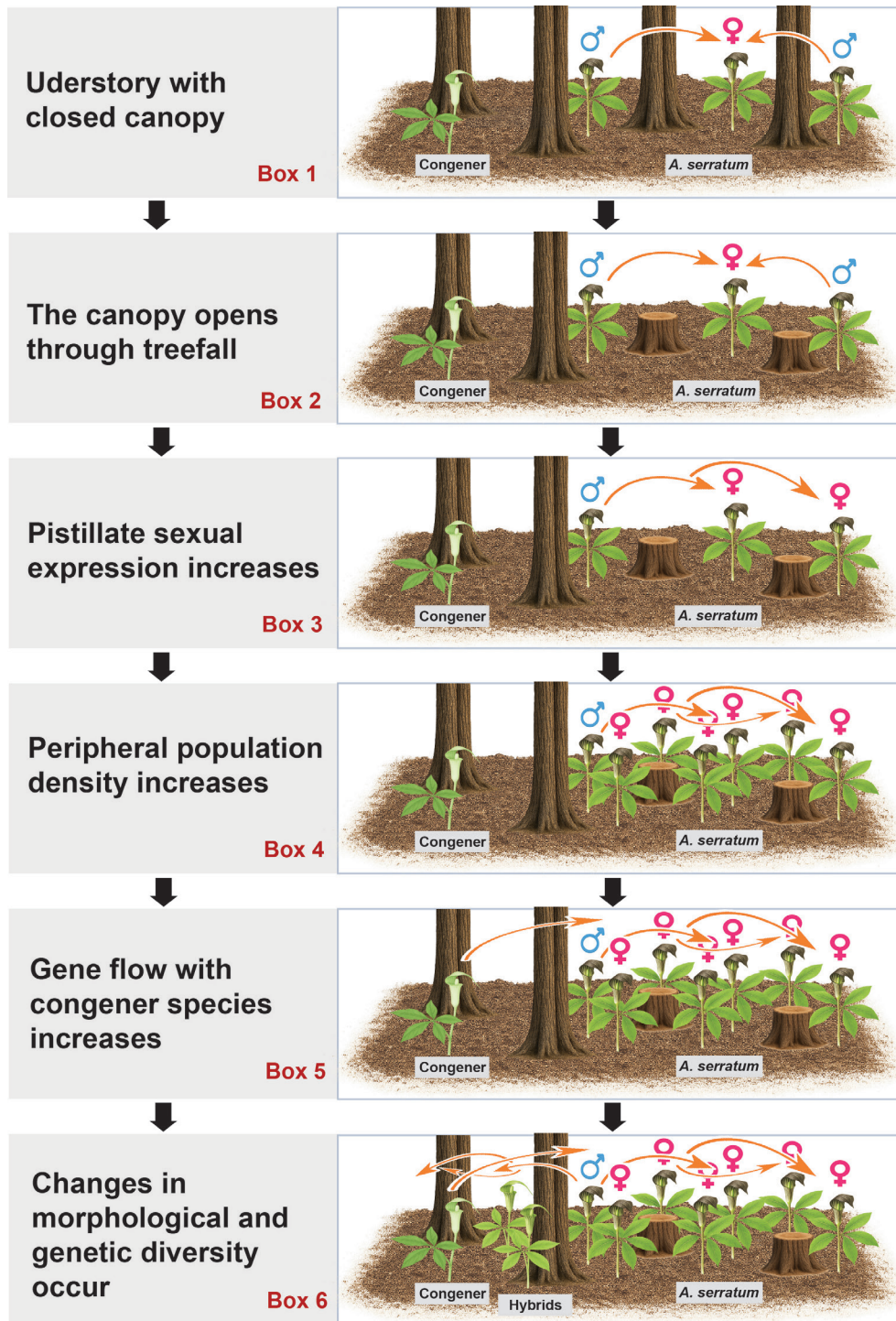


Figure 3. Conceptual model illustrating the ecological and evolutionary sequence linking light availability, reproductive plasticity, and hybridization in *Arisaema serratum*. Under a closed canopy, populations of *A. serratum* exhibit primarily staminate (male) expression, and interspecific contact with congeners is limited (Box 1). Following canopy disturbance (e.g. through treefall) (Box 2), increased light availability promotes a shift toward pistillate (female) expression (Box 3), enhancing local reproductive output and seed production. As population density increases in canopy gaps and forest edges (Box 4), spatial and temporal overlap with congeners expands, elevating opportunities for heterospecific pollen transfer (Box 5). Gene flow between *A. serratum* and sympatric species leads to introgression, generating morphological intermediates and increased genetic diversity (Box 6). Orange arrows represent gene flow between individuals. Staminate and pistillate individuals are noted by (♂) and (♀), respectively.

heterogeneity, reproductive plasticity, and gene flow. By decoupling the reproductive phase from plant size and coupling it instead to light availability, individuals of *A. serratum* can exploit brief periods of high irradiance within plantation forests, ensuring reproductive output under otherwise limiting conditions. This ecological strategy promotes persistence and expansion in anthropogenically altered habitats, while also facilitating contact and hybridization with congeners. The resulting feedback between ecological opportunity and genetic exchange likely contributes to the morphological diversity and phylogenetic complexity characteristic of this species. Future studies integrating demographic monitoring, pollinator observations, and expanded genomic datasets will be crucial for quantifying the fitness consequences of this flexibility and for understanding how human-modified environments continue to shape the evolutionary trajectories of *Arisaema* and other clonal, sexually plastic forest herbs.

CONCLUSIONS

Taken together, our results demonstrate that light availability, rather than plant size, is the dominant predictor of sex expression in *Arisaema serratum* populations on Shikoku Island. This shift from a size- to a light-dependent reproductive strategy likely reflects adaptation to the shaded, spatially heterogeneous conditions characteristic of evergreen conifer plantations. The decoupling of size and light effects suggests that reproductive transitions in *A. serratum* are triggered directly by environmental cues rather than by accumulated biomass, allowing individuals to reproduce under the variable light regimes typical of managed forests. These ecological patterns coincide with genomic evidence of allele sharing between *A. serratum* and several sympatric congeners, consistent with ongoing or historical introgression within plantation and secondary-forest mosaics. We therefore interpret reproductive plasticity as the initial driver of colonization in these modified habitats, with hybridization emerging as a secondary outcome of increased spatial overlap and flowering synchrony among species. Future work combining broader population sampling, experimental manipulations of light intensity, and higher-resolution genomic data will be necessary to test whether the observed plasticity is heritable, to quantify the direction and extent of gene flow, and to clarify how anthropogenic forest structure continues to influence reproductive and evolutionary dynamics in *Arisaema*.

DATA AVAILABILITY

All sample sequence data, the multisample VCF, and the logistic regression matrix are openly available for download from Zenodo (<https://doi.org/10.5281/zenodo.18163207>). Additional supporting information may be found online in Suppl. material 1.

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SUPPLEMENTARY MATERIAL

Supplementary material 1

Maximum likelihood phylogeny of *Arisaema* species. Support values are represented by bootstrap values. Branch lengths are proportional to the tree scale. Outgroup: *Pinellia tripartita*. “GF” refers to the green appendix morphotype of *A. ehimense*.

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