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RESEARCH ARTICLE

Range-wide study in a sexually polymorphic wild strawberry reveals climatic and soil associations of sex ratio, sexual dimorphism and sex chromosomes

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Abstract

1. Climatic and soil features influence resources and mate availability for plants. Because of different resource/mating demands of the male and female reproductive pathways, environmental variation can drive geographic patterns of sex-specific factors in sexually polymorphic species. Yet, the relationship between environment and sex, sexual dimorphism or sex chromosomes at the range-wide scale is underexamined.
2. Using ~7000 herbarium and iNaturalist specimens we generate a landscape-scale understanding of how sex ratio and sexual dimorphism vary with geographic, climatic and soil gradients in the sexually polymorphic wild strawberry (*Fragaria virginiana*) and test whether these conform to predictions from theory. Then, for ~300 specimens we use genotyping of the sex-determining region (SDR haplotypes) to reveal geographic and phenotypic patterns in sex chromosome types.
3. Across North America, the sex ratio was hermaphrodite/male-biased and was associated more with soil attributes than climate. Sex ratio-environment associations matched predictions for subdioecy in the West but for gynodioecy in the East. Climatic factors correlated with sexual dimorphism in traits related to carbon acquisition (leaf size and runnering while flowering) but not mate access (petal size, flowering time). Variation in sexual dimorphism was due to one sex being more responsive to the environmental variation than the other. Specifically, leaf length in females was more responsive to variation in precipitation than in hermaphrodite/males, but the probability of runnering while flowering in hermaphrodite/males was more responsive to variation in temperature than in females. The ancestral sex chromosome type was most common overall. But the frequency of the more derived sex chromosomes varied with environmental factors that differed between East–West regions.
4. *Synthesis*. A landscape-level perspective revealed that variation in soil and climate factors can explain geospatial variation in sex ratio and sexual dimorphism in a wild

strawberry. Variation in sex ratio was associated more with soil resources than climate, while variation in sexual dimorphism was the result of sex-differential responses to climate for vegetative traits but a similar response to abiotic factors in mate access traits. Finally, sex chromosome types were associated with soil moisture and precipitation in ways that could contribute to the evolution of sex determination.

KEYWORDS
community science, gynodioecy, herbarium, iNaturalist, landscape ecology, plant–climate interactions, sex chromosomes, subdioecy

1 | INTRODUCTION

Separate sexes (male and female) have evolved from hermaphroditism hundreds of times in flowering plant evolution (Renner, 2014), indicating that under some circumstances the benefits of sex specialization can outweigh the costs of reproductive uncertainty (reviewed in Pannell & Jordan, 2022; Spigler & Ashman, 2011). Indeed, there is a wide range of polymorphic sexual systems: gynodioecious (hermaphrodite and female), subdioecious (male, hermaphrodite and female) or dioecious (male and female), and these can form a continuum with intraspecific variation among populations within species (e.g. Costich & Meagher, 2001; Dorken & Barrett, 2003). Because the male and female reproductive pathways have different resource/mating demands, environmental variation can drive geographic patterns of sexual systems (reviewed in Varga & Soulsbury, 2020). Recent concerns over anthropogenic change in climate factors and soil fertility (IPCC, 2022; Penuelas et al., 2013; Singh et al., 2020) have led ecologists to call for a broader understanding of the abiotic drivers of the key features of sexually polymorphic populations, such as sex ratio, sexual dimorphism and sex determination (Hangartner et al., 2022; Hultine et al., 2016; Varga & Soulsbury, 2020). Yet, for most sexually polymorphic species the geographic relationship between-sex ratio and environment remains unexamined (Varga & Soulsbury, 2020) and evidence of clinal variation in sexual dimorphism or sex-determining factors is all but absent in plants (but see Bürli et al., 2022; Puixeu et al., 2019)—especially at the range-wide scale.

In sexually polymorphic plant populations, the sex ratio is determined by the reproductive fertility of each sex morph, the genetic mechanism of sex determination, and the degree of sex environmental lability (reviewed in Käfer et al., 2022; Schenkel et al., 2023; Spigler & Ashman, 2011). Given the lower energetic costs (Ashman, 1994; Obeso, 2002) of reproducing solely as a male (e.g. pollen production) than a female (e.g. ovule and seed production) in insect-pollinated plants, sex ratios can vary across gradients of environmental stressors (reviewed in Spigler & Ashman, 2011; Varga & Soulsbury, 2020). For instance, in gynodioecious species where hermaphrodites produce both pollen and as many seeds as females, they bear a higher reproductive cost than females. Female frequency is thus predicted to increase with increasing environmental stresses in gynodioecious species. For example, a survey of Illinois populations of *Lobelia spicata* demonstrated female frequency increased with increasing temperature stress (Ruffatto et al., 2015). Comparatively, in subdioecious and dioecious species, where males exist or hermaphrodites produce few seeds, the females bear the highest physiological demands of reproduction. In such systems, female frequency is predicted to decrease with increasing environmental stresses (Spigler & Ashman, 2011; Varga & Soulsbury, 2020). Yet across several subdioecious species, support for this hypothesis was mixed: female frequency increased with higher temperatures but lower water (Varga & Soulsbury, 2020). And while no environmental association was found in dioecious species (Varga & Soulsbury, 2020), higher female expenditure (and higher mortality [Marais & Lemaître, 2022]) can lead to a male-biased sex ratio, especially in long-lived iteroparous, clonal and fleshy-fruited dioecious species (Field et al., 2013). The difficulty of characterizing sexually polymorphic species as purely gynodioecious or subdioecious could also lead to murky environment-sex ratio associations. For these species, it is possible that a more complete landscape-wide view is needed to sufficiently capture variation that can reveal the underlying associations.

Sexual dimorphism of traits may also vary across environmental gradients because of sex-specific resource requirements. Sexual dimorphism arises from trait divergence in response to sex-specific adaptation or phenotypic plasticity, and these can also be context dependent (Ashman, 2005; Case & Ashman, 2007; Delph, 2019; Hangartner et al., 2022; Morgan & Ashman, 2003; Obeso, 2002). Specifically, traits may be under divergent selection through male and female fertility because of differential costs for these modes of reproduction (reviewed in Singh & Punzalan, 2018). For instance, females may be selected to invest more in leaves than males because more carbon is required to successfully mature fruit than to produce pollen (Ashman, 2005). In contrast, selection to ensure adequate mate access and pollen transfer may limit sexual dimorphism in traits like flowering time and petal sizes (Case & Ashman, 2007). When the local environment modifies the cost or the benefit of a given allocation pattern, selection and sexual dimorphism may change. For example, experimental modification of water availability impacts the direction of sex-specific selection on leaf size in dioecious *Silene latifolia* (Delph, 2019) and the degree of pollen limitation affects the strength of sex-specific selection on petals in experimental populations of *Fragaria virginiana* (Case & Ashman, 2007; Morgan & Ashman, 2003). Differences in sexual dimorphism can also arise when one sex is more responsive to environmental variation, and recent theory shows that sex-specific plasticity can promote population persistence (Hangartner et al., 2022). In *Vallisneria spinulosa*, females displayed higher plasticity than males in vegetative growth in response to water depth in aquatic mesocosms (Li et al., 2019), but *F. virginiana* hermaphrodite fruit production was more plastic than females' in response to experimentally manipulated resource availability (Spigler & Ashman, 2011). Either mechanism can lead to clinal variation in sexual dimorphism. For instance, in a common garden study, Puixeu et al. (2019) found genetic differentiation in sexual dimorphism in height and inflorescence size in *Rumex hastatulus* related to mean annual temperature at the source location. Likewise, Bürli et al. (2022) found that the degree of sexual dimorphism varied along climatic and elevation gradients in three wind-pollinated dioecious species due at times to greater environmental sensitivity of females.

Finally, environmental variation in sex ratio and sexual dimorphism could reflect geographic variation in genetic sex determination. Sex chromosomes are dynamic, and rapid changes in the sex-determining region ('SDR') can lead to polymorphism (types, haplotypes or races) within species (Palmer et al., 2019; Renner & Müller, 2021). Environmental stresses, such as those known to induce 'leaky' sex expression (e.g. temperature, drought, pollen limitation; Cossard & Pannell, 2021; Delph & Wolf, 2005) can also trigger evolutionary divergence of sex-determining mechanisms leading to variation along environmental gradients (Schenkel et al., 2023). Moreover, because sex chromosomes can be rich in variation for sex-specific adaptations (reviewed in Dean & Mank, 2014), sex chromosome variation may contribute to variation in sexual dimorphism. It is worth noting, however, that genes for sexually dimorphic traits can also be autosomal (Ashman, 2005; Lande, 1980; Spigler et al., 2011), and thus sexual dimorphism may not vary with sex chromosome type. Interestingly, Puixeu et al. (2019) found pronounced east–west geographic separation of the XY and XY1Y2 sex chromosome races of dioecious *Rumex hastatulus* as well as some, though not systemic, differences in sexual dimorphism between them. The majority of geographically widespread studies of intraspecific sex chromosome variation, however, have been performed in animals (e.g. Sniegula et al., 2022), leaving the generality of the Puixeu et al. (2019) findings an open question.

Most studies of environmental determinants of sex ratio or sexual dimorphism use only a small sample of geographically restricted sets of populations. A recent meta-analysis reported an average of six populations studied per plant species, and the highest sampled species (121 populations) did not cover the entire range (Varga & Soulsbury, 2020). These limited views restrict our ability to make inferences regarding sex-specific factors and their environmental drivers. Landscape-scale studies, however, can address how sex ratio and sexual dimorphism respond to climate and soil variation across broad and relevant sets of environmental gradients and thus offer a powerful means to address this limitation. Recent digitization of herbarium specimens and the exponential growth of iNaturalist observations have opened vast troves of data fit for novel range-wide exploration of these issues (Heberling, 2022; Heberling et al., 2021). Accordingly, we conducted the first ever continent-wide study of *Fragaria virginiana*, a widespread sexually polymorphic wild strawberry. We characterized ~15,000 herbarium and iNaturalist records to determine whether sex ratio and sexual dimorphism varied spatially or with climatic or soil gradients. We then used genotyping of SDR haplotypes to identify geographic and abiotic associations with known sex chromosome types for 172 herbarium samples, 47 germplasm accessions and 21 previously sequenced female plants. We explicitly tested hypotheses that (1) sex ratio (female frequency) correlates with environmental stress one of two ways: (a) increases (as predicted for gynodioecious species) or (b) decreases (as predicted for subdioecious species); (2) sexual dimorphism varies with environment in ways that reflect contrasting (resource acquisition) or similar (mate access) needs by the sexes; (3) SDR haplotypes are geospatially structured and contribute to observed variation in sex dimorphism and sex ratio.

2 | MATERIALS AND METHODS

2.1 | Study species

Fragaria virginiana (Rosaceae) is a widespread herbaceous perennial octoploid strawberry native to North America (Staudt, 1989). It vegetatively spreads via runners and sexually reproduces via 'seeds' (achenes) on fleshy animal-dispersed 'fruits' (receptacle tissue). It is sexually polymorphic, having populations that can contain combinations of females, hermaphrodites and/or males (hereafter the latter referred to collectively as 'hermaphrodite/male') and thus been described as subdioecious or gynodioecious (Ashman, 1999; Spigler et al., 2008). Female is the heterogametic sex (ZW), and sex is determined by a dominant female-specific male-sterility factor on W. Hermaphrodite/males are homogametic (ZZ) (Spigler et al., 2008). When hermaphrodite/males act as mother they only produce hermaphrodite/male progeny, while females produce 50:50 female: hermaphrodite/male progeny (Spigler et al., 2008). Sex chromosome turnover events led to three different chromosomes (VI-B2; VI-B1; VI-Av) housing three different SDR haplotypes (alpha, beta, gamma, respectively) (Tennessen et al., 2018). Their translocation history was inferred as alpha (ancestral, smallest), beta, and then gamma (most derived, largest) (Cauret et al., 2022; Tennessen et al., 2018). Sexual dimorphism in flower and vegetative traits has been observed in regional and greenhouse studies (Ashman, 1999; Spigler et al., 2011).

The most up-to-date taxonomic treatment (Hitchcock & Cronquist, 2018) recognizes two taxa: *F. virginiana* subspecies *virginiana* (including subsp. *grayana*) in eastern North America and *F. virginiana* subsp. *glauca* (including subsp. *platypetala*) in western North America. Moreover, south of the Canada/US border, the Great Plains create a natural gap in the distribution of *F. virginiana*, that likely also separates the two subspecies. Thus, we used the corresponding longitude of 102° W to distinguish the 'East' and 'West' regions of *F. virginiana*'s range but acknowledge that both genetics and environment contribute to any regional differences.

2.2 | Data collection

We collected data from two digital specimen sources: (1) herbarium sheets and (2) iNaturalist observations.

2.2.1 | Herbarium records

We collated imaged, digitized herbarium records of *F. virginiana* from online sources (iDigBio [https://www.idigbio.org/], the Global Biodiversity Information Facility [https://www.gbif.org/], Consortium of Intermountain Herbaria [https://intermountainbiota.org/] and the Consortium of Pacific Northwest Herbaria [https://www.pnwherbaria.org/]). All images were identified as *F. virginiana* and less than 5% had a subspecies designation. We recorded location information (GPS coordinates) and collection date. For records that did not have coordinates, we inferred location as the geographic centroid of the collection county. Centroids were generated by the *map* function within the *maps* package in R version 4.3.1 (Becker et al., 2022) and were extracted via the *centroid* function from the *geosphere* package (Hijmans et al., 2017). Records missing dates or location were excluded. In sum, we retained 6557 herbarium records collected between 1893 and 2021.

2.2.2 | iNaturalist records

We downloaded all research-grade observations of *F. virginiana* in North America between January 2017 and March 2022 with location information ($N=8302$). Observations on the iNaturalist platform are considered 'research-grade' when >2/3 of community users agree on the species identification. All images were identified as *F. virginiana* and less than 2% had a subspecies designation.

2.3 | Sex and phenotype scoring

2.3.1 | Plant sex

We classified specimens as flowering (having at least one open flower) or not (vegetative, budding, or fruiting). From the set of herbarium records that contained a flowering plant, we scored hermaphrodite/male or female based on the presence or absence of swollen, pollen-filled and/or dehiscing anther sacs, respectively (Figure 1b,d). For herbarium sheets with more than one specimen, we scored the plant in the upper left corner. If this was obstructed, then we scored the plant to its right. The iNaturalist observation images containing flowering plants were similarly classified (Figure 1a,c). From both data sets, we removed images where plant sex was ambiguous due to obstructed flowering structures or poor image quality, or in the case of iNaturalist, included more than one sex. In total, 3461 (51%) herbarium specimens and 3376 (49%) iNaturalist observations remained.

FIGURE 1 Images of living (from iNaturalist; a, c) and herbarium preserved (b, d) flowers of hermaphrodite/male (a, b) and female (c, d) *Fragaria virginiana*.

2.3.2 | Flowering phenology and runnering

To estimate flowering phenology, we recorded 'flowering day-of-year' (flowering DOY) as Julian date (1–365) from the collection date. For the herbarium specimens we also recorded whether a flowering plant had a new runner forming or not. Runners (stolons) are modified stems and represent investment in vegetative growth.

2.3.3 | Flower and leaf size

To characterize flower and leaf size, we selected samples from all the available flowering herbarium records. These included all the females and an equal number of geographically stratified, randomly chosen hermaphrodite/males leading to 1001 measurable specimens. We used ImageJ (Schneider et al., 2012) to measure the length and width (mm) of a fully unfurled petal and the central leaflet of the largest leaf of the same plant selected in the sex scoring above. Organ length and width were highly correlated (petal: $r=0.75$; central leaflet: $r=0.90$; $p<0.0001$; $N=633$), and thus, we only analysed lengths to represent size. Leaf size is an indicator of investment in carbon acquisition, while petal size reflects investment in pollinator attraction (Ashman, 1999; Case & Ashman, 2007). While specimen drying can shrink organs and collection practices may contribute bias, conclusions from comparisons across the vast sample sizes of herbarium studies are robust (Heberling, 2022).

2.4 | Environmental descriptors

We collected long-term climatic conditions (10-year pre-collection means) for each scored herbarium record from the CRUTS (Climatic Research Unit gridded Time Series; Harris et al., 2020). We used the CRUTS data set because it includes climate estimates back to 1900, allowing us to approximate climatic conditions for the oldest samples included in the study. We aggregated climate variables that Varga and Soulsbury (2020) identified as important drivers of sex ratio for gynodioecious and subdioecious plants across 342 species. Consequently, these sets of climatic drivers of sex ratio serve as a baseline expectation or a set of a priori hypotheses to compare other plant systems against. Specifically, we generated 10-year pre-collection means of temperature (°C) and precipitation (mm/monthly) of the driest, warmest and coldest quarters for each record. We likewise extracted soil properties identified as from SoilGrids, a global dataset of soil properties at 250 m^2 resolution. We extracted estimates of total soil nitrogen content and bulk density of the fine earth fraction for each record at 5–15 cm, a relevant depth for strawberry plants. The soil bulk density is the mass of oven-dried soil divided by the fresh soil volume and reflects the suitability of a soil for root growth and soil permeability. Specifically, high values of bulk density indicate harder, more compacted soils that are typically less permeable to water and plant roots (Houlbrooke et al., 1997; Sun et al., 2020).

2.5 | Sex chromosome detection

From the set of herbarium records that contained a flowering female *F. virginiana*, we selected 298 specimens collected between 1950 and 2022 and received leaflet samples from their hosting herbaria (Table S1). We also collected leaf tissue from 47 female *F. virginiana* maintained by the USDA ARS National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon. DNA was extracted from 0.5 cm^2 of leaf tissue by Ag-Biotech Inc. (Monterey CA). Additionally, 21 female plants with SDR determination from Tennessen et al. (2018) were included in analyses.

2.5.1 | SDR haplotype genotyping design

We designed primers to (1) determine the presence of the female-specific sex-determining region (SDR) and (2) differentiate the three SDR haplotypes.

First, we designed a presence/absence PCR assay (Table S2) based on the characterization of the RPP0W gene by Tennessen et al. (2018) and Cauret et al. (2022) with Primer3 (Untergasser et al., 2012) as implemented in Geneious 9.1.8. The female-specific gene 60S-RPP0W is a retrotransposed paralog of an autosomal 60S-RPP0 gene embedded in the SDR and shared among all three known SDR haplotypes (Tennessen et al., 2018). In the octoploid strawberry genome, there are multiple copies of the autosomal 60S-RPP0 gene. We designed the primer pair to span a missing intron in 60S-RPP0W allowing us to differentiate autosomal and W-specific gene copies by amplicon length (Figure S1). Since only females have the retrotransposed, intron-less, copy of 60S-RPP0, but both female and hermaphrodite/males have the intron-containing gene copies, this primer combination allowed us to confirm the presence of the SDR in female accessions. In amplifications, the presence of autosomal 60S-RPP0 paralogs acted as an internal positive control because it amplifies an approximately 1000 bp amplicon in both female and hermaphrodite/male individuals. For detailed PCR conditions, see Methods S1.

Second, because the sequentially translocating SDR gene cassette retained additional souvenir flanking sequence (Tennessen et al., 2018), we can use the accumulation of flanking sequence on either side of the gene cassette to differentiate the locations of the SDR, and thus their haplotypes, using two PCR assays. We designed two primer pairs to span the 'genomic junctions' created by the insertion of the SDR gene cassette into novel genomic locations on chromosome VI-B1 and VI-Av using Geneious. The presence or absence of an amplicon in PCR assays using these primers allowed us to differentiate the alpha, beta and gamma SDR haplotypes. To determine the SDR haplotype of female *F. virginiana* specimens, we used two PCR assays to determine the presence of junctions created by the successive transpositions of the SDR gene cassette. For details of these two PCR assays, a positive control, validation procedure and PCR genotyping workflow see Methods S1.

In sum, of the 345 samples with DNA extracted, 206 were successfully genotyped (112 failed to amplify) and 38 of the genotype calls with no evidence of RPP0W were rescored for sex by two additional observers, and 23 of these were determined to be hermaphrodite/male. In total, 240 (including 15 no-SDR [13 from this study and 3 from Tennessen et al., 2018]) females with SDR determination also had data on location and flowering day of year; 53 (22%) genotyped herbarium records had flower and leaf traits recorded.

2.6 | Statistical analysis

We used generalized mixed effect models with the 'glmmTMB' package in R version 4.3.1 (Brooks et al., 2017; R Core Team, 2021) to determine spatial and environmental drivers of variation in *F. virginiana* sex ratio and sexual dimorphism. We fit separate models containing spatial and environmental predictors to avoid multicollinearity stemming from the naturally close relationship between space and environment (e.g. latitude and temperature). We z-transformed each continuous predictor variable to reduce variance inflation and its effects on parameter estimates; parameter estimates for continuous predictors are consequently reported as change in response variable per unit standard deviation. We assessed the impact of geographic region by including region

(East or West) as a categorical variable in all models and controlled for the effect of time by including 'collection' year of each record as a random intercept in all models. When we included data type (herbarium or iNaturalist) in the analysis as a random factor, there was little difference in model coefficients and no change in the sign of coefficients (Table S3). Thus, we report only the results with collection year in the models.

We used an information theoretic approach (Burnham & Anderson, 2002) to select the most informative combinations of spatial or environmental predictors from full models using the *dredge* function from the 'MuMIn' package in R version 4.3.1 (Barton, 2023). Full spatial models included terms for latitude, elevation and their interactions with region. Full environmental models included terms for mean temperature and precipitation of the warmest and coldest quarters, mean soil nitrogen density at 5–15 cm, mean bulk density of the fine earth fraction at 5–15 cm and the interaction between each environmental model term and region. All continuous model terms were also z-transformed to reduce variance inflation. For models of sex ratio, we fit binomial models of sex (coded as 0 = hermaphrodite/males, 1 = females). For all models of sexual dimorphism (petal length, central leaflet length, flowering DOY and probability of runnering while flowering), we included interaction terms of sex interacting with all other predictor terms in the model. The main effect of sex can indicate sexual dimorphism, and the interaction between sex and other predictor variables can indicate shifting sexual dimorphism along spatial or environmental gradients. For models of petal length, we included an additional term for central leaflet length in all models to account for overall plant size variation (Ashman, 1999).

When AICc-based model selection resulted in more than one model with ΔAICc <2, we selected the model that contained only the predictors common to other models with ΔAICc <2, essentially yielding the most parsimonious model from among the 'best' ranked models (Burnham & Anderson, 2002). After model selection, we assessed potential effects of multicollinearity on model coefficients by inspecting variance inflation factors (VIF) of each model term. No model terms had excessively high VIF values (>10) other than interaction terms and their constitutive main effects, which can safely be ignored.

To ensure residuals from GLMMs were spatially independent, we ran Moran's *I* tests on simulated quantile model residuals for all top-selected models using the *testSpatialAutocorrelation* function from the DHARMa package in R version 4.3.1 (Hartig, 2022), which indicated no significant spatial autocorrelation among model residuals (Table S4).

3 | RESULTS

3.1 | Sex ratio

Across North America (Figure 2), the sex ratio was hermaphrodite/male-biased, with only 19% of plants scored as females (1319 females, 5518 hermaphrodite/males). This bias was consistent across specimen sources (herbarium vs. iNaturalist: 18% [603/3461] vs. 21% [716/3376]). Sex ratio varied with latitude ($p < 0.002$), the interaction between region and latitude ($p < 0.01$), and with the interaction of region and elevation ($p < 0.05$) (Table S5). Female frequency increased substantially with latitude in the West (β West = 0.187 ± 0.064) but not the East (β East = −0.036 ± 0.101) and decreased at higher elevations in the East (β East = −0.448 ± 0.238), but not the West (β West = 0.008 ± 0.077; Figure S2). Climate variables did not significantly correlate with sex ratio. However, soil properties influenced the probability of being female and this varied between regions (region-bulk density $p < 0.04$; region-nitrogen $p < 0.02$) (Table S3). In the West, the probability of being female increased rapidly with increasing soil nitrogen and increased subtly with increasing water availability (i.e. declining soil bulk density) (Figure 3a,b). In the East, comparatively, the probability of being female increased with decreasing water availability (i.e. increasing soil bulk density). In the environmental model, there was also a significant overall main effect of region ($p < 0.002$) reflecting the higher frequency of females in the East than the West (21% vs. 16%).

3.2 | Sexual dimorphism

Sexual dimorphism was pronounced (Table S6): Across all measured plants, petals of females were 28% smaller than hermaphrodite/males ($N = 1001$, $p < 0.0001$, $\beta = 1.55$), yet their leaves were 7% larger ($N = 1001$, $p < 0.001$, $\beta = -2.989$), they flowered 6 days earlier ($N = 6837$, $p < 0.0001$, $\beta = 3.184$; Tables S3 and S6) and had 75% greater probability of runnering while flowering ($N = 3376$, $p = 0.001$, $\beta = 1.138$; Table S3). Interestingly, only geospatial and climatic factors affected sexual dimorphism and only in resource acquisition traits (leaf size, runnering while flowering; Figure 4; Table S3). There was greater sexual dimorphism in leaf size under wetter summers because female leaf size increased with increasing summer precipitation, while that of hermaphrodite/males did not ($N = 1001$, $p = 0.036$; Figure 4a). Sexual dimorphism in runnering while flowering was greater at lower elevations ($p = 0.002$, $\beta = 0.821$; Figure 4b), and lower latitudes ($p = 0.024$, $\beta = 0.316$; Figure 4c) and at higher winter temperatures ($p = 0.016$, $\beta = -0.294$; Figure 4d). Specifically, at lower elevations and lower latitudes females were more likely to runner while flowering than hermaphrodite/males (Figure 4b,c). Hermaphrodite/males were more responsive to increasing winter temperatures than females and runnered more during flowering than females when preceded by cold winters but runnered less than females when preceded by warmer winters (Figure 4d). These patterns were observed across both regions, but sexual dimorphism in runnering while flowering is greater in the West (females were 83% more likely to runner while flowering than hermaphrodite/males) than in the East where they runnered more similarly ($N = 3376$, $p = 0.004$, $\beta = 1.583$). In contrast, sexual dimorphism in mate access traits was unaffected by geospatial or environmental factors because the sexes responded similarly

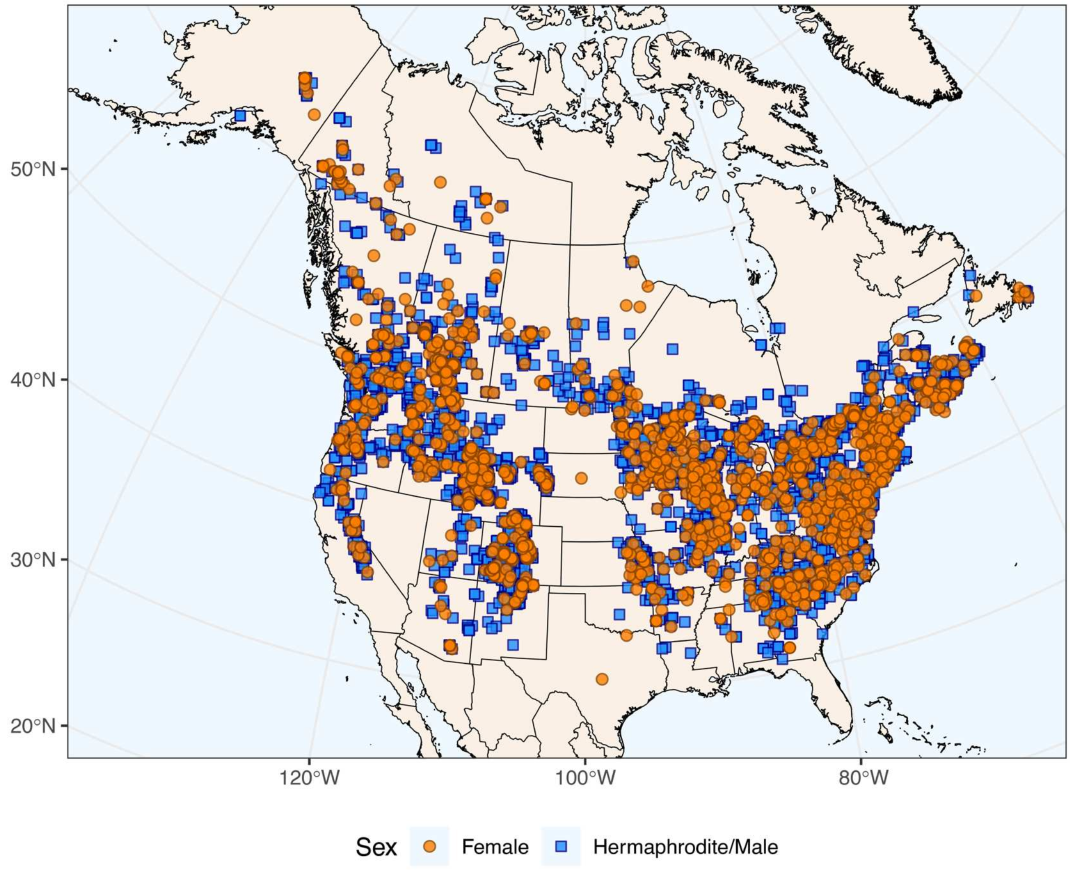

FIGURE 2 Distribution of hermaphrodite/male (blue square) and female (orange circle) *Fragaria virginiana* specimens in North America ($N = 6837$).

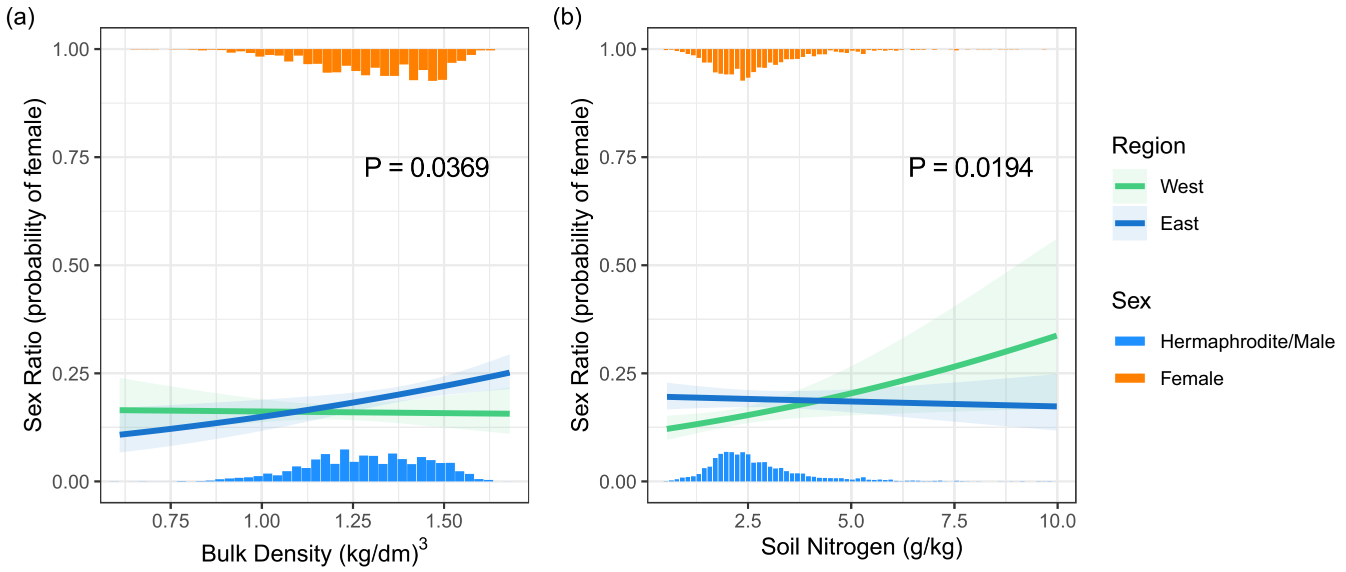

FIGURE 3 Relationships of sex ratio (probability of a specimen being female) with soil bulk density (i.e. decreasing water availability) (a), and soil nitrogen (b) by region (East: Dark blue; West: Green) of North America. Histograms along the top and bottom of plots indicate relative frequencies of females (orange), and hermaphrodites/males (blue) at each value of predictors; height of bars indicates only relative frequency of records and is not associated with values of the *y*-axis. *p*-values are for the region–environment interaction.

to this variation. Across both sexes, petal lengths increased with increasing nitrogen ($N = 1001$, $p = 0.047$, $\beta = 0.104$) and winter temperature ($N = 1001$, $p < 0.0001$, $\beta = 0.36$), but decreased with increasing elevation ($N = 1001$, $p < 0.0001$, $\beta = -0.446$; Table S3). Across both sexes, flowering day-of-year decreased with increases in all climatic and soil factors (Table S3).

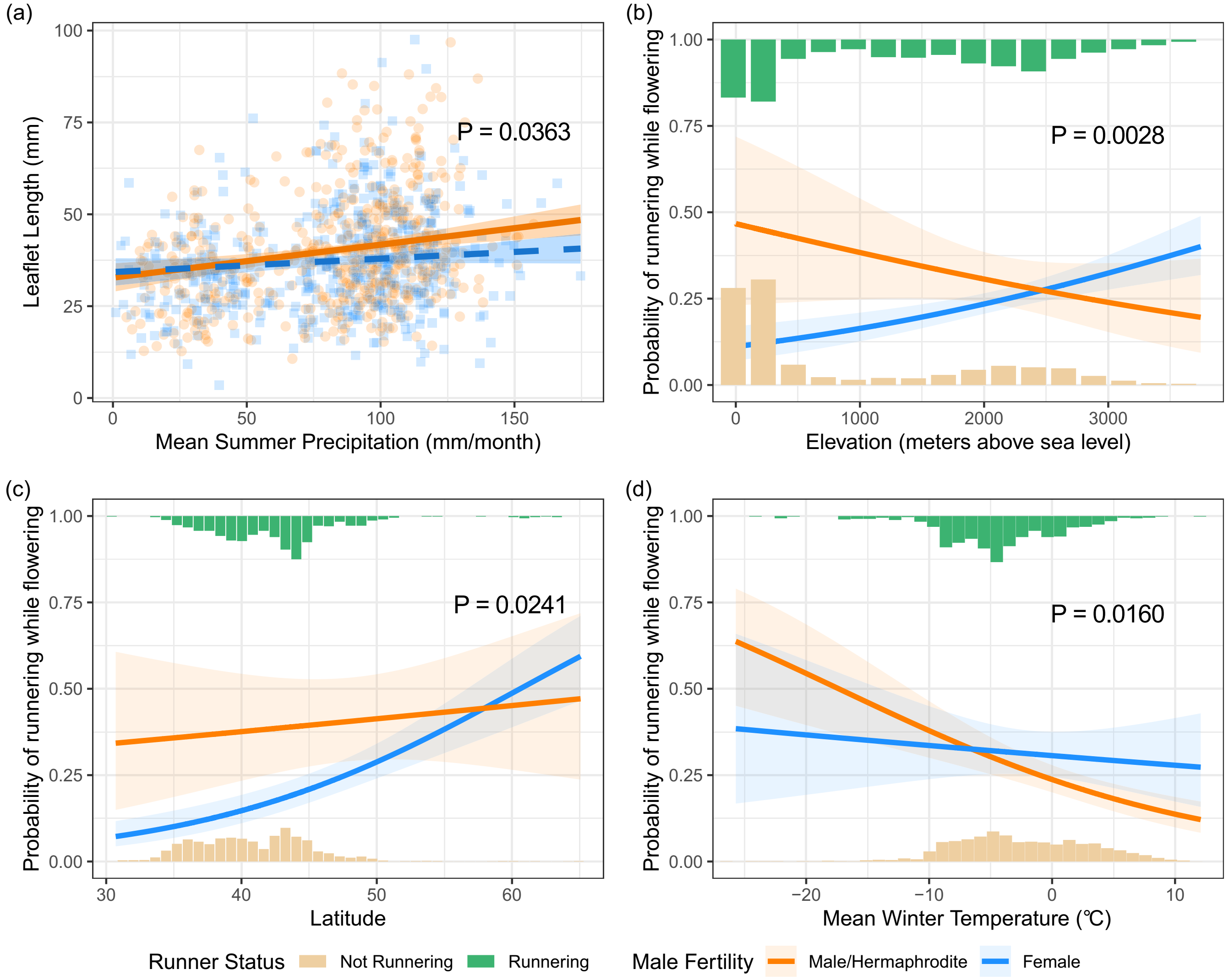

FIGURE 4 Pattern of sex dimorphism in leaf length (a) and runnering while flowering (b–d) varied with geospatial (elevation and latitude, b, d) and/or climatological factors (summer precipitation, a; winter temperature, d). Histograms of runnering [green] and not runnering [tan] are also shown (b–d). *p*-values are for the sex–environment interaction.

3.3 | Sex chromosomes

Of the females, successfully genotyped in this study 220 were resolved to a SDR haplotype. Successful genotyping was obtained for 58% of the 298 sampled herbarium specimens, including one collected in 1893. Thirteen of these were phenotypically confirmed to be female but had no evidence of RPP0W ('no-SDR females'). Across all the 240 genotyped females in our data set (including those from Tennessen et al., 2018), SDR haplotypes were biased toward alpha (70%) with beta and gamma each comprising 15% of genotyped females (Figure 5a). These showed a spatially heterogeneous pattern where the probability of a female having a gamma or beta haplotype was more likely in the West (37%) than in the East (26%) ($p < 0.005$) (Figure 5b). The probability that a female had a gamma or beta SDR haplotype also declined with increasing soil bulk density in the East but not in the West ($p = 0.015$) and declined with increasing summer precipitation in both regions but more dramatically in the West ($p < 0.005$; Figure 5c). The 15 no-SDR females were more common in the West than the East (10 vs. 5 out of 15) and appear to cluster to two mountainous regions, one in the Rocky Mountains and another in the southern Appalachian Mountains (Figure 5a). Only a portion of samples had phenotypic data (Tables S5 and S7), and although there appear to be some intriguing trends (e.g. gamma females flowered earliest, larger petals but smallest leaves; Table S7) statistical power is limited, and only flowering date was statistically testable and no significant variation among the SDR haplotypes was found (Table S5).

4 | DISCUSSION

In what we believe is the broadest and richest study of intraspecific variation in a sexually polymorphic plant, we revealed important range-wide associates of variation in sex ratio, sexual dimorphism and sex chromosomes. First, there is a general hermaphrodite/male bias in the sex ratio with spatial variation reflecting variation in soil attributes more than climatological ones. Second, while climatic

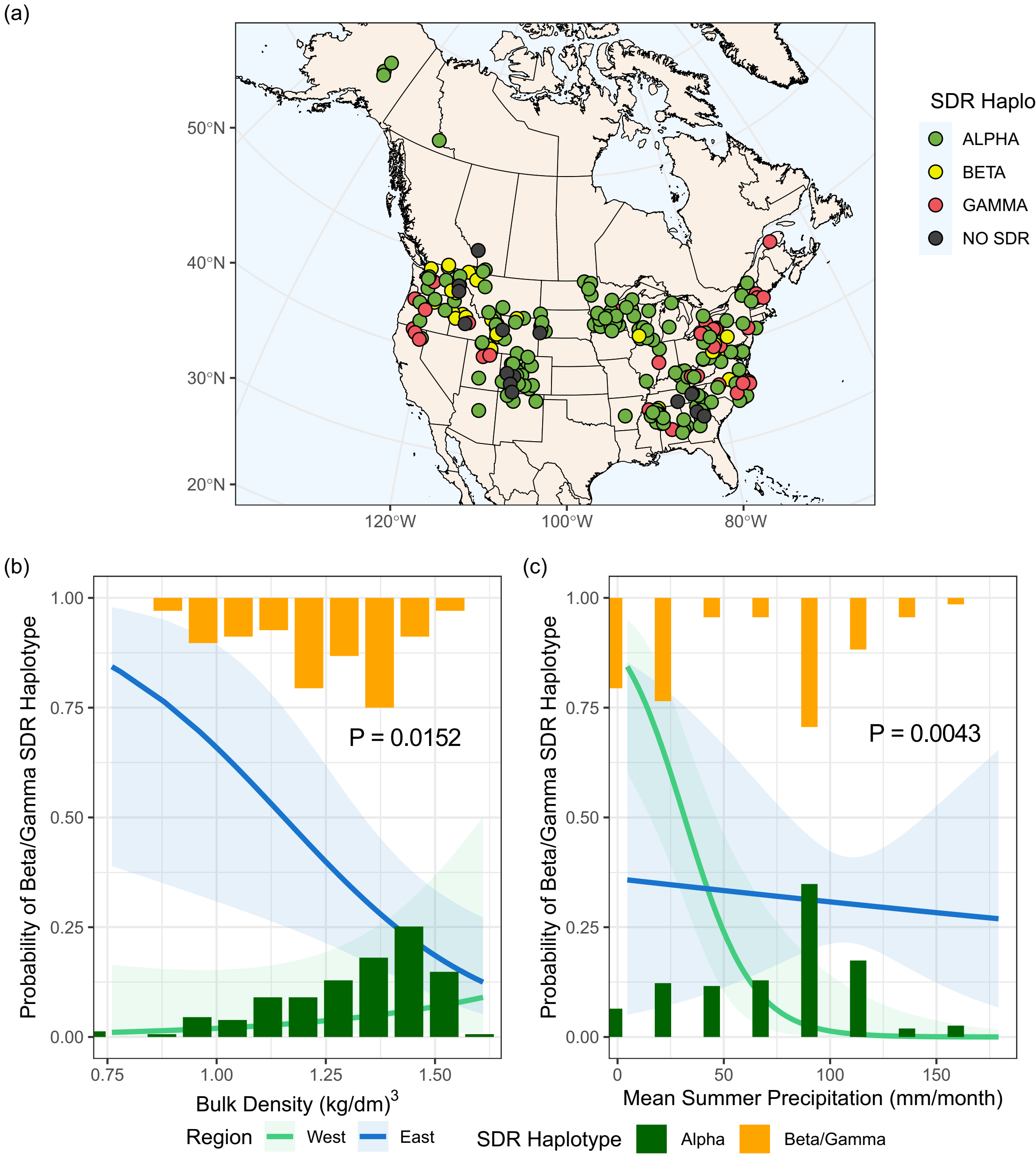

FIGURE 5 Spatial and environmental association of *F. virginiana*'s SDR haplotypes. Geographic distribution of SDR haplotypes (a) ($N=240$). Some point locations were slightly adjusted for visual clarity. Red = gamma; yellow = beta; green = alpha; 'no SDR' = black. Environmental associations of SDR haplotypes: Probability of being beta or gamma versus soil bulk density (b) and mean summer precipitation (c), by region (East: Blue; West: Green) of North America. Histograms of alpha [green] and beta or gamma [orange] are also shown (b, c). *p*-values are for the region–environment interaction.

factors lead to clines in sexual dimorphism in carbon acquisition traits (leaf size, runnering while flowering), those related to access to mates (petal size, flowering time) responded similarly to environment by the sexes leading to invariant sexual dimorphism. Third, landscape-level sampling of females exposed a SDR bias toward the alpha haplotype along with geographic and environmental associations for haplotype occurrence, and two clusters of females lacking the SDR. Yet we found no pronounced differences in traits among SDR haplotypes. We discuss each of these key findings in the context of theory. Finally, we consider how anthropogenic changes in these climate and soil features could impact sexually polymorphic species.

4.1 | Sex ratio

The sex ratio in *F. virginiana* across North America was hermaphrodite/male-biased (mean 19% female). Given that this is the first landscape-scale use of herbarium, germplasm and iNaturalist records for the purpose of sex ratio estimation, we compared our data to three studies that reported sex ratio from intensive population-level sampling: one of 17 populations in eastern Ohio and western Pennsylvania (Spigler & Ashman, 2011), one of 39 populations in Minnesota and Wisconsin (Stahler et al., 1995) and another with two populations in Oregon and Washington (Mortimer et al., unpublished data). Sex ratio from regional subsamples of our data centred on the locations of these studies (western Appalachia [PA/OH/WV; $N=956$], upper Midwest [MN and WI; $N=548$] was 24% females in each, and Pacific North West was 16% females), which are lower than the means of the population-level studies (29%; range of 16%–48% [Spigler & Ashman, 2011]; 35%; range 4%–75% [Stahler et al., 1995]; 26%; range of 25%–33% [Mortimer et al., unpublished data]). Individual discrepancies might be explained by local site features, for example, Spigler and Ashman (2011) sampled populations only along abandoned railroads, which tend to be drier sites and have more females than wetter sites (e.g. Wilk et al., 2009). But what is most important is that the relative ranks of the sex ratios among sites ($rs=1$) remain the same whether based on ground-truth population-level sex ratios or herbarium/iNaturalist data. Given that wild strawberry is an herbaceous perennial that can live decades, it makes sense that sex ratios may remain stable for many years. In fact, there was no temporal trend in the sampled data here (herbarium records: $p=0.432$, $\beta = -0.0011$, $z=-0.786$, $N=3376$; iNaturalist records: $p=0.834$, $\beta=0.0069$, $z=0.209$, $N=3461$). Proportion of females could simply be underestimated owing to the nature of herbarium specimens and iNaturalist observations (Heberling, 2022). And while Yang et al. (2022) surmised that herbarium collectors were likely biased toward early flowering (male) plants of *Lindera obtusiloba*, this explanation is not applicable here because females were the earlier flowering and the under-sampled sex (Tables S3 and S6). However, herbarium collectors and iNaturalist observers do typically document only a single or few individuals from any given locality, a practice that increases the likelihood of missing rarer, female plants that are observed when intensive site-level sampling is conducted. Thus, we acknowledge this as an additional note of caution to the use of specimen data for studies of morph frequency. However, we also want to emphasize the relationships of traits (and sex ratio) with environmental variables are of greatest interest and there is a robust literature demonstrating that iNaturalist and herbarium observations can accurately predict environmental trends in plant traits (e.g. Perez et al., 2020; Ramirez-Parada et al., 2022).

Both genetic and environmental variation can contribute to sex ratio variation. First, variation in genetic determinants of sex could be at play. This is because sex ratio bias is predicted to increase with sex chromosome differentiation (i.e. female bias resulting from degeneration of the Y in XY systems; male bias in ZW; Field et al., 2013). However, *F. virginiana* sex chromosomes can still recombine, and the extent of degeneration is not known. So while it is difficult to test this prediction directly, we do know the 'age' and order of turnover events of the SDR haplotypes impacts the SDR size and the degree of differentiation of W from Z. Specifically, alpha is the 'original' location/smallest on the least differentiated W chromosome and gamma is the youngest/largest on the most differentiated W chromosome with beta in the middle (Tennessen et al., 2018). And we did find both a lower probability of the SDR being gamma/beta (versus alpha) (26% vs. 37%), and a slightly less hermaphrodite/male-biased sex ratio (79% vs. 84%) in the East than in the West. Moreover, genetic variation in seed production in the pollen-bearing morph can contribute to sex ratio, but because we do not have genetic markers for the known quantitative variation in female function of the hermaphrodite/males (Spigler et al., 2011) and we had little ability to assess fruiting in herbarium and iNaturalist data, we cannot evaluate the genetic contribution of seed progeny by hermaphrodite/males to sex ratio. Nevertheless, both of these genetic features are ripe for future geospatial study in *F. virginiana*.

Second, the environment is predicted to impact the sex ratio in sexually polymorphic species, but differently for gynodioecious and subdioecious ones (Varga & Soulsbury, 2020). Interestingly, in *F. virginiana*, we found support for environmental correlates of sex ratio that differed between East/West regions (Table S5). In the East, female frequency increased with increasing water stress (i.e. higher soil bulk density leading to lower water availability). This regional pattern matches previous population-level studies of *F. virginiana* in the East (PA populations: Ashman, 1999; Spigler & Ashman, 2011) and also follows the general pattern observed for gynodioecious species where hermaphrodites bear a higher reproductive cost than females (Varga & Soulsbury, 2020). In contrast, in the West, female frequency increased with increasing nitrogen (and water availability, i.e., lower bulk density), which is indicative of less nutrient stress; this is more in line with predictions for subdioecious or dioecious species where females bear higher reproductive costs than hermaphrodite/males (Spigler & Ashman, 2011; Varga & Soulsbury, 2020). This divergent environmental pattern may reflect different prevailing sexual systems in each region, different gene–environment interactions and/or different selective forces. As noted above, the West shows a greater frequency of gamma/beta SDR haplotypes, and these are associated with drier summers (Figure 5). A greater frequency of the 'larger' most derived SDR (Tennessen et al., 2018) may confer a more subdioecious sexual system or the West may lead to a greater mortality rate for gamma/beta SDR females. Studies of environment-dependent and sex-linked mortality with *F. virginiana* females with characterized (gamma/beta or alpha) SDR haplotype would be powerful ways to disentangle these possibilities.

4.2 | Sexual dimorphism

We uncovered previously unknown effects of environmental variation in sexual dimorphism for traits related to resource acquisition and also environmental similarity for sexual dimorphism for traits related to mate access. Although it is difficult to disentangle plasticity from genetically based dimorphism by observing natural variation, the clines in sexual dimorphism seen here appear driven by sex-differential sensitivity to the environment as has been seen in other studies (Bürli et al., 2022; Puixeu et al., 2019). Specifically, our results join the handful of studies of bioclimatic variation in sexual dimorphism showing that it can reflect sex differences in response in ways that may reflect contrasting needs for sexual reproduction, especially in terms of resource acquisition traits. In *F. virginiana*, sexual dimorphism in leaf length and runnering while flowering increased with wetter summers and warmer winter temperatures, respectively. The former was due to females being more responsive to environmental variation and the latter due to hermaphrodite/males being more responsive. Puixeu et al. (2019) found that temperature-based variation in sexual dimorphism in leaf production in dioecious *Rumex hastatulus* resulted from a slower rate of decline in mean leaf production with increasing annual temperature in females than males. However, they also found that greater sexual dimorphism in plant height at peak flowering was due to males being more responsive to increasing temperature than females. Bürli et al. (2022) found that changes in sexual dimorphism in plant size in *Rumex lunaria* were due to female plant size declining faster than in males in response to both warmer and more variable temperatures. Whereas in both *Urtica dioica* and *Salix helvetica*, Bürli et al. (2022) found variation in sexual dimorphism in vegetative traits along environmental gradients was caused by a change in males combined with a lack of change in females. As an interesting counterpoint to variable sexual dimorphism in vegetative traits, in *F. virginiana* sexual dimorphism in petal size and flowering time was unaffected by environmental variation because the sexes responded similarly to multiple climate and soil factors (Table S5). Potential explanations include (1) strong selection to maintain flowering synchrony and pollinator attraction between the sexes or (2) these traits have stronger between-sex genetic covariation (Ashman, 2005). Common garden studies with material collected across this range will be key to disentangling sex-specific plasticity from genetic differentiation across the broad environmental gradients studied herein. Such studies with material from across the entire continent of the United States would be quite an undertaking, thus more profitably, the environmental drivers of variation identified from the continent-wide study can now be dissected to generate more specific mechanistic hypotheses that can be tested with experiments. To that end, we point out that studies on a smaller scale (3–5 populations) have revealed significant positive between-sex correlations for the studied traits (e.g. petal size and flowering time; Ashman, 1999, 2003) that were invariant with climate variation; while on the other hand other traits (e.g. runner number) showing climate responsiveness here had previously been shown to demonstrate plasticity in response to resource availability (Bishop et al., 2010). Thus, future work could be aimed at confirming sex-specific trait responses to water and temperature as well as determining whether environmental sensitivity itself has evolved across the range. Indeed, because sex-specific plasticity might be important in facilitating adaptation for specific traits and/or species to persist under changing environments (Hangartner et al., 2022), more studies including both resource acquisition and mate access traits are needed in plants.

4.3 | Sex chromosome variation

While interspecific sex chromosome variation is well known in plants (Palmer et al., 2019; Renner & Müller, 2021; Tennessen et al., 2018), intraspecific variation has not been explored in most species. In the broadest study to date, we demonstrate widespread polymorphism in SDR haplotypes, a bias toward the ancestral alpha SDR haplotype, and geographic and environmental patterns in SDR haplotype distribution (gamma/beta SDR haplotypes were more common in the West, declined with increasing summer precipitation and/or soil moisture). Puixeu et al. (2019) also found a pronounced east–west geographic pattern in the sex chromosome races of *Rumex hastatulus* in North America but they did not determine if there were environmental correlates of the distribution. Interestingly, neither Puixeu et al. (2019) in *R. hastatulus* nor our study in *F. virginiana* uncovered phenotypic differences among the sex chromosome variants within species, although we note that we only had females to compare (e.g. SDR haplotypes), and it is possible that traits of hermaphrodite/males from these same source populations could have differed. Given the paucity of studies, it is too early to generalize that such phenotypic variation is indeed absent. Larger and more balanced samples of *F. virginiana* SDR haplotypes and more species with intraspecific variation in sex chromosomes will be needed to know for sure.

Finally, we circumscribed the location of females with none of the currently characterized SDR haplotypes, an observation that previously was confined to a few individuals (Tennessen et al., 2018). This indicates an additional mechanism of male sterility in the species, perhaps due to interacting nuclear or cytoplasmic regions as seen in one of the progenitor diploid species (*F. vesca*; Tennessen et al., 2013) and in cultivated octoploid strawberry (Wada et al., 2020). This may not be surprising given the numerous ways male sterility can arise and given environment-dependent gene expression (Zerpa-Catanho et al., 2019). The clustered geography of non-SDR females (Figure 5), however, may reflect pockets of loss of the RPP0W SDR and the expression of the ancestral likely pentatricopeptide repeat (PPR) related sex-determining mechanism of *F. vesca* (Tennessen et al., 2013). Genetic mapping and genomic characterization of male sterility in non-SDR females is a priority for future research. A general point to be made here, however, is that the extensive sampling of SDRs uncovered hotspots of a novel sex-determining mechanism that might otherwise have remained cryptic. A result that encourages more broad surveys in other systems.

4.4 | Can these patterns inform on the impact of anthropogenic change?

All organisms are faced with the dramatic current changes in climate and soil fertility (IPCC, 2022; Penuelas et al., 2013; Singh et al., 2020), and while we acknowledge the limitations inherent to space–time substitutions, these can still be important tools for making predictions concerning the consequences of future change (Lovell et al., 2023). Based on the present results, we hypothesize that degradation of soil resources rather than via climate-driven flowering mismatches will affect reproduction and sex ratio. Whereas we hypothesize that changes in climatic conditions will impact sexual dimorphism in vegetative growth and, if this translates into sex-differential sexual or asexual reproduction, could impact sex ratio. Finally, if soil moisture and precipitation associations with SDR haplotypes reflect differences in ecological adaptation, then we speculate that climate change could contribute to the evolution of sex determination. Experimental manipulations of specific environmental factors and response monitored in females with known SDR haplotypes are needed to test these ideas. Nevertheless, this study provides a framework for more landscape-level studies across additional sexually polymorphic species, and together these provide a rich data source for formulating testable predictions on how anthropogenic change may impact these organisms.

AUTHOR CONTRIBUTIONS

Tia-Lynn Ashman, Nevin Cullen, Sebastian Mortimer and Aaron Liston designed the research; Ethan Richardson, Rachel Reeb, Trezalka Budinsky, Sebastian Mortimer and Nevin Cullen collected the data; Nevin Cullen conducted data analysis and produced visuals; and Tia-Lynn Ashman, Nevin Cullen, Sebastian Mortimer and Trezalka Budinsky wrote the first draft and all authors contributed to the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at Dryad Digital Repository https://doi.org/10.5061/dryad.zcrjdfnmc (Cullen et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Herbaria providing leaf tissue for SDR determination.

Table S2: Primer and amplicon details for SDR haplotype screening (*available in excel file*).

Table S3: Model summaries for analyses of best fit models of sex ratio and flowering (*available in excel file*).

Table S4: Moran's I test results for spatial autocorrelation (*available in excel file*).

Table S5: Model selection summaries for analyses of best fit models of SDR (*available in excel file*).

Table S6: Trait means ± SD for Female and Hermaphrodite/Male records.

Table S7: Trait means ± SD and sample sizes for SDR haplotype records.

Figure S1: Genomic structure of SDR haplotypes.

Figure S2: Sex Ratio varies with latitude and elevation by region.

Methods S1: Extended methods section, outlining the sex chromosome detection methods.

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