Soybean dependence on biotic pollination decreases with latitude

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ABSTRACT

Identifying large-scale patterns of variation in pollinator dependence (PD) in crops is important from both basic and applied perspectives. Evidence from wild plants indicates that this variation can be structured latitudinally. Individuals from populations at high latitudes may be more selfed and less dependent on pollinators due to higher environmental instability and overall lower temperatures, environmental conditions that may affect pollinator availability. However, whether this pattern is similarly present in crops remains unknown. Soybean (*Glycine max*), one of the most important crops globally, is partially self-pollinated and autogamous, exhibiting

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large variation in the extent of PD (from a 0 to ~50% decrease in yield in the absence of animal pollination). We examined latitudinal variation in soybean's PD using data from 28 independent studies distributed along a wide latitudinal gradient (4–43 degrees). We estimated PD by comparing yields between open-pollinated and pollinator-excluded plants. In the absence of pollinators, soybean yield was found to decrease by an average of ~30%. However, PD decreases abruptly at high latitudes, suggesting a relative increase in autogamous seed production. Pollinator supplementation does not seem to increase seed production at any latitude. We propose that latitudinal variation in PD in soybean may be driven by temperature and photoperiod affecting the expression of cleistogamy and androsterility. Therefore, an adaptive mating response to an unpredictable pollinator environment apparently common in wild plants can also be imprinted in highly domesticated and genetically-modified crops.

1. Introduction

Flowering plants exhibit a wide range of pollinator dependencies and mating systems (Barrett, 2003). Whereas an important fraction of plant species have the capacity of producing seeds after autonomous or vector-mediated self-pollination, thousands of others exhibit diverse genetic-based mechanisms or sexual dimorphisms that prevent self-fertilisation (Igic et al., 2008; Ollerton et al., 2011). This latter implies an obligate dependence on external vectors, mostly animals, to achieve pollen transfer between genetically distinct individuals. A recent analysis by Rodger et al. (2021) suggests that, in the absence of animal-mediated pollination, one third of flowering plant species would produce no seeds whilst half, including many crops, would have reduced seed set of 80% or more. Therefore, human-driven pollinator decline (Millard et al., 2021; Zattara and Aizen, 2021) has raised serious concerns about its consequences for biodiversity maintenance and agricultural production (IPBES, 2016; Potts et al., 2016), and thus the study of mating-system variation and distribution is even more relevant now than before. In fact, the relationship between plant mating and animal pollination has been a central theme in evolutionary biology, reflecting its implications for the diversification of flowering plants (Charlesworth, 2006; Eckert et al., 2010; Richards, 1986). In agriculture, the drivers of variability in mating systems and the mechanistic bases underlying this variation have been long-standing subjects of research because of their relevance for crop production, improvement and breeding (Bond, 1989; Muñoz-Sanz et al., 2020; Suso and del Río, 2015).

Reflecting variation in their pollinator dependence, many plant species show high levels of intraspecific variability in their capacity to self-pollinate autonomously (Kalisz and Vogler, 2003; Prasifka et al., 2018; Spigler and Maguiña, 2022). In fact, within-species variation in the extent of autonomous selfing is a characteristic of thousands of wild, mixed-mating species (Goodwillie et al., 2005). More recently, variation in the capacity for autonomous selfing has been also identified in several crops (Bishop and Nakagawa, 2021; Franceschinelli et al., 2022). While it is likely that varietal differences are a key predictor of autonomous selfing in crops, there is increasing evidence to suggest that more general environmental and bioclimatic factors may underpin this variation (Franceschinelli et al., 2022; Segers et al., 2022). From an applied perspective, understanding how autonomous selfing varies along major environmental gradients in crops may therefore provide information to manage pollination services more efficiently. Also, this type of study provides a unique opportunity to evaluate the persistence of evolutionarily-divergent or environmentally-plastic adaptive traits even after hundreds, or even thousands of years of domestication.

Pollinator availability, which affects flower visitation frequency, can act as a potent selection agent on plant mating systems (Arroyo and Squeo, 1990; Baker, 1955; Lloyd, 1980). This is hypothesised to be driven by the fitness benefit of autonomous selfing outweighing the costs of inbreeding depression in situations where the production of selfed seed is better than no seed production at all, i.e. the "reproductive assurance" hypothesis (Calviño and Galetto, 2003; Knight et al., 2005; Lloyd, 1980; Lloyd and Schoen, 1992). For this reason, a decrease in pollinator abundance and diversity, leading to increasing pollination limitation (Harder and Aizen, 2010), can favour shifts from outcrossing to autonomous selfing despite inbreeding costs (Eckert et al., 2010, 2009; Lloyd, 1992; Lloyd and Schoen, 1992; Teixido and Aizen, 2019). Several studies of wild plants have looked at changes in the incidence of selfing along spatial gradients characterised by variation in climatic factors, such as temperature, known to affect pollinator activity (Jones et al., 2013; Moeller et al., 2017; Razanajatovo et al., 2020). These have provided evidence that the incidence of autonomous selfing increases at high latitudes in agreement with the "pollinator availability" hypothesis (Grossenbacher et al., 2015; Koski et al., 2017; Yang et al., 2018). While the mating system of wild plants are subject to natural selection in response to pollinator availability, whether such response and latitudinal variation in mating system also occur in widely-cultivated and genetically-manipulated crops remain unknown. In fact, a similar latitudinal pattern might be seen in cultivated plants in which autogamy is favoured under conditions that may disfavour the plant-pollinator mutualism if such a response has a plastic component that has persisted despite the domestication process or if autogamy is correlated to other traits (e.g., phenology) that are under artificial selection. Therefore, the proposed exploration can provide insights into the origin, persistence, nature, and adaptive meaning of mating-system variation independent if a plant is wild or domesticated.

Soybean (*Glycine max* L. (Merr.) Fabaceae), a broadly genetically-modified commodity crop, is cultivated worldwide in temperate, subtropical, and tropical regions from ~50° N to ~40° S (Leff et al., 2004). In 2021, global production reached 363,863,000 tonnes, with most of it being grown in Brazil (37%), the United States (33%), Argentina (12%) and China (5%) (USDA Foreign Agricultural Services, 2022). Soybean is an annual crop grown mostly for its seeds, which are produced in pods containing 1–4 seeds each. The traditional view is that soybean is mostly or fully autogamous (Free, 1993; Roubik, 1995), but, more recently, the role of animal pollination in this crop has been debated (Chacoff et al., 2010; Giannini et al., 2015; Melathopoulos et al., 2015). This is partly because soybean has several characteristics of an animal-pollinated plant. Despite their small size (approximately 7 mm in length), soybean flowers produce volatiles and have nectaries and nectar guides (Erickson and Garment, 1979; Robacker et al., 1983). Several insect species, mostly bees, have been observed visiting their flowers (Gill and O'Neal, 2015; Levenson et al., 2022; Milfont et al., 2013; Monasterolo et al., 2015). Notwithstanding claims of full self-fertilization, plants in Mississippi produced up to 6.3% outcrossed seed (Ray et al., 2003), while outcrossing rates as high as 20% were found in wild soybean (Fujita et al., 1997). However, pollinators may be required not only for transferring outcross-pollen, but also for the transfer of self-pollen within flowers and among flowers within individuals. Several studies have evaluated the dependence on pollinators of cultivated soybean by comparing open-pollinated and pollinator-excluded flowers, and have shown that yield reductions ranging from zero up to about 50% can occur in the absence of pollinators (reviewed in Garibaldi et al., 2021). This wide range in the extent of soybean's pollinator dependence implies differences in its capacity for autonomous selfing that could vary along environmental gradients, beyond what may be expected from varietal differences.

In this study, we evaluated the overall extent of pollinator dependence in soybean and investigated whether variation in pollinator

dependence in one of the world's most important crops is related to latitude. Particularly, we tested whether soybean's dependence on pollinators decreases towards higher latitudes. To assess a latitudinal pattern in soybean's pollinator dependence, we compiled published and unpublished estimates of yield, or some of its components (e.g., seed or pod production), from open-pollinated vs. pollinator-excluded flowers available worldwide. However, a decreasing difference between these two treatments with increasing latitude could either reflect an increasing capacity for autonomous self-pollination and autogamous seed production (thereby increasing yield in pollinator-excluded treatments) or increasing pollen limitation due to reduced availability of pollinators (thereby decreasing yield in open-pollinated treatments). We disentangled these two possibilities by comparing the latitudinal trends in the difference in yield between open-pollinated vs. pollinator-excluded flowers with that estimated from studies where pollinator visitation was artificially increased by supplying managed bees. In brief, here we asked the following questions: (1) Does pollinator dependence in soybean decrease with increasing latitude? (2) Does the yield gap between open-pollinated and pollinator-excluded treatments increase with pollinator supplementation? (3) Does any potential effect of pollinator supplementation on the yield gap between open-pollinated and pollinator-excluded treatments depend on latitude?

2. Methods

We surveyed published studies that report the effect of the presence/absence of pollinators on soybean yield. We searched for articles in Google Scholar (last search conducted in February 2022) using different combinations of the following keywords: "soybean", "*Glycine max*", "pollinat* ", "isolat* ", "seed* ", "pod* ", "fruit* ", "yield", "production", "bees". Based on this search, we identified 25 studies that complied with our criteria of comparing yield or components of yield when pollinators were present vs. excluded. Our article compilation included 15 of the 16 studies listed in the recent review by Garibaldi we excluded one article on the perennial soybean G. wightii) et al. (2021) and 10 other studies not included in that review. Our database also included results from three unpublished studies (see details in Supplementary Methods). For each of the total of 41 field sites included in these 28 studies, we extracted their exact geographical coordinates (Fig. S1, Table S1, Supplementary Methods). In just two studies, sites were sampled over two different years. In these cases, we considered these locations as providing two sets of observations each. Lastly, a single soybean variety was used in each field, with at least 33 different soybean varieties used across the 41 fields. In four studies we could not get information on the soybean variety used. In such cases, we assumed that the farmers cultivated a site-specific variety not listed within the 33 soybean varieties. Therefore, overall the compiled dataset does not allow an estimation of pollinator dependence at the variety level independent of site.

There were differences among studies in how the effect of pollinators on soybean yield was estimated. Specifically, the effect of "pollinator presence" was measured through experiments that included at least one of three treatments: (1) "open", plots or individuals plants in soybean fields exposed to naturally occurring pollinators; (2) "open + honey bees", plots or individual plants in soybean fields exposed to naturally occurring pollinators plus managed honey bees from in-field deployed hives; (3) "enclosure + bees", soybean plants isolated by enclosures lined with mosquito mesh, but with supplemented pollination either from managed honey bees or leafcutter bees (*Megachile* spp.) within these enclosures. The consequence of these treatments on yield was measured from either (1) soybean plots, (2) individual plants or groups of plants, or (3) flowering branches that were isolated by using mesh bags or enclosures structures lined with mosquito mesh (Table S1).

Furthermore, given high variability in the reproductive variables measured across studies, we considered all the available estimates provided by each study as components of soybean yield (e.g., seeds, pods, seeds per pod, and yield itself). For the sake of simplicity, we grouped all

the different yield-related metrics into three categories: "seeds", which include seed number per pod, plant, area, or unit of weight or volume, and seed weight per seed, per a given number of seeds, or per pod; "pods", which include pod number per plant or area; and "yield" *sensu stricto*, which include total seed weight per plant or unit of area. Data from tables were downloaded directly to our database, whereas data from figures, when not available in tables, were extracted using ImageJ software (Schneider et al., 2012).

For each study j, we estimated the effect size i of the relative importance of animal pollination on yield (i.e., pollinator dependence, PD) as $\mathrm{logPD}_{ij} = \log_{10}\left(\frac{\overline{X}_{ij}^{P}}{\overline{X}_{ij}^{E}}\right)$, where $\overline{X}$ is the mean of a given yield metric i (e.g., number of seeds/pod, number of pods/plant, total seed weight/ha, etc.) corresponding to one of the three different pollinator-presence treatments, P (i.e., open pollination, open pollination + honey bees, or enclosure + bees) and the corresponding pollinator-absence treatment, E (Fig. S2). Whereas pollinator dependence is usually expressed as a percentage decrease in seed or pod production or in yield after pollinator exclusion (i.e., $\%\mathrm{PD}_{ij} = 100.\left(\frac{\overline{X}_{ij}^{P}-\overline{X}_{ij}^{E}}{\overline{X}_{ij}^{P}}\right)$), logPD_{ij} has the advantage of providing a standardised, unitless, symmetric measure of proportional change between open and exclusion treatments that ranges from $-\infty$ to $+\infty$ (Bishop and Nakagawa, 2021; Knight et al., 2005). However, to facilitate interpretation and for ease of communication, results of our models were transformed to %PD by means of $\%\mathrm{PD} = [100 \bullet (1 - 10^{-\mathrm{logPD}})]$ (Fernandez et al., 2021).

2.1. Data analysis

We evaluated the influence of latitude on the (log) ratio of soybean's pollinator dependence. We analysed data using general linear mixed-effects models (LMM), assuming a Gaussian error distribution and identity link function. Weighted meta-analysis was not used as the "weight" (i.e., the inverse of the variance) was not available for several of the studies. However, unweighted meta-analyses carried out using regular linear models tend to provide similar estimates as fully informed weighted analyses for model estimation (Fernandez et al., 2021; Kambach et al., 2020).

We tested the effect of latitude on pollinator dependence, *log*PD, using two models. The first, a main-factor model with the effect of latitude, pollinator-presence treatment (open, open + honey bees, enclosure + bee), and yield category (seeds, pods, and yield *sensu stricto*) included as fixed factors. The second model included the three main factors of the first model and a two-way interaction between latitude and pollinator-presence treatment and between latitude and yield category. These two interaction terms tested whether any latitudinal trend is modified when managed pollinators are supplied (see above) and whether there are differences in how different yield components change with latitude, respectively. In particular, comparisons among the three pollinator-presence treatments (i.e., open pollination, open pollination + honey bees, and enclosure + bees) in the first model allows inferring whether reduced yield could be ascribed to reduced pollination due to limited pollinator availability (Fig. S2). Furthermore, the pollinator-presence treatment x latitude interaction in the second model allows evaluating whether any potential pollination limitation vanishes at high latitude.

LMM models were implemented with the "*lmer*" function from the lme4 package (Bates et al., 2015) of the R software version 4.0.2 (R Core Team, 2021). Because many studies provided the opportunity of estimating more than one independent effect size, we included "study" as a random factor in all models. However, given the possibility of putative strong correlation among yield metrics within the same sampling unit, we tested different random structures nested within "study" to find the best random model that fitted our dataset (Zuur et al., 2009; see also Bishop and Nagawaka, 2021). For this, we compared a null model

without any random effect, with random models considering the following random structures: "study", "site" nested within "study", "soybean variety" nested within "study", and "site" nested within "soybean variety" nested within "study". We tested for the best model fit using differences in AIC (Akaike Information Criterion) and likelihood-ratio tests. In addition, the studies included in our analyses were unevenly distributed with the largest number of studies coming from the southern hemisphere (South America), followed by several studies from relatively high latitudes in North America and a few from equatorial Africa (Fig. S1). In this case, residual clustering could indicate an undue influence of these geographical factors in determining observed latitudinal patterns (Zuur et al., 2009). Nevertheless, residuals of none of the models indicating a latitudinal trend (i.e., the two models analysing latitudinal trends in pollinator dependence; Table 1) showed evidence of either negative/positive residual clustering (i.e., autocorrelation) at any spatial scale (Fig. S3). Because of apparent nonlinearity in latitudinal trends, we compared models with both linear and quadratic absolute latitude terms. We assessed the overall significance of the models by means of likelihood ratio tests, estimated parameters for the fixed factors of both models based on maximum-likelihood, and the statistical relevance of these factors assessed by Type-III Wald tests.

3. Results

Our dataset included a total of 28 independent studies that have examined the importance of animal pollination in soybean yield, with 19 studies from South America (nine from Argentina, nine from Brazil, and one from Uruguay), five from the USA, and four from Cameroon (see Fig. S1, Table S1). Many of these studies reported more than one yield metric, totalling 200 *log*PD estimates (102 for the open, 56 for the open + honey bees, and 42 for the enclosure + bees pollinator-presence treatments; and 59 for the pods, 71 for the seeds, and 70 for the yield *sensu stricto* categories). The absolute latitudinal range of *log*PD estimates ranged from 4.01 to 43.13 degrees.

3.1. The extent of pollinator dependence (PD)

The best random structure found was "site" nested within "study" (Table S2), and the quadratic latitudinal model with this random structure showed a better fit compared to the linear one (Likelihood-ratio test, Chisq = 4.12, P = 0.0424). Furthermore, both models were significantly different from the null expectation (model without interaction term, likelihood-ratio test, Chisq=17.612, P = 0.007; and model with interaction terms, likelihood-ratio test, Chisq=28.142,

Table 1
Anova tables summarising the overall statistical relevance of each fixed factor assessed, including (pollinator-presence) treatment, yield category, and latitude. Chi-square statistics and *P*-values correspond to Type-III Wald Tests.

	Model	Fixed effect	Chi-square	df	P-value	
(A)	*Pollinator dependence ~ Treatment + Yield category + abs (Latitude)^2*	Treatment	1.4978	2	0.47287	
		Yield category	7.0895	2	0.02888	*
		abs(Latitude)^2	10.9167	2	0.00426	* *
(B)	*Pollinator dependence ~ abs(Latitude)^2* Treatment + abs (Latitude)^2* Yield category*	Treatment	3.6277	4	0.16303	
		Yield category	5.5303	2	0.06297	
		abs(Latitude)^2	14.8179	2	0.00061	* * *
		abs(Latitude)^2:Yield category	5.1729	4	0.27001	
		abs(Latitude)^2:Treatment	4.8051	4	0.30788	

* $0.01 < P < 0.05$, * ** $P < 0.001$

P = 0.0136). Overall, our results showed that pollinators contribute substantially to soybean yield (Fig. 1A). Yield *sensu lato* (i.e., averaged over pollinator treatments and yield categories) decreased by an average of 28.85% (95%CI= [20.96%, 35.89%]) in the absence of pollinators. There was no evidence that the type of pollinator-presence treatment had an influence on the extent of the decrease in yield after pollinator exclusion (Table 1). In the absence of pollinators, pods, seeds and yield *sensu stricto* categories declined by 25.34%, 30.41% and 30.67%, respectively (Fig. 1B), with some indication that a reduction in pod production was the main, but probably not the only determinant of the observed reduction in yield (Tukey-adjusted tests: pods vs. seeds, $t_{162} = -2.304$, $P = 0.058$; pods vs. yield, Tukey-adjusted $t_{168} = -2.206$, $P = 0.073$).

3.2. Latitudinal trends in PD

Dependence on pollinators in soybean was related nonlinearly to latitude (Fig. 2, Table 1A). This relationship was best fitted with an overall quadratic function (centred at the mean latitude of the dataset 27.21 degrees) of the form $y = 0.147485 - 0.0074531x - 0.0003035x^2$, which has its maximum at 14.9 degrees latitude. In percentage terms, this equation predicted a maximum decrease in yield (*sensu lato*) in the absence of pollinators of 35.97% at this latitude, and a decrease in yield of 29.83% and 9.29% at the latitudinal range limits (respectively 4.0 and 43.1 degrees). In fact, our results revealed no evidence of any pollinator dependence at the highest latitudes. This can be seen as the predicted 95%CIs associated with the means of the different pollinator treatments and yield categories at these extremes overlapped with 0 (Fig. 2). We did not find evidence of an interaction between latitude and pollinator-presence treatment or yield category (Table 1B). Thus, our analyses provided no indication that the relationship between PD and latitude depended neither on the deployment of domesticated bees nor on whether yield has been estimated by counting pods, seeds, or by calculating yield *sensu stricto*.

4. Discussion

In many non-domesticated plant species, the complex interplay between genetic and ecological factors is likely to trigger local adaptation processes operating at the regional scale and shaping the mating responses of populations and metapopulations to specific environmental conditions. Such local adaptation may result in a shifting mating system across populations of the same species distributed along environmental gradients. The study of the relationship between latitude and mating systems has received attention under the light of the "pollinator availability" and "reproductive assurance" hypotheses (Lloyd, 1980; Moeller et al., 2017), which predict an increase in selfing and decreasing pollinator dependence with increasing latitude (Grossenbacher et al., 2015). Observed changes in pollinator dependence along a broad latitudinal gradient in cultivated soybean are mostly consistent with these expectations.

Our findings and those of Garibaldi et al. (2021) show that there is large variation in pollinator dependence for soybean. This contradicts popular thinking within the agricultural industry that soybean predominantly self-pollinates, producing mostly autogamous seeds (Delaplane and Mayer, 2000). Here we demonstrate that this variation is structured spatially. Specifically, we found that pollinator dependence in soybean decreases latitudinally, most abruptly at extratropical latitudes. The observed latitudinal pattern seems to be explained by variation in autogamous seed production, rather than by associated changes in pollinator abundance as we found no evidence of pollination limitation at any latitude. Therefore, these findings suggest that soybean plants developing under warmer temperatures and shorter photoperiods are more likely to benefit from increased pollination services, whereas plants growing at colder temperatures and longer photoperiods are more likely to rely on selfing. Even though the compiled dataset makes no
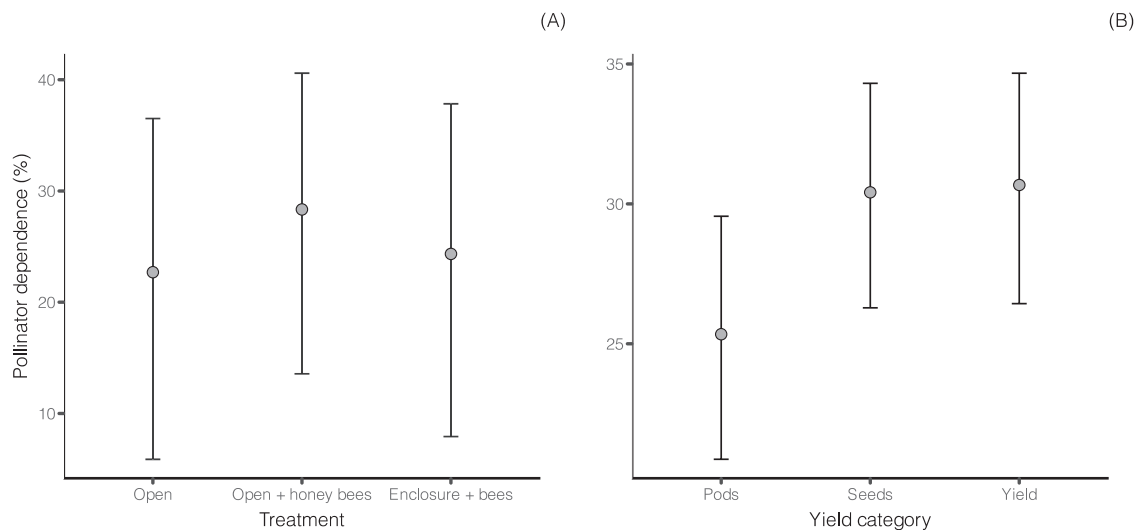
Fig. 1. Predicted least-squares means (+ 1 SE) of soybean pollinator dependence (%PD) for the different (A) pollinator-presence treatments and (B) yield categories according to the main factor model (Table 1).
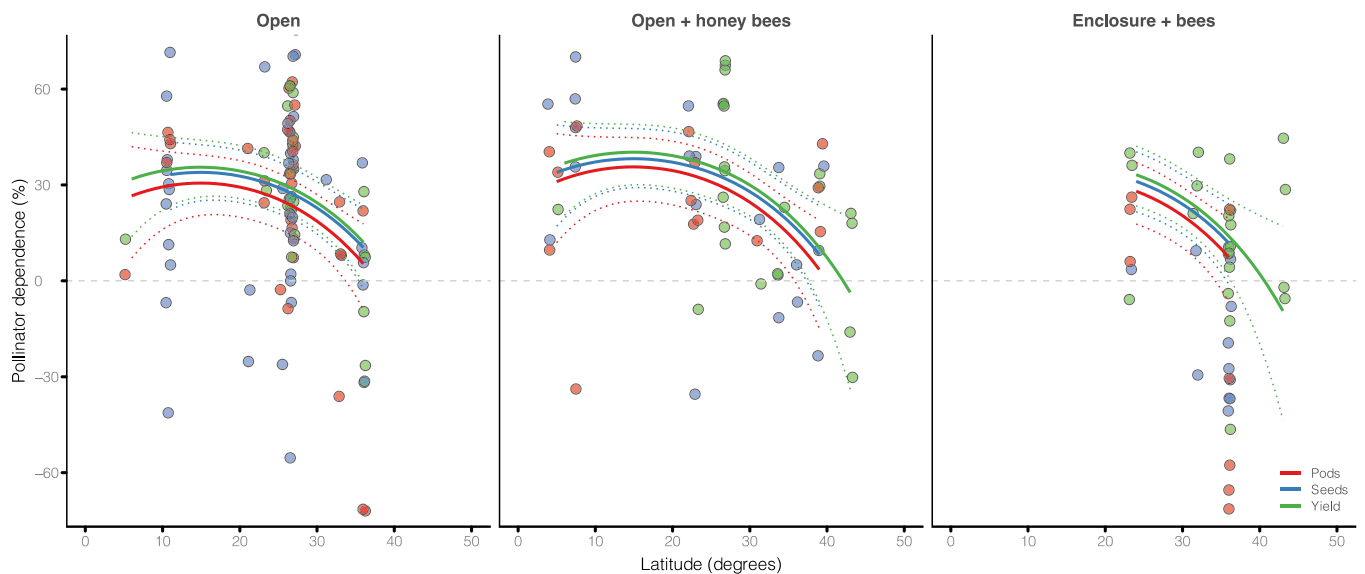

Fig. 2. Relations of soybean pollinator dependence (%PD) to absolute latitude for each pollinator-presence treatment and yield category according to the main factor model (Table 1). The continuous curves are the predicted quadratic trends and the dotted curves the 95% CIs.

possible to disentangle genetic from environmental effects in soybean owing to the fact that each field was mostly cultivated with a different soybean variety, both factors seem to be relevant in determining the observed latitudinal pattern in pollinator dependence.

Soybean is a "short-day" plant species (Jiang et al., 2011) characterised by a mixed mating system predicted by the production of both chasmogamous (open) and cleistogamous (closed) flowers (Benitez et al., 2010; Erickson, 1975; Khan et al., 2008). Soybean also varies in response to changes in the proportion of androsterile individuals/inflorescences resulting from cytoplasmic-nuclear male sterility (Graybosch and Palmer, 1988; Ortiz-Perez et al., 2006a, 2006b). Reduced cleistogamy and increased androsterility will both promote outcrossing. These floral changes can be plastically induced by several environmental factors, such as high temperatures, drought, and nutrient deficiency (Benitez et al., 2010; Blettler et al., 2018; Kaul, 1988; Sawhney and Shukla, 1994). As a consequence, mating can vary along environmental gradients so that plants can be more selfed if cleistogamous flowers are produced, or outcrossed if either more chasmogamous flowers are

produced or androsterility is expressed. Both mechanisms, by being connected (i.e., androsterily associated with the production of chasmogamous flowers) or by responding independently to specific environmental drivers (e.g., temperature), could be linked to latitudinal variation in pollinator dependence. Therefore, despite being a commodity crop developed through intensive breeding programmes, genetic modification, and the heavy use of external inputs (Chemeris et al., 2022), soybean seems to keep its capacity for plastic mating responses to its growing environment.

Independent of any effect on seed quantity or quality, a mixed-mating system involving partial autogamy predicts that autonomous self-pollen deposition will not saturate pollination function (Teixido and Aizen, 2019). In a study comparing stigmatic pollen loads in pollinator-excluded vs. pollinator-exposed soybean flowers, Huais et al. (2020) found that stigmas of pollinator-excluded flowers received, on average, about half the pollen grains as those of pollinator-exposed flowers. Also in that study, pollen was absent from only ~6% of the stigmas when pollinators were able to visit the flowers, but this

proportion increased to ~30% when pollinators were excluded. This evidence shows that despite extensive autonomous self-pollination and autogamous seed production in soybean, flower visitors can play a relevant role in soybean pollination either transferring self-pollen within flowers or among flowers within plants or cross pollen among plants.

The proposed flexible contribution of autonomous self-pollen deposition to yield could explain most variation in pollinator dependence in soybean. We found a mean yield gap value (the difference between open and pollinator enclosure treatments) of ~30%, an average contribution of animals to the production of soybean that can be considered moderate according to Klein et al. (2007). However, the variability of this dependence value was found to range from 0 to > 50% (Table S1; see also Garibaldi et al., 2021), suggesting that in some sites and varieties the economic impact of pollinators may be large, while in others almost negligible. This within-crop variability in pollinator dependence has been recently reported in other widespread crops, such as field beans (*Phaseolus vulgaris*) (Franceschinelli et al., 2022) and faba beans (*Vicia faba*) (Bishop and Nakagawa, 2021), and therefore might be a common feature of many leguminous and other herbaceous crops.

Along the latitudinal gradient studied, we observed the highest contribution of pollinators to soybean yield at lower latitudes where photoperiod (daylength) during the growing season is generally shorter (depending on the cropping season) and the air temperature was warmer compared to regions located further from the equator (Figs. S4 and S5). These two factors, daylength and temperature, can relate mechanistically to latitudinal changes in mating system and pollinator dependence, and even some non-linear dependence between them might eventually explain the apparent hump-shaped latitudinal gradient in pollinator dependence. Soybean flowering is particularly sensitive to the photoperiod length and decades of breeding efforts have resulted in a broad range of varieties and, more particularly, in selected maturing groups to best perform at different latitudes (Yang et al., 2018). Therefore, the genetic component of different varieties and maturing groups can play an essential role in plants' response to particular environmental conditions (Erickson, 1975), more specifically the response to the temperatures at which plants and particularly flowers will be exposed during development (Fig. S5), which in turn could affect both the extent of cleistogamy and androsterility.

Soybean cleistogamy is to some extent not genetically determined, but induced during development as a response to current environmental conditions (Erickson, 1975). This type of plastic cleistogamy allows a plant to develop closed flowers under unfavourable weather conditions and revert to the production of chasmogamous flowers when conditions improve (Culley and Klooster, 2007). Although environmental-induced cleistogamy occurs when conditions during floral development result in heteromorphic closed flowers or homomorphic flowers that remain closed, the specific response to photoperiod and correlated response to temperature is known to vary across varieties (Benitez et al., 2010; Erickson, 1975). However, Benitez et al. (2010) also documented the plasticity of soybean's cleistogamy, showing that varieties that produce cleistogamous flowers in northern regions will produce chasmogamous flowers at a lower latitude and under warmer conditions (see also Erickson, 1975). The effect of temperature on the number of facultatively outcrossing chasmogamous flowers was also observed in *Viola praemorsa* (Violaceae) subject to warmer temperatures (Jones et al., 2013). Therefore, the ratio of chasmogamous to cleistogamous flowers in soybean is expected to be the outcome of the interplay of genetic and ecological factors determining the plant response to changing environmental conditions of photoperiod and temperature during its development.

Similarly, androsterility, a phenomenon that enforces outcrossing and is common across flowering plants (Kaul, 1988), can be influenced by temperature. There are frequently-occurring reproductive mutations observed in soybean that induce the phenotypic expression of androsterility that results from either a difunctional anther tapetum or reduced pollen viability (Graybosch and Palmer, 1988). These mutations can arise spontaneously in field plantings and have been actively selected for the production of hybrid seeds (Palmer et al., 2004). Although little is known about the incidence of male-sterile plants in commercial soybean fields, it is known that the gene expression of androsterility commonly depends on either diurnal or nocturnal temperatures, with high temperatures during plant growth and flower development reducing or even eliminating male fertility (Graybosch and Palmer, 1988). It is clear that male-sterile inflorescences/individuals are fully dependent on flower-visiting insects to produce seeds (Ortiz-Perez et al., 2006a, 2006b), and the relation of androsterility to temperature could also explain increasing pollinator dependence at lower latitudes. However, the relative importance of this mechanism will depend on farmers resowing the previous year's harvested seeds for genotype replication and propagation, a practice that is known to occur despite contract regulations stipulating that it should not.

Either higher incidence of cleistogamy or lower incidence of androsterily could explain the pattern we observed in decreasing soybean's pollinator dependence with latitude. Interestingly, pollinator dependence in South America, the region providing most of the studies, seems to increase not only at low latitudes, but also inland where the highest summer temperatures in the continent are recorded (Fig. S4). Therefore, this unanticipated pattern strengthens a proposed link between temperature and pollinator dependence, a pattern that might call for active management of pollination services under climate change, where warmer weather might increase the risk of soybean yields being pollination limited. Despite this higher risk, our results do not provide evidence that soybean is limited by pollination overall and, more specifically, they do not support the expectation of higher pollination deficits with decreasing latitude due to increasing pollinator dependence. This could indicate that higher pollinator dependence at lower latitudes, or more generally under warmer conditions, could be compensated by higher pollinator abundance/activity (Arroyo et al., 1985; Primack and Inouye, 1993). In any event, our results do suggest that the use of domesticated honey bees or augmentation of wild bee abundance could assist in maximising yield in soybean in pollinator-devoid landscapes, particularly at lower latitudes.

5. Concluding remarks

The study of within-species variation in pollinator dependence and plant mating system along environmental gradients has been addressed in wild plant species (Moeller et al., 2017). This has, however, never been explored in crops, despite its agronomic relevance for pollination management and increasing yield. These studies also have important evolutionary implications when assessing the resilience of plant adaptations to the domestication process. For instance, although the proposed plant traits explaining the observed latitudinal pattern in pollinator dependence (i.e., cleistogamy and androsterility) might not have been targets of soybean's improvement programmes, their presence and environmental plasticity imply that adaptive traits underlying variation in mating system –likely present in soybean's wild ancestors – can persist in highly domesticated and genetically manipulated crops. In turn, reproductive traits determining pollinator dependence could play a key role in yield at any geographical location, stressing the importance of integrating both the pollinator and the plant response components when studying pollination services (Sáez et al., 2022). Based on our results, we also propose that the field of pollination services will benefit from both macroecological and mechanistic approaches to understand plant responses to changing environmental conditions (Aizen and Vázquez, 2006; Cunha et al., 2022). In particular, a deeper understanding of a plant's response to environmental conditions will help to better assess the relevance of plant-pollinator interactions for crop yield and open new perspectives and opportunities to breeders that need to contemplate current human-caused global impacts (e.g., climate change and pollinator decline) into their breeding programmes. The dependence of soybean on pollinators has been a subject of ongoing debate,

with farmers and agronomists generally assuming full autonomous self-fertilisation of the crop, thus neglecting any role of wild pollinators or domesticated honey bees on production (Garibaldi et al., 2021; Suso et al., 2016). Here we provide a macroecological resolution of this applied debate by examining this problem within the basic framework on how mating systems in plants, either wild or domesticated, respond to their environmental context.

Author Contributions

N.L. Cunha: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Visualization, Writing – review & editing. **N.P. Chacoff:** Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **A. Sáez:** Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **R. Schmucki**: Funding acquisition, Conceptualization, Formal analysis, Writing – original draft, Visualization, Writing – review & editing . **L. Galetto:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **M. Devoto:** Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **J. Carrasco:** Data curation, Writing – review & editing. **M.P. Mazzei:** Data curation, Writing – review & editing. **S.E. Castillo:** Data curation, Writing – review & editing. **T.P. Palacios:** Data curation, Writing – review & editing. **J. Vesprini:** Data curation, Writing – review & editing. **K. Agostini:** Writing – review & editing. **A. M. Saraiva:** Writing – review & editing. **B.A. Woodcock:** Writing – review & editing. **J. Ollerton:** Writing – review & editing. **M.A. Aizen:** Funding acquisition, Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data and code will be made available on a public GitHub repository, with a static release on Zenodo (doi:10.5281/zenodo.7565588). Data sources and metadata are available in supplementary material.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2023.108376.

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