

Anthropogenic land-use change decreases pollination and male and female fitness in terrestrial flowering plants

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Background and Aims: The majority of the earth's land area is currently occupied by humans.

Measuring how terrestrial plants reproduce in these pervasive environments is essential for understanding their long-term viability and their ability to adapt to changing environments.

Methods: We conducted hierarchical and phylogenetically-independent meta-analyses to assess the overall effects of anthropogenic land-use changes on pollination, and male and female fitness in terrestrial plants.

Key Results: We found negative global effects of land use change (i.e., mainly habitat loss and fragmentation) on pollination and on female and male fitness of terrestrial flowering plants. Negative effects were stronger in plants with self-incompatibility (SI) systems and pollinated by invertebrates, regardless of life form and sexual expression. Pollination and female fitness of pollination generalist and specialist plants were similarly negatively affected by land-use change, whereas male fitness of specialist plants showed no effects.

Conclusions: Our findings indicate that angiosperm populations remaining in fragmented habitats negatively affect pollination, and female and male fitness, which will likely decrease the recruitment, survival, and long-term viability of plant populations remaining in fragmented landscapes. We underline the main current gaps of knowledge for future research agendas and call out not only for a decrease in the current rates of land-use changes across the world but also to embark on active restoration efforts to increase the area and connectivity of remaining natural habitats.

KEYWORDS: Habitat fragmentation, reproductive success, siring success, paternity correlation, seed production, seed-set, fruit production, fruit-set, mating systems, pollinators decline, pollination, plant-pollinator interactions

INTRODUCTION

Land-use change imposed by human activities is the most important global driver of biodiversity loss (Sala *et al.*, 2000; Haddad *et al.*, 2015). Those changes lead to structural modifications at the landscape scale, including the loss of native habitat, a reduction in functional connectivity among remaining patches (i.e., habitat fragmentation), and an increase in habitat homogeneity and degradation (Bartlett *et al.*, 2016). These changes reduce population sizes and increase isolation among the remaining populations, which are surrounded by anthropogenic matrices in fragmented landscapes (Haddad *et al.*, 2015). Empirical evidence indicates, in accordance with the species-area ecological theory, that a reduction in habitat availability leads to the extinction of populations and species (Pimm and Askins, 1995; He and Hubbell, 2011; Chase *et al.* 2020).

Species survival in fragmented landscapes declines as a result of changes in biotic interactions and loss of genetic diversity resulting in a reduction in the progeny vigour (Hanski *et al.*, 2013; Aguilar *et al.*, 2019). A recent debate on the effects of land-use change and the resulting habitat loss and fragmentation on biodiversity loss focuses primarily on species diversity (Fahrig, 2003, 2017; Fletcher *et al.*, 2018), ignoring the key role of biotic interactions (e.g., mutualistic and antagonistic interactions) on population recruitment and long-term viability. According to Fahrig (2017), habitat fragmentation at a landscape-scale has nil or even positive effects on biodiversity. However, a reduction in habitat size decreases the number of individuals of a species in a given area, with potential negative effects on population viability (Haddad *et al.*, 2015; Hadley and Betts, 2012). Therefore, regardless of landscape-scale, habitat fragmentation will isolate and reduce populations, which will affect both mutualistic and antagonistic interactions. In the case of angiosperms, more than 90% of species rely on animal

pollinators for sexual reproduction and gene flow (Quesada *et al.*, 2011; Rosas-Guerrero *et al.*, 2014; Tong *et al.*, 2023). As a result of habitat loss and fragmentation, plant and pollinator population sizes decrease, affecting the mobility and feeding behaviour of pollinators, which may diminish pollen flow and outcrossing rates, decreasing the reproductive output, genetic diversity, and progeny vigour of plant populations (i.e., reduced number and quality of sires; Aguilar *et al.*, 2006, 2008, 2019).

The effects of land-use change on plant reproduction have been studied extensively over the past three decades. The first systematic review and meta-analysis of habitat fragmentation effects on pollination and plant reproduction was conducted almost two decades ago (Aguilar *et al.*, 2006), and documented an overall decrease in seed and fruit production in angiosperm plant populations across 89 plant species growing in fragmented habitats. This first quantitative review also found that self-incompatible (SI) species, which are highly dependent on pollinators for successful reproduction, were the most negatively affected (Aguilar *et al.*, 2006). This study also showed, using a subset of 50 species, that lower reproductive output in fragmented habitats is mostly related to pollen limitation (Aguilar *et al.*, 2006). Since the publication of this first meta-analysis, abundant new information has become available in the scientific literature on the effects of anthropogenic land use-change on pollination, female (fruit and seed production) and male (pollen removal, pollen performance, siring success) fitness components of terrestrial flowering plants. The current study represents a new, updated synthesis on the effects of land-use change on pollination and angiosperms reproductive success, incorporating novel meta-analytical tools to account for the phylogenetic relationships among plant species.

Sexual reproduction in flowering plants depends on four principal components: (1) pollination, the movement of male gametes from the anthers to the stigma of conspecific flowers

by pollinators or abiotic vectors; (2) fertilisation, the germination of compatible pollen grains on conspecific stigmas and the growth of pollen tubes through the style to fertilise the ovules to produce seeds, which involves overcoming pre- and post-zigotic barriers; (3) the development of fertilised ovules and fruits until seeds are matured; and (4) seed germination and establishment. Historically, plant reproductive success has been conceptually assessed and viewed in terms of the female components of plant fitness (i.e., fruit and seed production). However, angiosperms are able to maximise fitness through both female function and male function (i.e., seeds sired) (Schaeffer *et al.*, 2013). Male fitness may be estimated through pollen production and size (Stephenson *et al.*, 1992; Lau and Stephenson, 1993, 1994), pollen removal (Stanton *et al.*, 1992; Queller 1997; Aguilar and Galetto, 2004), pollen dispersal (Campbell, 1991; Cristóbal-Pérez *et al.*, 2020), and pollen performance associated to the number and quality of successful sires (Quesada *et al.* 1991, 1993, 2001; Stephenson *et al.*, 1992; Breed *et al.*, 2012, 2015; Cristóbal-Pérez *et al.*, 2020, 2021; Aguilar-Aguilar *et al.*, 2023). A quantitative review documented that male and female fitness may vary in response to differences in environmental factors (e.g., light, nutrients, water availability) and biotic interactions (i.e., mutualistic and antagonistic interactions) (Schaeffer *et al.* 2013). However, a thorough review of how land-use change affects overall plant fitness (i. e. integrating male and female fitness) is missing.

Land-use change may affect some or all phases of the plant reproductive cycle. Changes in the abiotic environment might limit resources for the production of flowers, fruits, and pollen quantity and quality (Stephenson *et al.*, 1992; Lau and Stephenson, 1994; Quesada *et al.*, 1995; Schaeffer *et al.*, 2013; Chen and Zuo, 2018). A reduction in plant and pollinator population sizes and changes in pollinator foraging behaviour are likely to affect total reproductive output (i.e., male and female). A decrease in the number of local mating partners increases the probability of

inbreeding in self-compatible (SC) species, limits pollen availability for outcrossing, or reduces the quantity and quality of sires involved in seed production (Charlesworth and Charlesworth, 1987; Ellstrand and Elam, 1993; Fuchs *et al.* 2003). These negative effects will be exacerbated in self-incompatible (SI) plants that are highly dependent on pollination from outcross pollen donors (Aguilar et al., 2006, 2012). Similarly, the sexual expression of plants, the type of pollinator vectors or the degree of pollination specialization may also influence the reproductive response to land-use changes. For example, pollination-generalist plants with vertebrate pollinators and hermaphrodite flowers may be reproductively less susceptible to land-use changes than pollination-specialist plants with invertebrate pollinators and dioecious or monoecious flowers.

Here, we present an updated systematic and quantitative meta-analysis of the effects of land-use change on pollination and on male and female fitness in terrestrial flowering plants. For the first time, are incorporated both hierarchical and phylogenetic-independent meta-analyses to study the effects of fragmentation on plant fitness. Specifically, we address the following questions: What are the global effects of land-use changes on pollination and on female and male fitness in angiosperms? What are the most relevant ecological and life-history traits of angiosperms explaining reproductive susceptibility to land-use changes?

MATERIALS AND METHODS

Literature search

We conducted two systematic literature searches in the multidisciplinary online database SCOPUS, comprising the period between 1900 and 2023. In order to recruit studies analysing land-use change effects on female and male fitness, we used two different strings of keyword

combinations. To search for studies assessing land-use change effects on female fitness, we used: “plant*” AND (“fragmentat*” OR “habitat fragment*” OR “habitat loss” OR “forest fragment*” OR “forest loss” OR “deforest*” OR “landscape fragment*” “land-use change*” OR “population size” OR “isolat*” OR tillage OR grazing OR agriculture OR mining OR urbaniz*) AND (“female fitness” OR “reproductive success” OR “reproductive output*” OR “seed set” OR “fruit set” OR “seed*” OR “fruit*”). Second, to find studies assessing land-use change effects on male fitness, we used: “plant*” AND (“fragmentat*” OR “habitat fragment*” OR “habitat loss” OR “forest fragment*” OR “forest loss” OR “deforest*” OR “landscape fragment*” “land-use change*” OR “population size” OR “isolat*” OR tillage OR grazing OR agriculture OR mining OR urbaniz*) AND (“male gametop*” OR “male fitness” OR “male success” OR male reproductive success” OR “pollen export*” OR “pollen dispers*” OR paternity OR “sired seed*” OR siring OR “seed* sired”). Each search yielded 1203 and 461 papers, respectively, which were subsequently examined for suitability in our synthesis (see PRISMA in Fig. S1). We only included studies that complied with the following criteria: a) assessed some anthropogenic land-use change driver such as habitat loss (including all the factors associated with the structural changes imposed by it, namely: amount of habitat, population size, degree of isolation, fragment area, etc.), tillage, agriculture, grazing, mining, urbanisation; and b) provided numerical data on pollination (e.g., pollinator visitation, number of pollen grains deposited on stigmas), female fitness (e.g., number of fruits or seeds, fruit-set, seed-set), and male fitness (e.g., correlation of paternity, number of pollen donors, pollen removal), such as means, standard deviations, sample sizes, or correlation coefficients. Thus, we included studies with both categorical approaches (e.g., comparing fragmented versus continuous habitats, isolated versus non-isolated populations,

populations within agricultural lands versus natural habitats) and correlational approaches (e.g., gradients of fragment area or degrees of isolation).

For each plant species and study, we also included relevant information on some ecological and life-history traits that were suspected to influence the magnitude of effect sizes (see Aguilar *et al.*, 2006; 2019): i) Compatibility systems, split in plants able to set seeds with either self- or outcross-pollen (self-compatible) and plants only able to set seeds with outcross pollen (self-incompatible); ii) Pollination vector, split in four broad categories linked to their potential mobility through the landscape, such as vertebrate (bats and birds), invertebrate (insects), both (vertebrate and invertebrate) or wind; iii) Pollination specialisation, with plants considered generalists when pollinated by several or many different animal species of different taxa and specialists if pollinated by one or a few taxonomically related pollinators; iv) sexual expression of plants, with three broad groups: plants with hermaphroditic flowers; plants with monoecious flowers; and dioecious plants. We also analysed two other traits that could be partially associated with compatibility systems and pollinator specialisation: v) life forms split in herbs, shrubs and trees; and vi) the geographical region where a species occurs in two broad groups, tropical or extra-tropical. These characteristics were obtained either from the source articles or from additional papers (Table S1) and were used as moderator categorical variables to assess their influence in the pollination and male and female reproductive response to land-use changes (see Data Analysis). By establishing broad characterizations of these traits for each plant species, we make sure to attain sufficiently large sample sizes within each subcategory to compare their relative effects. Additionally, within the final list of selected studies assessing the effects of land-use changes on male and female fitness, we further searched for those that had

also measured variables related to the pollination process (e.g., pollinator visitation frequency, pollen loads on stigmas, or pollen tubes in the style).

Data analysis

We calculated Hedges' d , as the common effect size measure across all studies, which has the advantage of being unbiased by a small sample size (Gurevitch *et al.*, 2001). Hedges' d is an estimate of the unbiased standardised mean difference between pollination, female or male fitness response under control and treatment habitat conditions for each studied species. Hedges' d can be interpreted as the inverse-variance-weighted difference in pollination, female or male fitness between natural and disturbed conditions, measured in units of standard deviations. To calculate Hedges' d , we obtained, either from text or tables, the mean values, sample sizes and standard deviations of different measures for either pollination (effective visitation by pollinators, number of pollen grains deposited on stigmas, number of pollen tubes growing in styles), female (number of fruits, number of seeds, fruit-set, or seed-set) or male fitness (correlation of paternity, number of pollen donors and pollen removal) in each of two contrasting landscape conditions: a control where habitats had none or little anthropogenic intervention, and a treatment where habitats were subjected to moderate or high anthropogenic intervention. Several studies assessed land-use change effects on different female (or male or pollination) response variables (e.g., seed set and fruit set) within the same study. In these cases, we calculated a single effect size across all these variables within the same study to prevent idiosyncratic selection of one variable over the other one. When these fitness response variables were shown in graphs, we took the exact values using the software Data Thief III (www.datathief.org). There were some cases where some of these values were not reported, so we used different statistics of parametric tests (e.g., ANOVAs, Chi-square, t -tests) to assess

anthropogenic land-use effects on female or male fitness variables. These statistics were mathematically transformed into Hedges' d (Borenstein *et al.*, 2009; Lajeunesse *et al.*, 2013). When studies evaluated land-use change effects with correlational approaches (e.g., fragment area size-gradients, degree of isolation), we took Pearson's correlation coefficients (r) or the coefficients of determination (R^2) and sample sizes and used these parameters to obtain Hedges' d using mathematical transformations (see Borenstein *et al.*, 2009; Lajeunesse *et al.*, 2013). Complete databases for each meta-analysis (pollination, female and male fitness) with all calculated Hedges' d values were then uploaded onto *metafor* package to run the analyses (Viechtbauer, 2010; R Core Team, 2022). Positive values of the effect size (d) imply that anthropogenic land-use increases pollination, female or male fitness, whereas negative d values imply anthropogenic land-use decreases these parameters in comparison with undisturbed, well-preserved habitats. In the case of male fitness, increased pollen removal and number of pollen donors in either landscape condition implies increased male fitness. However, increased correlated paternity implies reduced average male fitness (i.e., fewer pollen donors per maternal plant; e.g., Breed *et al.* 2015). Thus, for this particular operational variable (correlated paternity) we changed the sign of Hedges' d to be able to properly incorporate the same biological meaning across all the male fitness operational variables. Studies using this parameter refer to it as an estimate of male reproductive success (Ritland 2002, Cascante *et al.* 2002, Fuchs *et al.* 2003, Breed *et al.* 2012, 2015).

We conducted hierarchical mixed effects meta-analyses for each conceptual variable (pollination, female and male fitness), which included all operational variables, for example fruit number, seed set within the female fitness conceptual variable. Mixed effects models assume that studies within a class (i.e., a moderator variable such as compatibility system) share a common

effect but that there is also random variation among studies in a class in addition to within-study sampling variation (Borenstein *et al.*, 2009; Koricheva *et al.*, 2013). The models also took into account the hierarchical dependence in our data due to cases where multiple data points (i.e., effect sizes) were obtained from the same paper by including a publication-level (i.e., study identity) random effect as a nesting factor (i.e., effect size identity nested within study identity: `random=list(~1|study_id/es_id)`). Heterogeneity among effect sizes was assessed with Q statistics, which are weighted sums of squares tested against a χ^2 distribution (Hedges and Olkin, 1985). Specifically, we examined the P values of Q_M statistics that describe the variation in effect sizes that can be attributed to differences among subcategories of each moderator variable. Moderator categorical variables were the ecological and life-history traits of the plant species, which were suspected to influence the magnitude of effect sizes: compatibility systems, pollination vectors, pollination specialization, life forms, sexual expression and also the geographical region. Effect sizes were considered significantly different from zero if their 95% bias-corrected bootstrap confidence intervals (CI) did not overlap with zero (Borenstein *et al.*, 2009; Koricheva *et al.*, 2013). All the analyses were conducted in R using the *metafor* package (Viechtbauer, 2010; R Core Team, 2022).

Phylogenetic-independent meta-analyses

When effect sizes are calculated at the species level in a meta-analysis, the assumption of independence of data points can be violated because of shared evolutionary history among related species (Lajeunesse, 2009; Chamberlain, *et al.* 2012). This introduces a hierarchical correlated error structure that reflects common ancestry (Symonds and, Blomberg 2014), which can be accounted for by explicitly incorporating the phylogenetic relations among species. To control for such potential bias, we compared the results of our first hierarchical meta-analytical

model with a phylogenetically explicit model that only considers one effect size estimate per plant species (Lajeunesse, 2009).

To run phylogenetic meta-analyses (PMA), we first built a phylogenetic tree with the plant species included in each of the three meta-analyses (pollination, female and male fitness). To do this, we used the megatree GBOTB.extended.WP.tre (Jin and Qian, 2022) as a phylogenetic backbone, which was derived from the megatree of seed plants reported by Smith and Brown (2018). We used the *U.PhyloMaker* R package (Jin and Qian, 2023) to upload the megatrees and then prune them to generate the phylogenetic tree only with those species present in each meta-analysis. Phylogenetic trees are given in the supplementary online material (Fig. S2a-c). In cases where more than one effect per species was calculated (e.g., fruit and seed number, for female fitness), we had to estimate a single effect size per plant species by pooling multiple effect sizes per species using a traditional meta-analysis with a fixed effects model (Koricheva *et al.*, 2013). We used *PhyloMeta v1.3* (Lajeunesse, 2011) to calculate the overall effect size of each phylogenetically-independent meta-analysis. Additionally, we calculated the phylogenetic signal (Blomberg's K) (Blomberg *et al.*, 2003) in effect sizes for each of the three PMAs with the *phylosig* function from the R package *phytools* (Revell, 2012). Estimated K-values close to zero indicate that effect sizes are not phylogenetically related.

Publication bias

An intrinsic problem common to any quantitative systematic review is the potential presence of publication bias: studies reporting significant results may have a higher chance of being published than those reporting non-significant results. To detect the existence of publication bias in our dataset, we ran Kendall's rank correlation tests between effect sizes and

sample sizes across studies. If publication bias exists, then studies with small or null effect sizes are missing, and the correlation tests are significant. We also performed the ‘trim and fill’ method, which recalculates the estimated mean effect size by trimming the smaller studies from one side of the funnel plot and refilling it on the other side of the funnel plot until the funnel is fully symmetric. The ‘trim and fill’ method provides an estimate of how the overall effect size would change if we were able to incorporate all potential missing studies (Jennions and Møller, 2002). Finally, we calculated the weighted fail-safe number (Rosenberg, 2005), which indicates the number of non-significant, unpublished, or missing studies that would need to be added to a meta-analysis in order to nullify the overall effect sizes (Rosenthal, 1979). If the calculated fail-safe number is greater than $5n+10$, where n is the number of studies, then publication bias may be safely ignored (Rosenberg, 2005).

RESULTS

Generalities of the articles and species included in the review

We were able to extract data from 235 publications assessing land-use changes on female fitness responses, allowing the estimation of 312 effect sizes for 263 plant species (Fig S1, Table S1) distributed around the planet (Fig. 1). From the second literature search, we included a total of 79 publications that analysed land-use change effects on male fitness, estimating 105 effect sizes from 93 plant species (Fig S1, Table S1). The vast majority of studies assessed the effects of habitat loss and fragmentation on female (96%) or male fitness (99%). Only a handful of studies evaluated other land-use change factors such as agriculture, grazing, logging and urbanisation (Table S1). Herbs (59%), trees (21%), shrubs (15%), and hermaphrodite flowers (92%) with insect pollination (84%) made up the majority of the plants studied for female fitness. The compatibility system of these species was relatively evenly distributed (49% SI and 41% SC;

Table S1). Studies examining male fitness typically took place in trees (60%), herbs (18%), and shrubs (17%). These species had hermaphroditic (78%) and monoecious (17%) sexual expressions and also showed a similar distribution of compatibility systems (42% SI and 52% SC; Table S1). For both the female and male fitness, only 5% of the studied plant species were epiphytes, hemiparasites and vines (Table S1). Bees were the main pollinators (pollination studies =74%; female fitness= 54%; male fitness=51%), followed by flies (pollination studies =32%; female fitness= 30%; male fitness=30%) of the studied plant species (Fig. 2a-c). Plants pollinated by beetles, moths and bats are the least studied (Fig. 2a-c). Plants pollinated by both vertebrates and invertebrates comprised only 3.8% of the data, thus the pollination vector category “both” was not included in the analyses.

Effects of land use change on pollination and female and male fitness

From the studies assessing either female or male fitness, we gathered 83 effect sizes from 75 unique plant species that also evaluated land-use change effects on the pollination process. We found a significant overall negative effect on pollination (Hedges' $d = -0.3448$, Fig. 3A). The overall heterogeneity of effect sizes for pollination was large and statistically significant ($Q_{\text{total}} (df = 82) = 217.28, p < .0001$). Self-incompatible plants showed significant negative land-use change effects on pollination, whereas SC showed no effects (Fig. 3B). The pollination levels of plants pollinated by vertebrates were unaffected by land-use change (Fig. 3C), whereas the pollination levels of plants pollinated by invertebrates decreased significantly. Pollinator generalist and specialist plants showed similar negative effects on the pollination process (Fig. 3D). Neither life forms, sexual expression nor geographical region of plants explained any variation in pollination responses to land-use changes ($Q_M < 1.47; p > 0.224$; not shown).

Anthropogenic land-use changes, mainly habitat loss and fragmentation, showed significant overall negative effects on both female (Hedges' $d = -0.6248$, Fig. 4A) and male fitness (Hedges' $d = -0.4894$, Fig. 5A). The overall heterogeneity of effect sizes for both female and male fitness was large and statistically significant ($Q_{\text{total}} (df = 311 \text{ and } 104) > 391.72, p < .0001$). The compatibility system of plants significantly explained variation in the effects of land-use change on female fitness, with SI species exhibiting more pronounced reproductive declines than SC species ($Q_M = 4.78; p = 0.028$; Fig. 4B). However, male fitness responses of SI species were comparable to SC species ($Q_M = 1.81; p = 0.178$; Fig. 5B). Plants pollinated by different pollination vectors showed similar negative effects on female fitness (Fig. 4C). However, for male fitness, only plants pollinated by invertebrates showed a significant decrease, whereas vertebrate- and wind-pollinated plants showed non-significant land-use change effects (Fig. 5C). Plants pollinated by specialists and generalists exhibited comparable negative effects of land-use change on female fitness ($Q_M = 3.54; p = 0.082$, Fig. 4D). For male fitness, however, plants pollinated by generalists showed stronger negative effects on male fitness, whereas specialist plants showed non-significant effects ($Q_M = 6.18; p = 0.013$, Fig. 5D). Life forms and sexual expression as well as geographical region of plants fail to explain any variation in female or male fitness responses to land-use changes ($Q_M < 2.41; p > 0.143$).

Correlation between pollination and fitness

Finally, we conducted correlation analyses within the group of studies that simultaneously assessed land-use change effects of pollination on female or male fitness in the same species. We found a positive and significant correlation between land-use change effects on pollination and female fitness ($r = 0.421; p < 0.01; N = 82$; Fig. 6) and no correlation between the effects of land-use change on pollination and male fitness ($r = 0.07; p = 0.769; N = 18$; not shown).

Phylogenetic-independent meta-analyses

The inclusion of phylogenetic relationships among species produced similar overall results as those from the hierarchical meta-analyses (Table S2). In light of this, we present only the results from the conventional hierarchical meta-analysis, which yielded larger sample sizes (312 versus 262 input values for female fitness, 105 versus 93 for male fitness 83 versus 75 for pollination) and thus more accurate estimations. In addition, we found no significant phylogenetic signals in any of the three PMAs (Table S2).

Publication bias

Rank correlation tests between effect sizes and sample sizes across studies assessing land-use change effects on female fitness and pollination were statistically significant (Table S3), suggesting the potential presence of publication bias, whereby studies with small or nil effects might be missing from our sample. However, the ‘trim and fill’ method showed that after correcting such initial asymmetry in the funnel plot (which led to a significant rank correlation test), the overall effect size did not change, remaining negative and significantly different from zero (Table S3). We did not observe a significant correlation in the male fitness meta-analysis. Finally, the calculated weighted fail-safe numbers were always much larger than $5n+10$, implying that publication bias may be safely ignored in our database (Table S3).

DISCUSSION

Nearly two decades after the first review of habitat fragmentation effects on sexual plant reproduction (Aguilar et al., 2006), we present an updated version that includes three times more species than the previous review. This study also incorporates land-use effects on male fitness and a phylogenetically-independent meta-analysis of pollination and plant fitness traits. The reproducibility of our synthesis confirms previous results on pollination and female fitness, and expands the notion of negative habitat fragmentation effects on plant male fitness. To synthesize the main findings of this review, we present a graphical summary that integrates pollination and female and male fitness in Fig. 7. Our study showed that plants in non-fragmented habitats (Figs. 7A and 7C), have greater pollination (pollinator visitation, number of pollen grains and tubes), female fitness (seed production), and male fitness (number of sires) than plants in fragmented habitats (Figs. 7B and 7D). In fragmented habitats, insect-pollinated self-incompatible plants have the lowest female and male fitness. The pollination intensity of SC plants in fragmented habitats (Fig. 7D) is similar to that of SC and SI plants in non-fragmented habitats (Fig. 7A and 7C); however, less seeds are produced in SC plants in fragmented habitats (Fig. 7D).

Pollination

Lower pollinator abundance and visitation in fragmented habitats reduces the number of pollen grains deposited on stigmas of SI species, resulting in a reduction of fruit and seed production. Plants pollinated by invertebrates, mainly bees, are the most susceptible to habitat fragmentation (Fig. 2A-C). It is known that species richness and abundance of invertebrate pollinators are negatively affected by habitat fragmentation (Aizen and Feinsinger, 1994; Winfree *et al.*, 2009, 2011; Quesada *et al.*, 2011; Xiao *et al.*, 2016; Cortés-Flores *et al.*, 2023), reducing the amount

and quality of pollen deposited on stigmas (Aizen and Feinsinger, 1994; Aizen and Harder, 2007; Quesada *et al.*, 2011; Tommasi *et al.*, 2022). Small invertebrate pollinators are most susceptible to habitat fragmentation because of their limited flying and foraging capabilities, being unable to fly among remnant fragments (Greenleaf *et al.*, 2007; Cristóbal-Pérez *et al.*, 2021; Kendall *et al.*, 2022). Plants pollinated by vertebrates and wind showed a non-significant trend of decreased pollination in fragmented habitats, implying that vertebrate pollinators and wind may be able to connect plant populations in fragmented landscapes. A reduction in the density of conspecific plants in fragmented habitats will also limit the amount and quality of pollen transported among compatible plants (Bernhardt *et al.*, 2008; Shao *et al.*, 2008; Johnson *et al.*, 2009). In addition, small pollinator population sizes due to the reduced availability of floral resources in forest fragments will decrease pollen movement among flowers, and may increase the number of geitonogamous and self-crosses (Quesada *et al.*, 2003, 2013; Lobo *et al.*, 2005; Breed *et al.*, 2015; Cuénin *et al.*, 2019).

Results showed a positive correlation between the effects of land-use change on pollination and female fitness measured on the same plant species (Fig. 6). This positive correlation implies that increased pollen limitation (either in quantity and/or quality) in fragmented and disturbed habitats would be the main cause of the observed decrease in female fitness (Fig. 6). Limitation of compatible pollen in SI species can also be contingent on pollen quality; for example, fragmentation may reduce the number of unrelated reproductive partners in fragments or small populations (Fuchs *et al.*, 2003; Llorens *et al.*, 2012). Consequently, even if pollen loads on stigmas are adequate (Johnson *et al.*, 2009), an increase in genetically related and self-pollen transfer may cause reduced pollination success (Johnson *et al.*, 2009; Suarez-Gonzalez and Good, 2014; Cristóbal-Pérez *et al.* 2021).

Female fitness

Our study found a negative overall effect of land-use change on fruit and seed production, regardless of life form or sexual expression. Several mechanisms can negatively affect female fitness in fragmented habitats. Reduced effective population size of conspecific plants in isolated plant populations can increase pollen limitation, due to decreased pollinator visitation and/or reduced pollination quality (Aguilar *et al.*, 2006, 2012). Moreover, changes in habitat quality can reduce maternal resources available for flower development, seed and fruit production (Stephenson, 1992; Oostermeijer *et al.*, 1998; Vergeer *et al.*, 2003; de Vere *et al.*, 2009), increasing fruit and seed abortion (Stephenson, 1981; Obeso, 2002).

As previously observed, we found that habitat fragmentation has a pronounced effect on female fitness of self-incompatible species (Aguilar *et al.*, 2006, 2012). Self-incompatibility is mediated by pre-zygotic barriers that recognise and hinder the growth of self-pollen and ovule penetration in some SI systems (e.g., gametophytic) (Gibbs, 2014). In SI species, reduced effective population sizes can increase the likelihood of incompatible crosses due to a reduction in the diversity of compatible alleles, thereby reducing ovule fertilisation via the limitation of compatible pollen (Young *et al.*, 2000; Pickup and Young, 2008; Young and Pickup, 2010). A study by Young and Pickup (2010) showed that small populations of *Rutidosia leptorrhynchoidea* have low S-allele diversity and mate availability and exhibit significant reductions in seed-set compared to larger populations (>1000 plants) with higher numbers of S-alleles, despite maintaining pollinator services. Short-distance matings between genetically related individuals increase the relatedness of seeds within fruits (Stacy *et al.*, 1996); this results in higher rates of seed abortion due to selfing or biparental inbreeding with negative effects on seed-set and fruit-set (Hufford and Hamrick, 2003; Ishihama *et al.*, 2006). Between 40-50 % of all angiosperms on

the planet are self-incompatible (Zhao *et al.*, 2022; Ferrer *et al.*, this issue). Therefore, half of the flowering species are vulnerable to the ongoing land-use changes at global scale. Self-pollination may ensure female reproductive success in certain SC species, despite the negative effects of inbreeding depression and seed and pollen discounting (Busch and Delph, 2012; Delmas *et al.*, 2016; Spigler and Kalisz, 2017). However, inbreeding depression may have negative consequences on the performance and genetic diversity of offspring (Aguilar *et al.*, 2012, 2019). More studies that analyse long-term performance and establishment of self-pollinated progeny under field conditions are required.

Male fitness

We present the first synthesis of habitat fragmentation effects on male fitness, a forgotten generation that has been overlooked in studies of human-modified landscapes (Heslop-Harrison, 1979; Aguilar and Galetto, 2004; Schaeffer *et al.*, 2013; Minnaar *et al.*, 2019). Our synthesis shows that male fitness significantly decreased in fragmented landscapes. The general lower diversity and abundance of floral visitors in fragmented habitats (Xiao *et al.*, 2016) is likely one of the main causes for the lower pollen removal and delivery, with negative effects on siring success. This assertion is in line with the positive (although non-significant) correlation between fragmentation effects on pollination and male fitness. The lack of significance is likely due to the low number of studies that actually assessed land-use changes effects on both pollination and male fitness on the same species. The number of sires is systematically greater in non-fragmented than fragmented habitats. Siring success can be limited by the reduced number of cross-compatible and unrelated conspecific individuals in forest fragments (Rosas *et al.*, 2011; Quesada *et al.*, 2013; Delnevo *et al.*, 2020; Cristóbal-Pérez *et al.*, 2021). A reduced number of

sires also increases paternity correlation within fruits, reducing the genetic diversity of the progeny (Cascante *et al.*, 2002; Fuchs *et al.*, 2003; Aguilar *et al.*, 2019; Cristóbal-Pérez *et al.*, 2021; Aguilar-Aguilar *et al.* 2023), with negative consequences on early progenie performance (Aguilar *et al.*, 2019; 2023).

Habitat fragmentation has also been associated with reduced habitat quality due to the effects of increased radiation, higher temperatures, desiccation, wind currents, and edge effects (Aguirre-Acosta *et al.*, 2014; Bartlett *et al.*, 2016). These factors may reduce the amount and quality of nutrients available for plants in fragmented and disturbed habitats. Experimental studies that have evaluated the effect of soil nutrient limitation on male function, have found negative effects on pollen quality, production, and performance (Stephenson *et al.*, 1992; Lau and Stephenson, 1994). Furthermore, abiotic stresses (e.g., temperature, humidity and pesticides), influence physiological plant processes involved in pollen development, affecting pollen production, viability, and performance (Delph *et al.*, 1997; Brittain and Potts, 2011; Sage *et al.*, 2015; Borghi *et al.*, 2019), through alteration of metabolic pathways (Borghi *et al.*, 2019) and genetic (Stephenson *et al.*, 1992; Delph *et al.*, 1997) or epigenetic changes (Chen *et al.*, 2016). However, the effects of these abiotic factors on male fitness have not been studied in the context of anthropogenic land-use changes.

Land-use change showed negative effects on the male fitness of plants pollinated by generalists, whereas no significant effects were observed in male fitness of pollinator specialists. Vertebrates comprised 51% of the pollinator assemblages of pollination specialist plants assessed in this review. Vertebrates (birds and bats) are usually able to move longer distances than insect pollinators; thus, they may be more efficient at moving pollen across longer distances,

maintaining similar levels of pollen dispersal and siring success under fragmented and non-fragmented landscape conditions. In line with these findings, a previous meta-analysis found no fragmentation effects on the progeny vigor of plants pollinated by vertebrates (Aguilar *et al.*, 2019). In contrast, all the pollination generalist plant species considered in this review were exclusively pollinated by insects, which, as previously mentioned, are particularly affected by anthropogenic land-use changes (Winfree *et al.*, 2009, 2011; Quesada *et al.* 2011; Xiao *et al.*, 2016; Cortés-Flores *et al.*, 2023). In fact, negative effects of fragmentation on male fitness were only recorded in plants pollinated by invertebrates.

Future research

Our systematic review reveals several knowledge gaps and research biases when assessing anthropogenic land-use change effects on pollination and plant reproduction. Below we describe the most relevant research gaps:

1. Land-use changes are usually linked to the loss and fragmentation of native habitats, but there are several other associated human activities like agriculture, selective logging, urbanisation, mining activities, among others. The effects of these other human disturbances on pollination and plant reproduction have been little explored.
2. Many susceptible plant life-forms, such as epiphytes and vines, are particularly underrepresented in land-use change studies; few studies show consistent negative effects of forest fragmentation on epiphyte reproduction (e.g., Sáyago *et al.* 2018).
3. Environmental abiotic drivers may also affect flowering phenology. Floral synchrony among conspecifics is decoupled and flowering begins earlier in fragmented and disturbed habitats as compared to undisturbed habitats, which negatively affects plant reproductive

success (e.g., Fuchs *et al.*, 2003; Herrerías-Diego *et al.*, 2006). Yet, land-use effects on the phenology of pollinators and on plant-pollinator interactions is limited and requires more attention.

4. Self-incompatibility mechanisms may also be disrupted as a result of reduced plant density and changes in abiotic conditions following human land-use changes (see Ferrer *et al.* this issue). Plants are able to switch to partial self-incompatibility in cases of polyploidy or gene duplication (de Nettancourt 2001), pleiotropic effects of modifying genes (Levin, 1996; Good-Avila and Stephenson, 2002; Good-Avila and Stephenson, 2003), or temporal plasticity of self-incompatibility proteins in natural populations (Vogler *et al.*, 1998), but analyses in disturbed conditions are scarce. Forest fragmentation and habitat disturbance can switch the mating system of plants (e.g., Quesada *et al.*, 2001; Fuchs *et al.*, 2003). In addition to the evolution of self-compatibility we may also expect an increase in the selfing rate of self-compatible plants in fragmented habitats. Clearly more studies are needed to understand the consequences of changes in plant mating systems in disturbed and fragmented landscapes.

5. Land-use change and other synergistic drivers, such as agriculture intensification, pesticide use, and climate change, have a significant impact on pollinator decline (Potts *et al.*, 2010; Goulson *et al.*, 2015; Guedes *et al.*, 2016). Although the direct relationship between pollination and plant reproductive success has been demonstrated, few studies have examined the effect of changes in pollinator assemblages on plant reproduction and the structure of plant pollinator networks in human disturbed fragmented habitats (Astegiano *et al.*, 2015; Cortés-Flores *et al.*, 2023). Finally, more research is required in threatened ecosystems where poorly studied pollinators such as flies are the most frequent (Cristóbal-Pérez *et al.*, 2023).

6. The potential synergism between climate change and land use change may strongly shift the geographic distribution of plant and pollinator species, affecting plant-pollinator interactions by modifying and decoupling plant and pollinator assemblages. Only a few studies have assessed how these shifts affect pollinators and plant reproduction (e.g., Stephan *et al.*, 2021).
7. Over the past two decades, the most severe forest fragmentation has occurred in the tropics (Ma *et al.*, 2023), precisely where the planet's biodiversity is greatest (Raven *et al.*, 2020) and where, according to our review, scientific studies on plant-pollinator interactions are scant. Even old mature tropical forests and contiguous 40-year old restored secondary-growth landscapes are losing the insect rich diversity with unknown consequences for the future of plant-pollinator interactions (Janzen and Hallwachs, 2019, 2021). Therefore, increased attention is required for tropical ecosystems (Lambin *et al.*, 2003; Costantini, 2015; Janzen and Hallwachs, 2019, 2021; Ma *et al.*, 2023).

Conclusions

Our findings indicate that angiosperm populations remaining in fragmented habitats undergo reduced pollination service and thus produce, on average, lower quantity of progeny as both female and male fitness are significantly depleted. In addition, progeny quality is also significantly reduced in fragmented landscapes (Aguilar *et al.* 2019). Such conditions will likely reduce the recruitment, survival and long-term viability of plant populations remaining in fragmented landscapes. Our results suggest that plant species in most current fragmented landscapes may be subjected to different levels of extinction debts, whereby species currently present will not be able to survive and become locally extinct in the near future (Tilman *et al.*,

1994; Kuussaari *et al.*, 2009; Aguilar *et al.*, 2018). We argue that it is of paramount importance not only to decrease the current rates of land-use changes across the world, but to embark on active restoration efforts for increasing the area and connectivity of natural habitats. Such an endeavor may initially focus on increasing connectivity among fragmented populations by improving the characteristics of the surrounding matrices, which strongly influence the quality and resilience of forest remnants (Lindborg and Eriksson, 2004; Frate *et al.*, 2015; Aguilar *et al.* 2018). By allowing the movement of pollinators across the landscape we may ensure increased pollination quality and quantity, which will increase male and female reproductive success of plant populations remaining in fragmented habitats.

DATA AVAILABILITY

The data-set used in the meta-analyses is available in Table S1.

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CAPTION OF FIGURES

Fig. 1. Distribution of studies that analyses the effect of land use change on pollination and male and female fitness. The colour of the circle represents the variable analysed. The size of the circle represents the number of effect sizes.

Fig. 2. Number of plant species visited per pollinator group in studies analysing land-use change effects on (A) pollination, (B) female fitness, and (C) male fitness. Plant species with more than one pollinator group were added as many times as their functional groups.

Fig. 3. (A) Overall weighted-mean effect size and 95% bias-corrected confidence intervals of anthropogenic land-use changes on pollination for plant species with different (B) compatibility systems, (C) pollination vectors and (D) degree of pollination specialisation. SI (Self-incompatible), SC (Self-compatible). k shows the number of effect sizes and in parenthesis the number of studies.

Fig. 4. (A) Overall weighted-mean effect size and 95% bias-corrected confidence intervals of anthropogenic land-use changes on female fitness and for plant species with different (B) compatibility systems, (C) pollination vectors and (D) degree of pollination specialisation. SI (Self-incompatible), SC (Self-compatible). k shows the number of effect sizes and in parenthesis the number of studies.

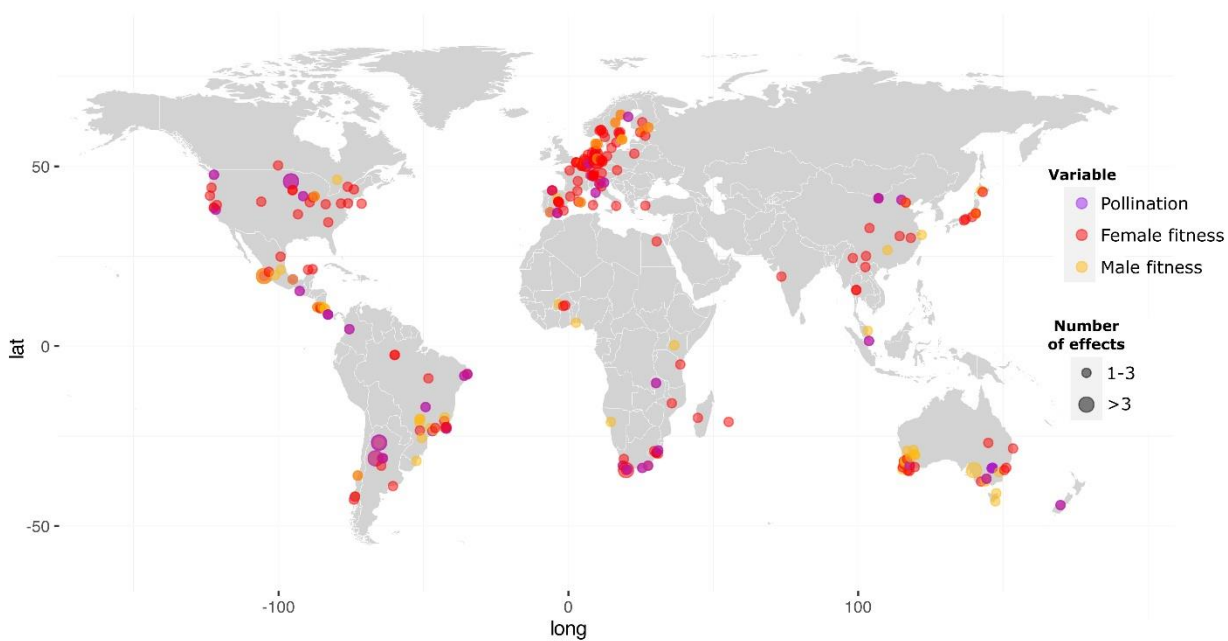
Fig. 5. (A) Overall weighted-mean effect size and 95% bias-corrected confidence intervals of anthropogenic land-use changes on male fitness and for plant species with different (B) compatibility systems, (C) pollination vectors and (D) degree of pollination specialisation. SI (Self-incompatible), SC (Self-compatible). k shows the number of effect sizes and in parenthesis the number of studies.

Fig. 6. Relationship between land-use change effects on pollination and female fitness. Each point represents a unique plant species where land-use change effects were assessed simultaneously on both pollination and female fitness. Dotted lines indicate values of zero for the effect sizes. Pearson's $r = 0.421$; $p < 0.01$. Colours of the points indicated compatibility systems of the species: ND: Non-determined compatibility system, SC: Self-compatible species, SI: Self-incompatible species.

Figure 7. Graphical summary that integrates the effects of human land-use change (i.e habitat fragmentation) on pollination and female and male fitness. Each figure represents the pistil of flowers of self-incompatible (SI) (A and B) and self-compatible (SC) species (C and D) from Non-fragmented (A and C) and Fragmented (B and D) habitats. Six aspects are represented in Figure 7 (A,B,C,D): 1-Pistils of flowers of black recipient plants, 2-Pollen grains of different colors representing different pollen donors (sires) of different genotypes (purple, green, red and blue). Self-pollen grains are black, 3- Lines inside the styles represent pollen tubes generated by the pollen grains from the different pollen donors. 4- Fertilized seeds of developing fruits at the

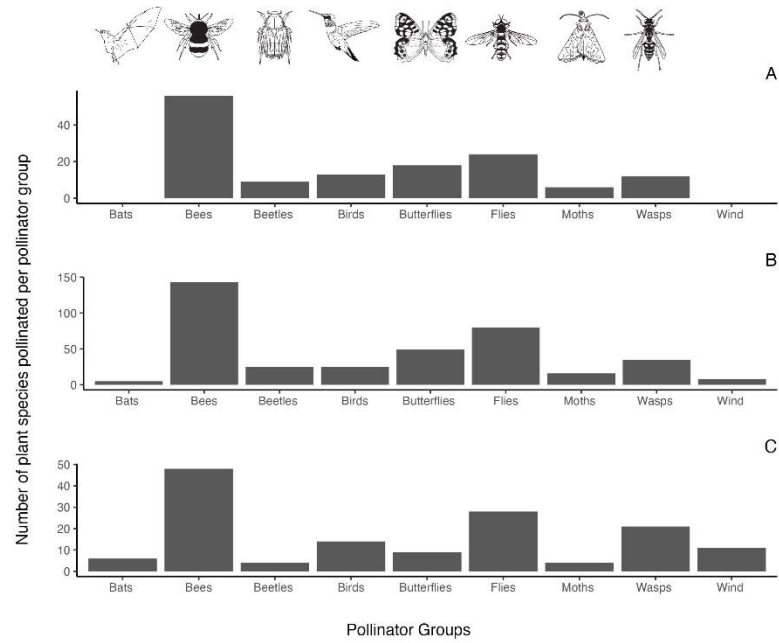
base of each pistil in different colors representing the paternity of each seed from each pollen donor, 5-Deformed seeds in gray represent abortions, 6- In white, unfertilized ovules. In Non-Fragmented habitats, plants from (A) Self-Incompatible (SI) and (C) Self-Compatible (SC) species have higher pollen loads and pollen tubes in styles, a greater diversity of sires, and higher seed set than plants in fragmented habitats (B, D). In fragmented habitats, (B) Self- Incompatible (SI) species have the lowest seed set due to the limited number of pollen grains and sires received on stigmas and to their inability to produce selfed seed. In contrast, (D) Self-Compatible (SC) species may produce seeds by self-pollination ensuring reproduction when outcross pollen transfer is low.

Figure 1



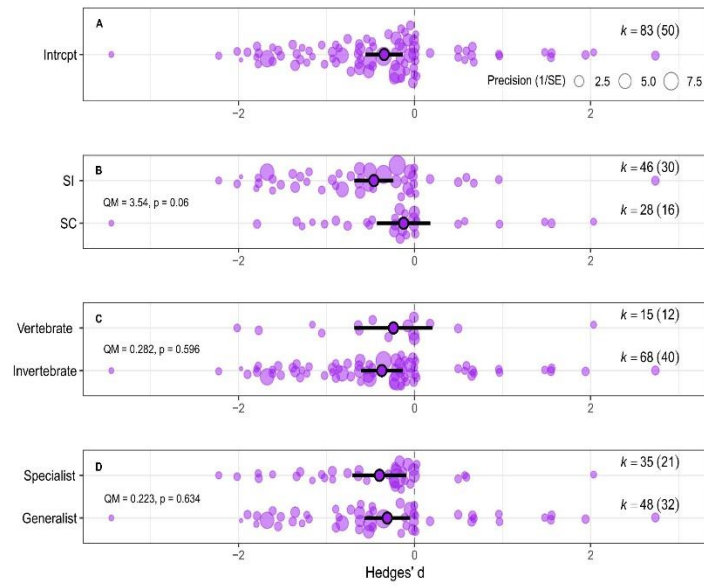
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Figure 2



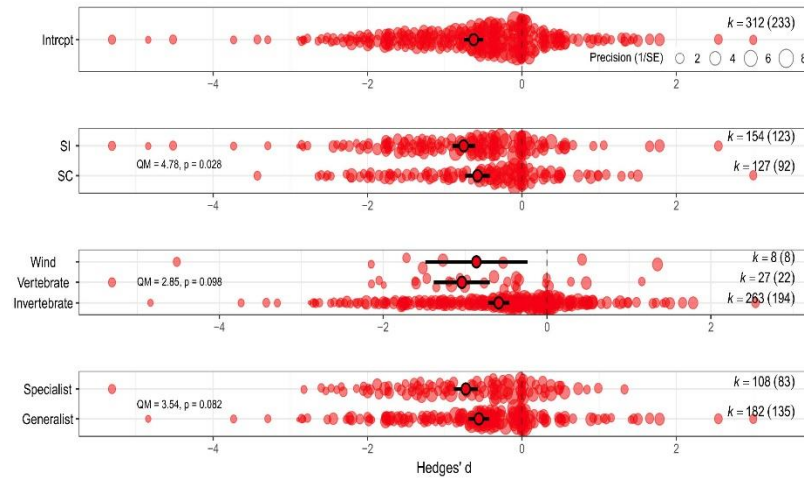
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Figure 3



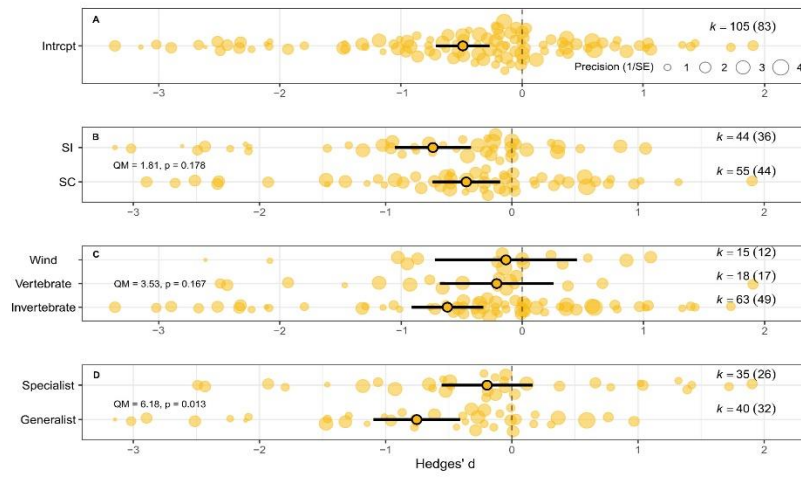
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Figure 4



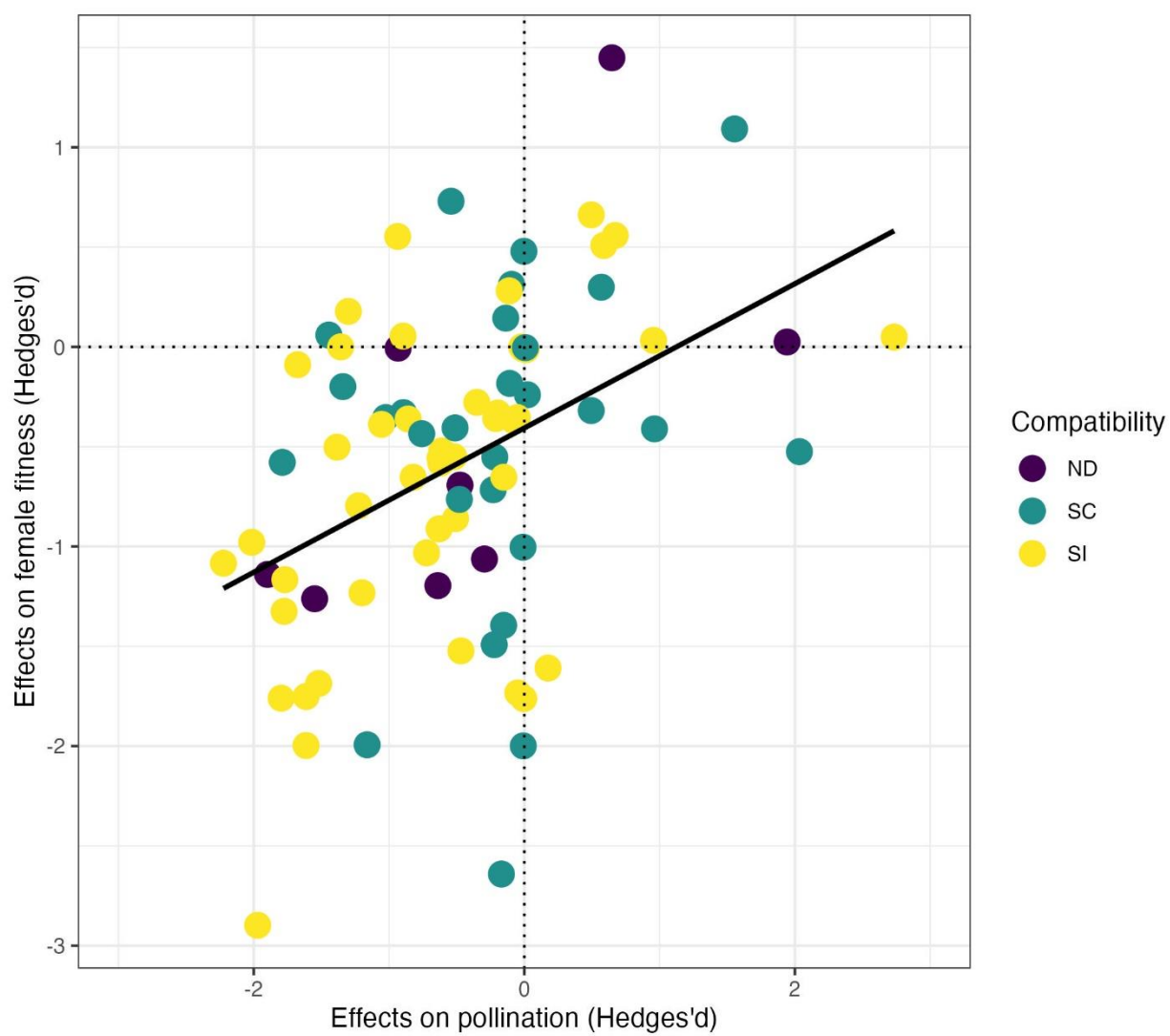
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Figure 5



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Figure 6



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Figure 7

