



Pollination bottlenecks in glory lily (*Gloriosa superba* L., Colchicaceae): Implications for reproductive success and conservation

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Abstract

Gloriosa superba L. (Colchicaceae), a tuberous geophyte of tropical Africa and Asia and the principal commercial source of colchicine, depends on biotic vectors for seed production. Its floral architecture enforces outcrossing: a trifid stigma on a style deflexed at 90° to the ovary, six radiating exerted stamens and a pollen-to-ovule ratio of 58333 : 1 together produce approach herkogamy and a predominantly xenogamous breeding system. This review applies a pollination bottleneck framework to the species, tracing sequential constraints on reproduction and distinguishing those demonstrated empirically from those inferred from comparable taxa. Fifty-nine sources retained from a structured search of five databases were assigned to four evidence tiers. Pollen delivery, rather than stigma availability, emerges as the binding constraint: stigmas remain receptive for 3–4 days while viable pollen persists for hours and comparative experiments show fruit set rising from 59.55 % under open pollination to 80.94 % under blower-assisted and 87.31 % under hand pollination. The deficit extends to seed quality, with hand-pollinated seed attaining 54 % germination and a vigour index of 1252. Although assessed globally as least concern (LC), the species is subject to documented regional depletion, with some 75 % of raw material demand being met from wild collection. Mitigation options nonetheless exist: supplemental hand- and blower-assisted pollination, pollen cryopreservation to widen the transfer window, assisted gene flow between characterised ecotypes and cultivation substitution together offer a practical route to improving reproductive output and maintaining genetic diversity. Density thresholds, isolation effects, phenological mismatch and floral volatile chemistry remain inferred rather than demonstrated and are identified as research priorities.

Keywords: assisted gene flow; dichogamy; geophyte conservation; herkogamy; pollen limitation; supplemental pollination

Abbreviations: CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora; F1: filial 1 (first filial generation); G_{ST}: coefficient of genetic differentiation; Hs: within-population genetic diversity; IAA: indole-3-acetic acid; IKI: iodine-potassium iodide; ISSR: inter-simple sequence repeat; IUCN: International Union for Conservation of Nature; MVP: minimum viable population; N_m: gene flow (number of migrants per generation); P/O: pollen-to-ovule; RADseq: restriction site-associated DNA sequencing; RAPD: random amplified polymorphic DNA; UV: ultraviolet; VOCs: volatile organic compounds

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1. Introduction

Animal pollination underpins sexual reproduction in roughly 87 % of angiosperm species, with dependence highest in tropical communities, where about 94 % of plants rely on biotic vectors (1). Where vector service is inadequate, plants experience pollen limitation, a reduction in reproductive success caused by insufficient pollen quantity or quality (2, 3). Documented declines in pollinator abundance and diversity, driven by habitat loss and fragmentation, agrochemical exposure, pathogen spread, climatic change and invasive species,

have converted this latent constraint into an active threat (4–7). The burden falls disproportionately on plants with specialised pollination systems and obligate outcrossing, which possess neither an alternative vector nor an autogamous fallback when their mutualists fail. *Gloriosa superba* L. is such a species, a tuberous geophyte of the Colchicaceae and the principal commercial source of colchicine, it is assessed globally as least concern (LC) (8), but sustained tuber harvest has driven local depletion across parts of its Indian range and prompted recommendations for monitoring harvesting levels where populations may become locally

threatened (8–10). Its floral architecture enforces outcrossing: a trifold stigma borne on a style deflexed at 90° to the ovary, six radiating exerted stamens and a pollen-to-ovule ratio in the tens of thousands together produce pronounced herkogamy and a xenogamous breeding system in which pollen transfer depends on a narrow set of large-bodied vectors (11, 12). Seed production in this species is therefore not merely assisted by pollinators but overwhelmingly dependent on them (11, 13) and its reproductive ecology remains the least studied aspect of its biology (11). Prior work has concentrated on medicinal chemistry, tissue culture, descriptive floral biology and visitor behaviour (9, 13). Pollinator identity and the influence of nectar traits on foraging remain poorly understood across much of the range, including the Northwestern Himalaya (13). Although low natural seed set is widely acknowledged, the ecological and physiological constraints producing it have not been integrated into conservation planning (13, 14). This review addresses that gap by developing and applying a pollination bottleneck framework to *G. superba* (15). Rather than treating reproductive traits and environmental pressures separately, as previous accounts have done (9, 10, 16), the framework traces sequential constraints across three stages: pre-pollination, pollination and post-pollination and asks at which stage reproductive output is lost. This organisation makes it possible to connect local pollen limitation to the wider consequences of genetic erosion and population vulnerability and to distinguish constraints that are empirically demonstrated in this species from those inferred from comparable taxa. Three questions structure the synthesis: (i) which stage of the reproductive sequence imposes the binding constraint on seed output; (ii) which of the constraints identified are established for *G. superba* and which are extrapolated from comparable taxa; and (iii) which interventions have demonstrated the capacity to relieve them. The review first establishes the study system, floral biology and pollinator assemblage, then examines the bottlenecks in sequence, assesses the reproductive and genetic consequences that follow from them and finally evaluates mitigation and management options.

Methodology

Five databases - Scopus, Web of Science, PubMed, ScienceDirect and Google Scholar - were searched on 15 February 2026 for literature published from 2000 up to the search date, using PRISMA 2020 principles adapted to the narrative synthesis design. Three concept blocks were combined with AND and terms within each block were combined with OR: the taxon ("*Gloriosa superba*" OR "glory lily" OR "flame lily" OR "*Gloriosa*"); the process ("pollinator" OR "breeding system" OR "floral biology" OR "seed set" OR

"herkogamy" OR "dichogamy"); and the constraint ("pollen limitation" OR "habitat fragmentation" OR "phenological mismatch" OR "pollinator decline" OR "genetic erosion"). Reference lists of retained articles were also hand-searched. Eligible records reported primary data, or syntheses grounded in primary data, on reproductive ecology, pollination biology, breeding systems, population genetics or conservation status, in peer-reviewed journals, edited volumes or intergovernmental assessments. Excluded records included phytochemical, pharmacological and agronomic studies without reproductive-ecological content, conference abstracts, theses, preprints, patents, non-English sources and non-peer-reviewed web material. Database searching identified 342 records; removal of 117 duplicates left 225 records for screening, of which 168 were excluded, yielding 57 sources from the database-search pathway (Fig. 1). Two additional sources were identified through other methods during revision: one primary study identified through supplementary searching and the International Union for Conservation of Nature (IUCN) Red List assessment for *G. superba*, obtained through targeted consultation of the IUCN Red List. The final qualitative synthesis comprised 59 sources. Because the species-specific evidence base is small, sources were assigned to four evidence tiers, signalled in the text wherever a claim rests on anything other than Tier 1: direct empirical studies of *G. superba* (Tier 1, $n = 19$); reviews of the species (Tier 2, $n = 4$); studies of other taxa used to model mechanisms undocumented in *G. superba* (Tier 3, $n = 12$), claims from which are identified as inferences throughout; and theoretical, ecological and policy literature (Tier 4, $n = 24$). Pollination evidence was classified separately at three levels - observed visitation, potential pollination inferred from behaviour or floral contact and effectiveness demonstrated experimentally - to avoid equating visitor frequency with pollination effectiveness. The schemes are independent: a Tier 1 study may report taxa at any level. Within Tier 1, evidence was appraised according to replication, reporting of variance and duration. Only two Tier 1 studies incorporated field observations across multiple flowering seasons, so inter-annual variation in pollinator abundance and reproductive output remains poorly constrained. The Tier 1 corpus is geographically clustered in South Africa and four Indian states, with no data from East or West Africa, Sri Lanka, Southeast Asia or most of peninsular India. Pollinator effectiveness has also been assessed using different methods in the two best-studied regions, limiting direct comparison. Where findings conflict, both are presented and the methodological basis of the discrepancy is examined rather than averaged.

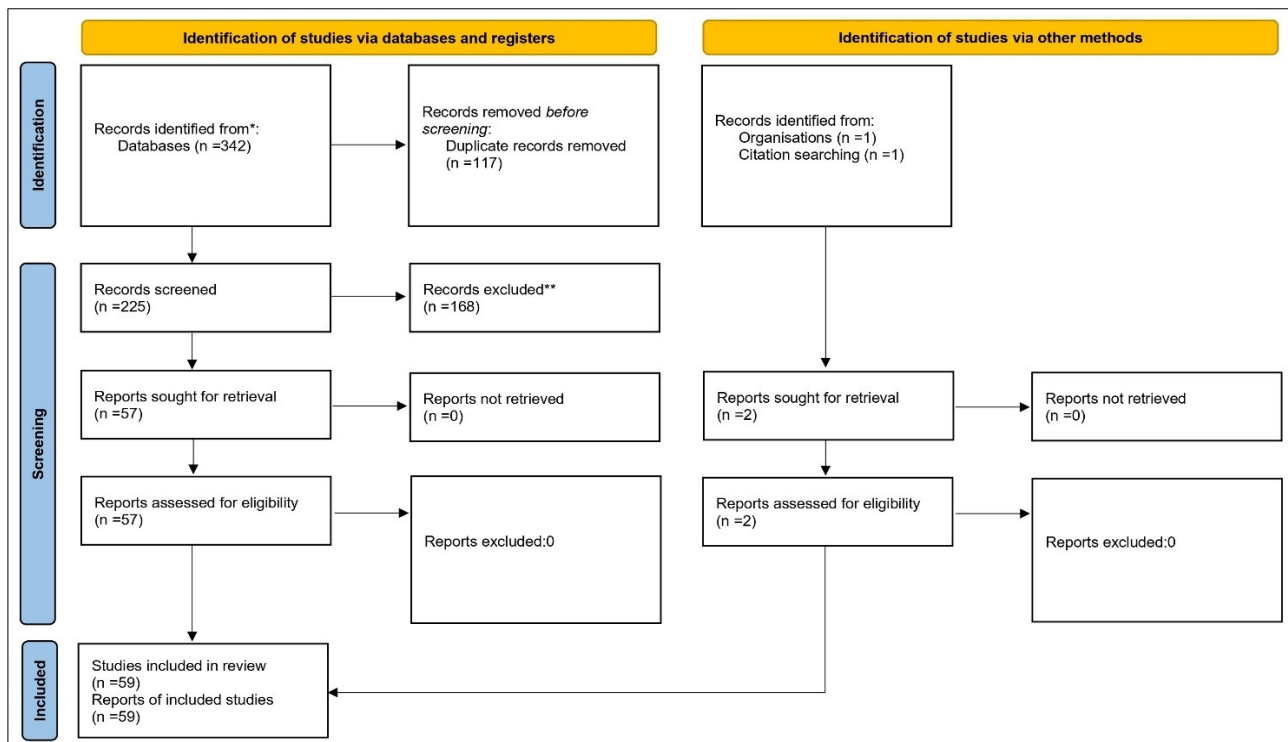


Fig. 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA)-style flow diagram of the identification, screening and inclusion of sources.

The study system: Distribution, habitat and conservation status

Gloriosa superba is a perennial tuberous geophyte with a scrambling, tendril-climbing habit reaching 4–6 m, native across tropical and subtropical Africa and Asia, extending from Senegal east to Somalia and south to South Africa and through the Indian subcontinent, Sri Lanka and Southeast Asia (9, 16, 17). This exceptionally wide and disjunct range is directly consequential for the present synthesis, because the floral visitors recorded at its extremes are not equivalent: South African populations are visited principally by large lepidopterans, with sunbirds also recorded (11), whereas Indian populations are dominated by hymenopteran visitors (18). No single pollination syndrome can therefore be attributed to the species and every generalisation below is qualified by provenance. Three further attributes of the system bear on reproductive success. First, habitat: the species occupies open woodland, grassland, forest-savanna ecotone, thicket margins and anthropogenically disturbed scrub and field surveys describe wild occurrences as small, scattered stands of few individuals (16, 17, 19). A species that is naturally sparse and edge-associated is, by construction, exposed to the isolation and edge effects examined below. Second, phenology: growth and flowering are strictly seasonal and monsoon-cued, with anthesis following the onset of heavy rain (9, 16). Flowering runs from July to September in the Northwestern Himalaya and from December to April in South Africa (13, 17). A reproductive window this narrow and this tightly coupled to a single climatic trigger, is the precondition for the

phenological-mismatch risk assessed below. Third, propagation: tubers permit clonal persistence independent of seed (9, 16), which allows established stands to survive reproductive failure while ceasing to recruit. Standing plant abundance is therefore a poor proxy for population viability in this species and censuses that count ramets will systematically understate decline. The species is assessed globally as LC (8), a status reflecting its wide distribution across Africa and Asia; regional accounts nonetheless report severe depletion of Indian populations during the 1980s through indiscriminate tuber harvest (10, 16) and the global assessment itself records local over-collection in India (8). Despite the availability of cultivated material, an estimated 75 % of pharmaceutical raw-material demand is still met from wild-collected tubers (9), sustaining a harvest pressure that removes reproductive individuals from precisely the small, isolated populations least able to absorb the loss. Harvest pressure and pollination failure are therefore not independent threats but compounding ones, a point developed later in this review.

Floral biology and morphological adaptations for pollination

Floral architecture and structural adaptations

The floral architecture of *G. superba* displays several structural modifications that facilitate biotic pollination (11). Flowers are solitary and large, with six perianth segments (tepals) that are rigidly reflexed and conspicuously crinkled along their margins (11). During anthesis, the tepals undergo a marked colour transition, from light green or yellow through orange to deep scarlet or crimson (11). Nectaries are located within

tubular structures along the midline of the adaxial tepal surface and each flower is organised into six functional pollination units or merantha (11). Morphometric measurements give a mean tepal length of 6.69 cm and width of 1.45 cm, borne on an elongated pedicel averaging 10.32 cm (13). The reproductive organs are arranged with a spatial precision that enforces outcrossing. The androecium comprises six hypogynous, exerted stamens bearing versatile linear anthers approximately 1.26 cm long, which dehisce extrorsely by longitudinal splitting to release abundant yellow pollen (10, 13). The gynoecium is superior, tricarpellary and trilocular, with numerous anatropous ovules borne in two rows on axile placentae (10). A defining structural feature is the style, which bears a trifid stigma and is sharply deflexed at a 90° angle at its point of attachment to the ovary (11). This orientation, combined with the radiating arrangement of the stamens, produces approach herkogamy - the spatial separation of anthers and stigma that reduces the probability of autonomous self-pollination. The deflexed style typically projects into open space within the

surrounding vegetation, where it intercepts approaching visitors: papilionid butterflies foraging at the nectaries deposit and collect pollen on the ventral surfaces of their wings (11). These structural adaptations are illustrated in Fig. 2.

Colour dynamics and visual signalling

Tepal colour changes progressively through floral development, from light green in bud, to yellow at opening, to orange-red or golden-red and finally to deep scarlet or crimson at full bloom (16). During the stigma-receptive phase, the tepals are typically bicoloured, with a scarlet apex, yellow midsection and greenish base (10). This pattern is reinforced by ultraviolet (UV) reflectance: the distal orange-red half of each tepal reflects approximately 40 % of incident UV, while the yellow base is UV-absorbing (11). The junction of the two zones forms a high-contrast, V-shaped mark oriented toward the nectary - a configuration that functions as a nectar guide for pierid butterflies (11). These bicoloured cues attract both indigenous and naturalised pollinators in South African populations (11).

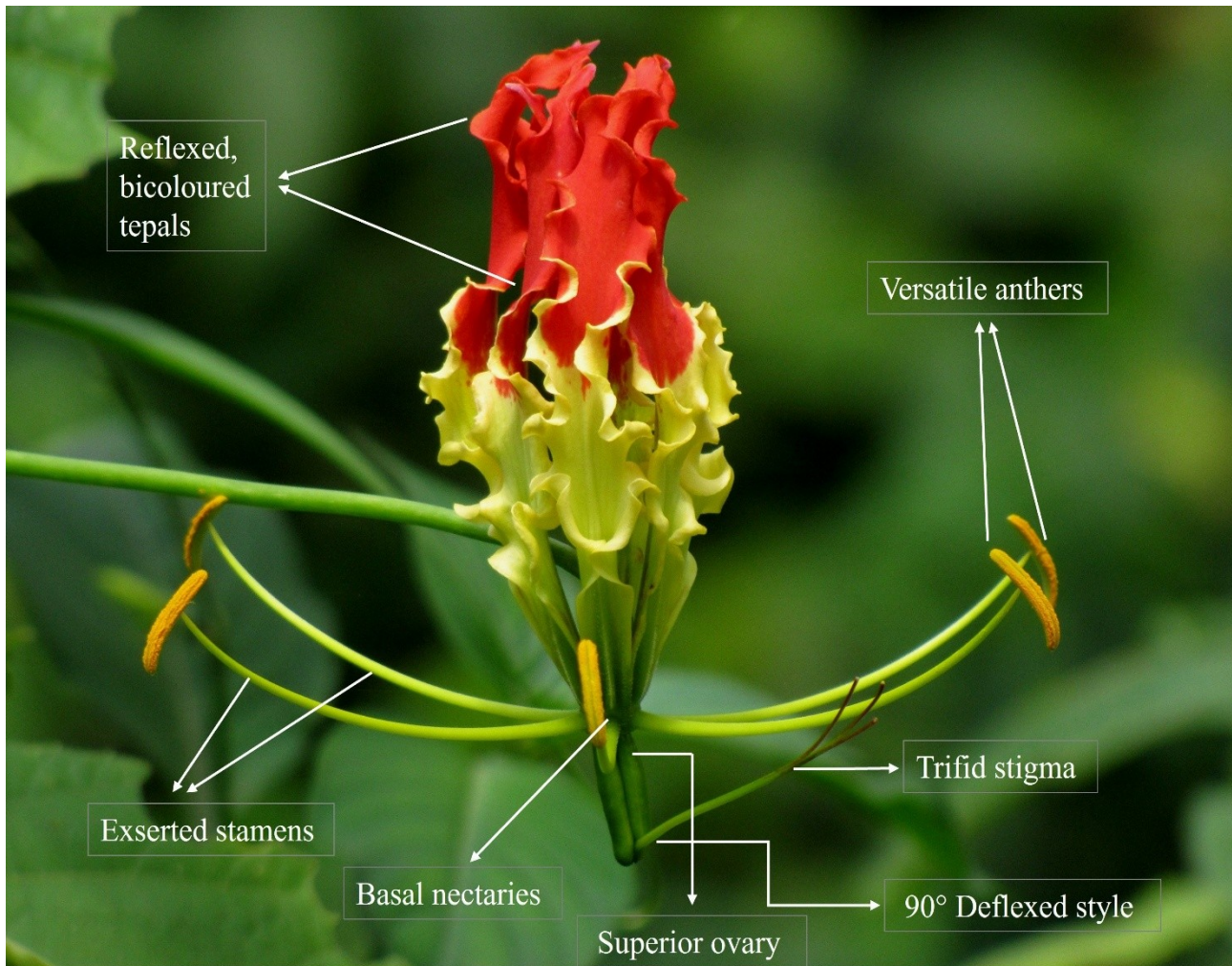


Fig. 2. Floral architecture of *Gloriosa superba* showing reflexed bicoloured tepals, basal nectaries, six exerted stamens with versatile anthers, superior ovary, deflexed style and trifid stigma. The spatial separation of anthers and stigma produces approach herkogamy, restricting autonomous self-pollination. Photograph by Vaikoover, 2011 (Wikimedia Commons, CC BY 3.0, <https://creativecommons.org/licenses/by/3.0/>); labels added by the authors.

Nectar biology

Nectar secretion in *G. superba* follows a periodic pattern closely synchronised with floral development and with the activity of its pollinators. Nectar production began after floral stage 4 and peaked at stage 6. From stages 5–8, nectar secretion ranged from 4.13 ± 0.28 to $33.04 \mu\text{L flower}^{-1}$ in 2021 and from 3.78 ± 0.33 to $32.10 \pm 0.54 \mu\text{L flower}^{-1}$ in 2022, with nectar accumulation ceasing after stage 8 (18). Supply also fluctuates within the day: mean volumes are substantially higher in the morning ($1.75 \mu\text{L}$) than in the afternoon ($0.94 \mu\text{L}$), a difference attributable either to depletion by visitors or to a diurnal decline in secretion rate (11). Qualitative analysis identifies sucrose, glucose, fructose and raffinose as the principal sugars (13). Dried nectar sugar reaches approximately $94.70 \mu\text{g}$ per flower at stage 6, coinciding with peak stigma receptivity and with the foraging activity of the principal bee and butterfly visitors (13). Nectar sugar concentration varied across developmental stages, with the maximum concentration recorded at stage 7 (18). Reported sugar concentrations vary widely across regions, from 29.8–78.04 % (11, 13). This range is unusually broad for a single species and is unlikely to reflect geographic variation alone: differences in sampling time relative to anthesis, in the control of evaporative concentration and in whether flowers were bagged before sampling would each shift the measured value and the source studies do not report these parameters in a form that permits direct comparison. The upper values in particular warrant confirmation under standardised sampling protocols. Because nectar robbing occurs in wild populations and can substantially deplete standing crop, experimental studies typically cage flowers before collection in order to separate secretion from removal (13).

Pollen biology and morphology

Pollen viability is initially high but declines rapidly. Freshly dehiscent pollen tested with acetocarmine or iodine-potassium iodide (IKI) shows viability of 88.40–96.22 % (12, 13). Viability is maximal during the first 4 hr after dehiscence and is retained for approximately 14 hr (20), falling to about 49.85 % by the second day after anthesis (13). Loss of pollen moisture accelerates this decline and desiccation is the principal environmental determinant of viability (20). *In vitro* germination is optimal at 25 °C under humidity sufficient to prevent desiccation (20). Sucrose and boron promote pollen tube growth: a 5–10 % sucrose solution yields germination of up to 98.86 %, whereas concentrations of 20 % suppress germination to below 10 % (12). The six exerted, radiating stamens bear versatile anthers that dehisce between 07:30 and 09:30 (12). A single anther produces approximately 350000 pollen grains, giving a pollen-to-ovule ratio of 58333.33 : 1 and indicating an obligately xenogamous breeding system (12). Set against this very high pollen investment, the rapid post-dehiscence decline in viability means the effective window for successful transfer is narrow, a constraint examined among the pollination bottlenecks below.

Anthesis, dichogamy and stigma receptivity

Anthesis in *G. superba* is morning-oriented and light-associated (12). Bud opening begins between 06:30 and 07:30, peaks between 08:30 and 10:30 and declines sharply through the late morning as irradiance increases (12). Floral stages 1–5 constitute the pre-pollination phase, with peak nectar accumulation and reproductive activity occurring from stages 6–8 (18). Protogyny is the predominant form of dichogamy observed across investigated populations of *G. superba* (18). Maximum mean seed set is obtained when pollination is carried out 1 day before anther dehiscence, confirming that the stigma attains receptivity in advance of pollen release (18). Anther dehiscence follows between 07:30 and 09:30 on the day of anthesis or in some documented Indian populations 1 day after anthesis (12). Congeners including *G. minor* are reported as protandrous; therefore the protogynous condition in *G. superba* is not a generic feature of the genus but a species-level mechanism promoting xenogamy (12). Temporal separation of the sexual phases acts together with the structural herkogamy described above, so that self-pollination is constrained both in time and in space (11, 18). Stigma receptivity is assessed by artificial pollination followed by pod set analysis and extends over 3–4 days, from 1 day before anthesis, through the day of anthesis, to 2 days afterwards (18). Effective pollen transfer is concentrated between 07:00 and 11:00, when nectar availability and pollen viability are both greatest (18). The two sexual phases are therefore markedly asymmetric in duration. The female phase remains open for 3–4 days, whereas viable pollen is available for a matter of hours before the decline in pollen viability described above begins. Reproductive failure in this species is consequently more likely to arise from failure of pollen delivery than from unavailability of receptive stigmas - a distinction that determines which interventions can succeed and one returned to below. The floral, nectar and reproductive characteristics determining pollination success in *G. superba* are summarised in Table 1.

Floral scent and volatile compounds

The floral traits of *G. superba* - large, showy, brightly coloured, diurnally open flowers visited by butterflies that effect pollen transfer on their wings - have been interpreted as conforming to the psychophilous syndrome (11). Two considerations qualify this assignment. First, it rests principally on South African observations and sits uneasily with the hymenopteran dominance reported from Indian populations (18). Second, the diagnostic criteria for butterfly pollination are themselves contested: nectar quantity, nectar guides and the strength of floral scent have all been variously included in or excluded from definitions of the syndrome and no comprehensive set of characters has been widely adopted (21). Syndrome classification for this species should therefore be treated as provisional. Scent remains uncharacterised. No volatile organic compound (VOC) profile has been published for *G. superba* and reduced fragrance, although commonly listed as a component of the psychophilous syndrome, has not been verified



instrumentally in this species. The comparisons that follow are drawn from other taxa and are offered as hypotheses about mechanisms that might operate in *G. superba*, not as evidence that they do. In the butterfly-pollinated orchid *Platycoryne mediocris*, scent emission is minimal, at approximately 0.55 ng flower⁻¹ hr⁻¹, compared with up to 2551 ng flower⁻¹ hr⁻¹ in moth-pollinated relatives within the same *Habenaria* clade, which depend on strong nocturnal odour cues (22). Its trace emissions comprise the benzenoid benzaldehyde (68.2 %), the terpenoid linalool (12.0 %) and phenylethyl alcohol (22). The oil palm *Elaeis guineensis* Jacq., by contrast, produces a strong fragrance dominated by estragole, palmitic acid, farnesol and squalene, which attracts its specific weevil pollinators (23). In sexually deceptive orchids of the genus *Ophrys*, floral volatiles form highly specific odour bouquets that mimic female sex pheromones and maintain reproductive isolation between closely related lineages (24). These systems

establish that low or absent emission is itself a functional trait in butterfly-pollinated taxa rather than a null condition and that trace compounds can carry substantial signalling load. The potential for floral scent chemistry to diverge from predictions based on syndrome alone is illustrated by the sand verbena *Abronia ameliae* Lundell, which is pollinated principally by diurnal visitors, although nocturnal pollinators also contribute considerably to reproduction; the species nevertheless emits a volatile profile dominated by compounds associated with nocturnal moth attraction (21). Whether comparable patterns occur in *G. superba* remains unknown. Direct characterisation of *G. superba* floral volatiles across the diurnal cycle and across geographically distinct populations would establish whether scent contributes to visitor attraction and whether volatile profiles vary between the South African and Indian assemblages. This is identified below as a research priority.

Table 1. Floral, nectar and reproductive characteristics of *Gloriosa superba* influencing pollination success

Trait category	Specific characteristics and parameters	Reproductive function	References
Floral architecture	○ Large, solitary flowers ○ Six crisped, reflexed tepals ○ Pedicel c. 10.32 cm ○ Tepal 6.69 × 1.45 cm	Conspicuous display intercepting large-bodied biotic vectors	(11, 13)
Visual signalling	○ Colour transition: green → yellow → orange → crimson ○ UV reflectance c. 40 % on distal half ○ UV-absorbing base	High-contrast V-shaped nectar guide directed toward the nectary	(10, 11, 16)
Breeding system	○ Pollen 350000 grains per anther ○ P/O ratio 58333.33 : 1 ○ Predominantly xenogamous	High pollen investment per ovule, indicating inefficient transfer and obligate outcrossing	(12)
Structural herkogamy	○ Trifid stigma ○ Six exerted, radiating stamens ○ Style deflexed 90° at ovary attachment	Approach herkogamy restricting autonomous self-pollination; transfer via ventral wing surfaces	(11)
Nectar dynamics	○ Secretion from stage 4, peak at stage 6 ○ Volume 4.13–33.04 µL (2021) and 3.78–32.10 µL (2022) per flower ○ Sugars: sucrose, glucose, fructose, raffinose ○ Concentration 29.8–78.04 %	Reward synchronised with stigma receptivity and peak visitor activity	(11, 13, 18)
Pollen viability	○ Initial viability 88.40–96.22 % ○ Maximal within 4 hr of dehiscence ○ 49.85 % by day 2 ○ Desiccation-sensitive	Narrow transfer window (07:30–09:30) requiring prompt visitation after anthesis	(12, 13, 20)

Pollinator diversity, specificity and visitation patterns

Pollinator diversity and visitation patterns

Floral visitors to *G. superba* differ markedly between the African and Asian portions of its range. In South African populations the flowers attract the sunbird *Cinnyris chalybeus*, but large-winged lepidopterans are the principal effective vectors: *Eronia cleodora* and *Papilio demodocus* contact the anthers and stigma while manoeuvring about the flower during nectar feeding, carrying pollen on their ventral wing surfaces (11). At Nauni, Solan, Himachal Pradesh, hymenopteran visitors were prominent, including *Apis cerana*, *Apis mellifera* and *Bombus haemorrhoidalis* (13, 18). Experimental pollination treatments further demonstrated that honeybees can contribute to reproductive output in *G. superba*, although hand and blower-assisted pollination produced higher values for several reproductive traits (13). The apparent contrast between the South African and Indian systems should not be interpreted simply as a difference between butterfly and bee pollination. The combined visitor observation with controlled pollination, pollen-deposition analysis and pollinator-exclusion experiments, providing direct evidence for effective butterfly-mediated pollen transfer (11). The Indian work characterised visitor abundance and foraging behaviour and evaluated the reproductive output of alternative pollination treatments (13, 18). The two bodies of work differ in experimental approach and in the endpoints measured, which makes direct comparison of pollinator effectiveness difficult. Nectar exploitation has additionally been reported in *G. superba* (11, 25), so visitor identity alone cannot establish whether a given visit results in effective pollen transfer. Resolving how much of this regional difference represents true variation in pollinator effectiveness will require equivalent measures across populations - pollinator-exclusion treatments, single-visit pollen-deposition measurements and direct observation of pollen placement on different visitor taxa. Until such data are available across the range, the pollination system of *G. superba* is better described as regionally variable and incompletely characterised than as a single syndrome operating uniformly throughout its distribution. Vector identity may also govern the spatial scale of pollen movement, since large butterflies move between plants over distances that differ from typical bee foraging bouts - a distinction of particular consequence for a species that is partially self-incompatible and reliant on among-plant transfer for full seed set (11, 12).

Pollination effectiveness and floral visitor impacts

Pollination effectiveness in *G. superba* depends on the interaction between visitor behaviour and floral architecture and varies among visitor taxa and regions. In South Africa the principal effective pollinators are the large-winged lepidopterans *E. cleodora* and *P. demodocus*, which transfer pollen through contact between their ventral wing surfaces and the widely separated anthers and stigma (11). The mechanism is supported by a positive relationship between butterfly wing-scale deposition on stigmas and the number of conspecific pollen grains they receive, quantified below

(11). In Indian populations hymenopterans also contribute, honeybee pollination yielding 45.57 % fruit set, below the 59.55 % recorded under open pollination, while blower-assisted and hand pollination reached 80.94 % and 87.31 % respectively (13). The pollination system may therefore be more functionally diverse than the South African evidence alone would imply. The enhanced reproductive response under assisted pollination suggests that natural pollen delivery may be insufficient to saturate stigmatic pollen demand, providing an experimental link between pollinator effectiveness and the pollen-limitation bottleneck examined below and an empirical basis for the interventions evaluated later in this review. Antagonistic floral visitors - such as nectar robbers and non-pollinating insects that consume rewards without effecting pollination - represent a potentially important but insufficiently resolved component of this system. Honeybees and ants have been reported extracting nectar from the basal nectaries without contacting the anthers and such removal could reduce the reward available to subsequent legitimate visitors (11, 25). Nectar depletion should not, however, be equated with reduced seed production. Establishing that effect would require demonstrating that nectar removal alters subsequent visitation by effective pollinators, stigmatic pollen deposition, or final fruit and seed set. The relationship between nectar exploitation and reproductive success therefore remains a testable hypothesis rather than an established mechanism. The dependence on biotic vectors is demonstrated directly, though its magnitude depends on the treatment applied. Emasculated flowers excluded from pollinators produced no fruit, while unbagged flowers in some populations reached fruit set of up to 100 % (11); pollinator exclusion without reported emasculation yielded 28.53 %, against 59.55 % under open pollination (13). Taken together, the evidence indicates that *G. superba* is strongly pollinator-dependent but not necessarily reliant on a single pollinator guild throughout its range.

Pollination bottlenecks

The constraints examined below act at two points in the reproductive sequence. The first group limits the availability and effectiveness of pollen vectors before transfer can occur: declining pollinator populations, habitat fragmentation and isolation, phenological asynchrony, competition from introduced and invasive species, abiotic effects on floral rewards and anthropogenic disturbance at the microhabitat scale. The second concerns transfer itself - whether sufficient compatible pollen reaches receptive stigmas - together with the population-level threshold below which vector service becomes unreliable. Post-pollination constraints on fruit and seed development and their genetic consequences, are treated in the two sections that follow. Throughout, constraints demonstrated empirically in *G. superba* are distinguished from those inferred from comparable taxa, following the evidence tiers set out in the review methodology.

Population density, pollination thresholds and Allee effects

Theoretical treatments of pollination failure indicate that reproductive bottlenecks can be particularly severe in specialised plant–pollinator interactions, where environmental change may generate spatial or temporal mismatches between mutualistic partners (26, 27). *Gloriosa superba* has a floral architecture that promotes specialised pollen transfer by particular visitor types, while some wild occurrences are small and spatially fragmented. No minimum viable population (MVP) size or pollination-density threshold has been determined empirically for *G. superba*. Evidence from other animal-pollinated species illustrates the form such a constraint could take. In fragmented populations of *Haloxylon ammodendron*, pollen limitation was an important constraint on seed set and higher pollinator visitation was associated with greater seed production, although wind pollination also contributed substantially in that species (28). In the monocarpic Haleakalā silversword, percent seed set measured over five years was significantly correlated with the number of plants

flowering annually: individuals flowering in low-flowering years set significantly less seed than those flowering in high-flowering years and asynchronously flowering plants were pollen-limited whereas synchronously flowering plants were not, suggesting a pollinator-mediated Allee effect (29). That species is strongly self-incompatible, however, whereas *G. superba* retains partial self-compatibility, so the strength of any comparable density dependence in *G. superba* cannot be assumed from this case. Whether *G. superba* exhibits a comparable density-dependent reproductive threshold remains untested. Establishing this would require replicated measurements of local flowering-plant density, pollinator visitation, pollen deposition and per-plant fruit and seed set across a gradient of population densities within the same region. Given the regional depletion and continuing wild harvest documented above, determining whether reduced population density generates a pollination-mediated Allee effect represents an important conservation research gap, identified below as a priority. The principal reproductive bottlenecks in *G. superba* and their consequences are summarised in Fig. 3.

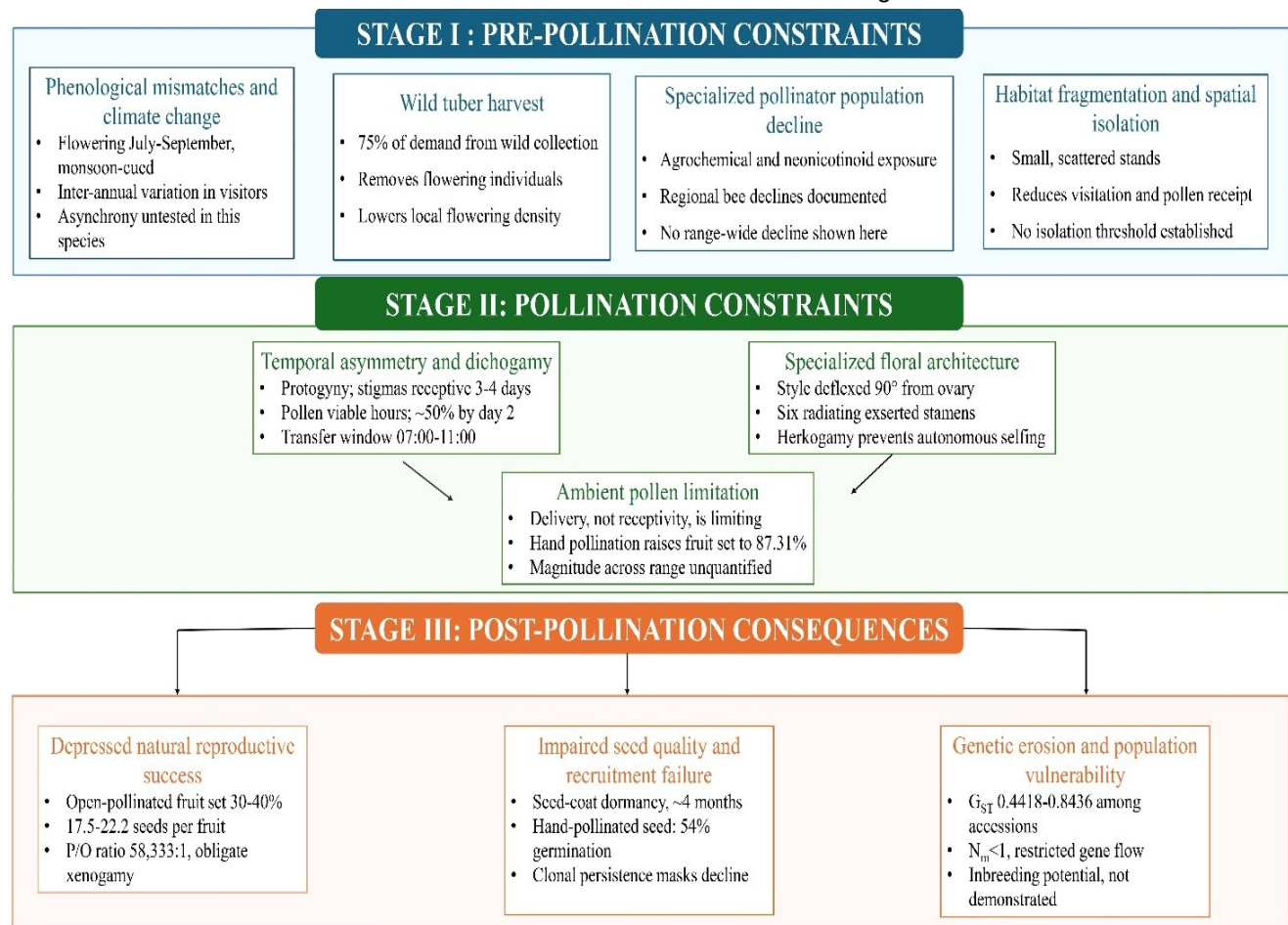


Fig. 3. Sequential pollination bottleneck framework for *Gloriosa superba*. Pre-pollination constraints act on the availability and effectiveness of pollen vectors (Stage I); these combine with floral and temporal traits enforcing outcrossing to produce pollen limitation at the point of transfer (Stage II); and reduced pollen delivery propagates into depressed fruit and seed set, impaired seed quality and restricted gene flow (Stage III). Constraints demonstrated empirically in *G. superba* are distinguished from those inferred from comparable taxa throughout this review.

Pollinator population decline

Because *G. superba* depends on animal-mediated pollen transfer for seed production, as demonstrated experimentally in South African populations (11), any reduction in effective pollinator service would be expected to reduce reproductive output. Declines in pollinator abundance and diversity have been documented across multiple taxa, with habitat destruction, degradation and fragmentation among the major proposed drivers (4, 27). Analyses of historical records found significant declines in local bee species richness in 52 % of British and approximately 67 % of Dutch 10 × 10 km grid cells after 1980 (7). Pathogen transmission at shared floral resources and exposure to agrochemicals, including neonicotinoids, have also been identified as contributors to pollinator decline (5). Evidence from other plant–pollinator systems indicates that reductions in pollinator abundance can reduce fruit and seed production (30). For *G. superba* specifically, the evidence remains regional and does not yet demonstrate a range-wide decline in pollinators. Studies have established dependence on animal-mediated pollen transfer for seed production in South African populations (11), while other studies have documented diverse floral visitors and experimentally evaluated pollination treatments at Nauni, Himachal Pradesh (13, 18). These studies demonstrate the importance of pollinator-mediated reproduction but do not establish a long-term decline in pollinator abundance for the species. Pollinator decline should therefore be regarded as a plausible but currently unquantified pressure, distinct from the better-documented threat of wild tuber harvesting and not addressable by regulation of harvest alone.

Habitat fragmentation and isolation

Habitat fragmentation reduces the abundance, diversity and activity of pollinators and consequent reductions in pollen delivery are among the most frequently reported effects on animal-pollinated plants (31). In *Hedysarum scoparium* Fisch. & C.A.Mey., reduced vegetation cover and increased isolation between patches were associated with lower pollen loads on stigmas and reduced reproductive success (32). In a once-common Australian grassland daisy, severe fragmentation produced concurrent declines in genetic variation, mate availability and reproductive success in small remnant populations (33). These cases identify two distinct mechanisms - loss of pollinator service and loss of compatible mates - both of which would be expected to operate in a species that is partially self-incompatible and dependent on among-plant pollen transfer. No isolation-distance threshold has been established for *G. superba* and no study has related patch size, connectivity or edge proximity to pollen deposition or seed set in this species. The expectation that

fragmentation constrains its reproduction is therefore an inference from comparable systems rather than a documented finding. The inference is nonetheless grounded in the species' ecology: *G. superba* characteristically occupies forest edges, thicket margins and anthropogenically disturbed scrub and occurs as small, scattered stands, so its populations already possess the spatial configuration in which fragmentation effects are most pronounced. Testing this would require measurement of pollinator visitation, stigmatic pollen load and seed set across a gradient of patch sizes and isolation distances within a single region - the same design framework required for the density threshold discussed above.

Phenological mismatch and climate change

Flowering in *G. superba* is narrowly seasonal and monsoon-cued, a configuration that in principle exposes the species to asynchrony with pollinator activity under changing climatic conditions. At Nauni, Himachal Pradesh, flowering occurs from the second fortnight of July–September, coinciding with the monsoon-associated period. Within individual flowers, nectar production begins after stage 4 and peaks at stage 6, while nectar sugar concentration is highest at stage 7 (18). Inter-annual variation in the visitor assemblage has also been observed at these sites, including differences between years in the representation of *Vespa* species (18), indicating that visitor composition is not fixed from season to season. Because both flowering and nectar availability vary temporally, shifts in the timing or duration of either floral development or pollinator activity could alter the period of effective interaction; however, such climate-driven mismatch has not been demonstrated experimentally in *G. superba*. No long-term record of *G. superba* flowering phenology has been published and no study has tested whether climate-induced asynchrony reduces its reproductive output. The risk must therefore be extrapolated from general models and from other species. Plant–pollinator mismatches arising from differential responses to warming are predicted to reduce seed production where a plant depends on a narrow set of vectors (26) and phenological asynchrony has been identified as a mechanism of population decline in mountain plant species facing climatic change (34). Because pollen transfer in *G. superba* depends on a restricted set of effective vectors, even modest shifts in the timing of vector activity relative to anthesis could reduce pollen delivery and fruit set (11, 18). A hypothetical narrowing of the plant–pollinator interaction window under climate change is illustrated in Fig. 4. Validating this risk would require multi-decadal phenological records for both the plant and its principal vectors, together with projections under defined climate scenarios; neither currently exists for this species.

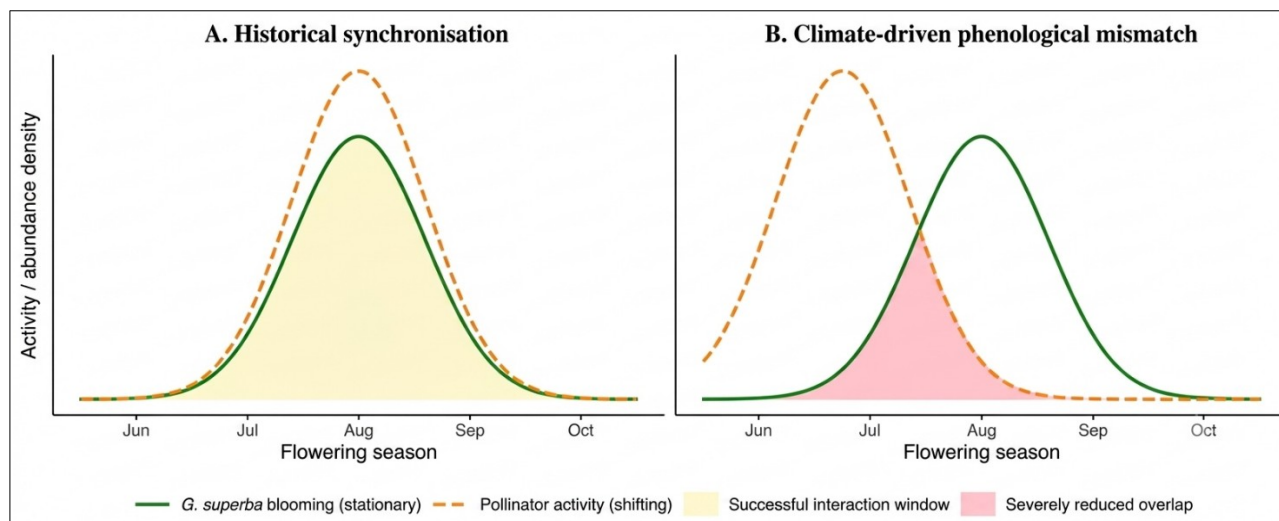


Fig. 4. Hypothetical narrowing of the plant–pollinator interaction window in *Gloriosa superba* under climate-driven phenological change. (A) Historical synchronisation, in which flowering and pollinator activity coincide across the monsoon-associated flowering period. (B) A scenario in which pollinator activity advances while monsoon-cued flowering remains stationary, reducing the period of effective interaction. The illustration does not represent measured data: no long-term phenological record has been published for *G. superba* and climate-driven asynchrony has not been demonstrated in this species.

Pollen limitation

Pollen limitation - reduced seed production arising from insufficient delivery of compatible pollen - is the point at which the constraints described above register in reproductive output. For *G. superba*, direct evidence comes from comparative pollination experiments at Nauni, Himachal Pradesh, where fruit set rose from 59.55 % under open pollination to 80.94 % under blower-assisted and 87.31 % under hand pollination (19; Fig. 5). The finding that supplementing natural pollen delivery improves reproductive output indicates that delivery under ambient conditions is not saturating. This is consistent with the temporal asymmetry identified above. Stigmas remain receptive for 3–4 days, whereas viable pollen is available for a matter of hours, so the quantity of pollen arriving within the receptive window - rather than the availability of receptive stigmas - is the limiting quantity. The magnitude of pollen limitation across the species' range nonetheless remains unquantified. Reported seed set is low and variable and covaries with the local abundance of effective vectors (11, 25), but no study has calculated a pollen limitation index across a series of populations differing in size or isolation. Evidence from comparable specialised systems indicates that the deficit intensifies with increasing isolation as fewer pollen grains reach receptive stigmas (32). Quantifying this for *G. superba* would require paired supplemental and open pollination treatments applied to the same plants across multiple populations - the standard design for estimating pollen limitation and one not yet applied to this species.

Competition from introduced and invasive species

Invasive plants alter pollinator foraging patterns and the structure of pollination networks and their removal has been shown to strengthen network resilience and improve reproductive function in native species (35). No study has examined invasive plant competition in *G. superba* communities, so any effect on this species remains undocumented. Introduced floral visitors present a more directly relevant consideration, though one whose direction is contested. High densities of *A. mellifera* have been shown to disrupt the structure and functionality of plant-pollinator networks, reducing native pollinator diversity and altering community composition (36). Other analyses conclude that honeybees are quantitatively important pollinators in natural habitats worldwide and, where native pollinator diversity is depleted, may partially compensate through high visitation frequency (37). The outcome that predominates appears to be context-dependent and neither has been tested in *G. superba*. The species' biogeography complicates this further. *Apis mellifera* is native across the African portion of the *G. superba* range but introduced in India, where it occurs alongside the native *A. cerana*. Any competitive displacement of native flower visitors would therefore be anticipated in Indian populations but not in South African ones and the two regions cannot be treated as equivalent in this respect. Whether the wing-mediated transfer mechanism characteristic of the large lepidopteran vectors affords *G. superba* any insulation from generalist visitor displacement is untested.

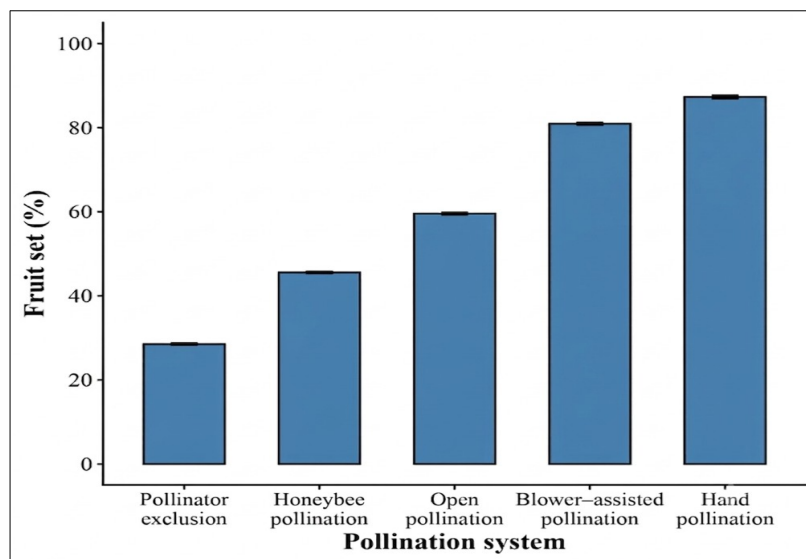


Fig. 5. Effect of pollination system on fruit set in *Gloriosa superba* at Nauni, Himachal Pradesh, India. Values are means $\pm$ SE. All treatments differed significantly at $p \leq 0.05$ by Duncan's multiple range test. Data from Reference (13).

Abiotic stressors affecting pollination

Abiotic conditions influence both the quantity and quality of floral rewards and the viability of pollen and therefore act on pollination success independently of vector availability. Elevated temperature and drought stress reduce nectar availability and global change is predicted to alter the expression of floral traits that mediate pollinator attraction (38). Nectar volumes reported for *G. superba* differ among study sites. One study reported approximately 5.40 μL per flower in South African populations, whereas another study reported up to 33.04 μL per flower in the Nauni population, with nectar volume varying across floral developmental stages (18). These values should be interpreted cautiously because nectar volume is influenced by floral developmental stage, sampling time and environmental conditions, making direct geographic attribution to climate inappropriate without standardised sampling. Where reward availability is reduced during the morning transfer window, the correspondence between peak nectar availability and pollinator activity described above may be disrupted, with consequences for visitation rate and per-visit reward (18, 38). Style orientation appears not to track the solar path but is instead directed towards gaps in the surrounding vegetation, a developmental characteristic that plausibly improves visitor access and may protect the reproductive organs from physical disturbance (11). Direct data are lacking on the effects of rainfall on pollen dispersal and nectar dilution and on wind speed as a determinant of pollinator flight activity and floral orientation in *G. superba*.

Anthropogenic disturbance at the microhabitat scale

Gloriosa superba frequently occupies forest-savanna boundaries, thicket margins and roadsides, habitats subject to sustained human use. Bee communities respond negatively to anthropogenic disturbance across a wide range of habitat types and disturbance

categories (39) and landscape-scale reductions in pollinator availability translate into reduced visitation and fruit set in dependent plants (31). For a species whose floral architecture constrains autonomous self-pollination, such reductions cannot be buffered by selfing. Agrochemical exposure is among the better characterised of these pressures. Neonicotinoid exposure reduces foraging success and survival in honeybees (40), impairs the pollination services that bumblebees provide (41) and reduces bumblebee colony growth and queen production (42); these effects act in combination with pathogen pressure and floral resource scarcity (5). Artificial light at night reduces nocturnal pollinator visitation and fruit set, with consequences propagating to diurnal visitors (43) and may therefore affect the moth and sunbird components of the *G. superba* visitor assemblage. Species specific data are largely absent. No published study quantifies the effects of trampling by livestock or visitors on *G. superba* habitat, the influence of firewood and fodder collection on flowering density, or the impact of roadside pollution on floral volatile signalling. Establishing the relative contribution of these stressors to reproductive failure in this species is identified below as a research priority.

Reproductive success - fruit set, seed set and pollen-ovule ratios

Natural fruit set, seed set and pollen-ovule ratios

Natural open-pollinated fruit set varies considerably between regions, from 30.01–39.94 % across six populations in the Siwalik sub-Himalayan range of Jammu and Kashmir (44) to 59.55 % at Nauni, Himachal Pradesh (13). This disparity is consistent with the regionally variable and incompletely characterised pollination system described above, although differences in habitat, season and measurement protocol may also contribute. Reduced output is not confined to fruit set but extends to seed development within maturing fruits: in South African wild-type

populations, seed number per fruit averages 17.5–22.2 and varies with plant size (25). Total seasonal output varies correspondingly, with plants of 60–65 cm producing a mean of 258 seeds per season compared with 128 in plants of 45–50 cm (25), indicating a positive association between individual plant vigour and reproductive output. Across regions and across metrics, ambient pollination falls short of the values achieved under supplemental pollination, which forms the basis of the pollen-limitation argument developed above. These figures come from northern Indian and South African populations and no systematic comparison of reproductive success across the species' wider range has been published. Differences between reported values therefore cannot be separated from variation in habitat, season and measurement protocol. The pollen-to-ovule (P/O) ratio provides an independent line of evidence for the breeding system. At 58333.33 : 1, the P/O ratio falls well within the range diagnostic of obligate xenogamy because a high pollen investment per ovule is required precisely because transfer between plants is inefficient and self-pollination is structurally constrained by the 90° deflexed style and radiating anthers (11, 12). The P/O ratio thus quantifies the species' dependence on effective vectors rather than indicating that pollen supply is adequate. No published data compare P/O ratios in *G. superba* with those of related Colchicaceae, nor assess the consistency of the ratio across populations and seasons. Whether population size affects per-plant reproductive success in this species remains untested (11, 18). In comparable animal-pollinated species occupying fragmented landscapes, habitat degradation and isolation reduce stigmatic pollen receipt and generate reproductive bottlenecks (32); given the dependence of *G. superba* on a restricted set of vectors, disturbance to local pollinator communities would be expected to act similarly here.

Hand pollination experiments and breeding system

Although *G. superba* is capable of self-fertilisation, a partial pre-zygotic self-incompatibility mechanism operates, such that cross-pollinated flowers set more seed than self-pollinated ones (11, 25). Pollinator exclusion experiments confirm the requirement for external vectors: emasculated flowers denied access to pollinators produced no fruit, whereas unbagged flowers in some populations reached fruit set of up to 100 % (11). A methodological distinction between the two studies accounts for an apparent discrepancy in baseline reproductive potential. Complete reproductive failure was recorded where emasculation was combined with pollinator exclusion (11), whereas pollinator exclusion alone yielded 28.53 % fruit set (13); the latter study does not report emasculation of the excluded flowers. Autonomous self-pollination therefore appears to contribute measurably to fruit set in *G. superba*, though at levels well below those achieved under open or assisted pollination. The substantial difference in fruit set between hand-pollinated and open-pollinated plants indicates that pollen transfer, rather than resource availability, constrains natural reproductive success (18, 25). Fruit set under the different pollination treatments is

shown in Fig. 5. The morphology of lepidopteran visitors is associated with pollination efficacy: the number of pollen grains deposited on a stigma correlates positively with the number of butterfly wing scales left on it ($R^2 = 0.15$, $p = 0.0346$) (11). The relationship is statistically significant but explains only about 15 % of the variance in stigmatic pollen load, so wing-scale deposition should be regarded as corroborating the transfer mechanism rather than as a quantitative predictor of pollen receipt. Reproductive output also varies within an inflorescence: proximal flowers produce more seeds per fruit than terminal flowers, consistent with a resource gradient or with differences in the timing of anthesis along the inflorescence (25). Occasional ovule abortion has been attributed to disrupted polarity within the embryo sac or to partial incompatibility (25). Published data are lacking on the effects of heterospecific pollen interference on stigma receptivity and fertilisation success and on the composition and quality of natural stigmatic pollen loads.

Seed quality, viability and germination

Natural regeneration in *Gloriosa superba* is constrained by morphological and physiological properties of the seed that limit viability and germination. The seeds are tegmic and enclosed by a prominent, brightly coloured sarcotesta, an adaptation for endozoochorous dispersal by birds and other animals (25). Internally, an aleurone-rich endosperm supports survival under unfavourable conditions, while a thick-walled tegmen provides mechanical protection (25). In the Northwestern Himalayan region, mean seed test weight is 2.02 ± 0.01 g and pods average 7.24 ± 0.23 cm in length (18). Germination is strongly dependent on seed treatment. The species exhibits seed-coat-imposed dormancy and a dry storage period of approximately 4 months is typically required before germination will proceed (45). The sarcotesta and endosperm restrict water uptake, while mechanical removal of the sarcotesta followed by water soaking substantially improves germination (45); seeds retaining the sarcotesta frequently fail to germinate and are susceptible to fungal contamination (45). Germination is also temperature-sensitive: following sarcotesta removal, one study reported the highest germination at 23 ± 2 °C after 31 days, although overall germination remained low and irregular (25). More recent work demonstrated that mechanical scarification followed by 24 hr water soaking substantially improved germination to 85 % *in vitro* and 81 % *in vivo*, compared with 23 % in untreated controls (45). Tuber formation is typically observed after 120 days; higher temperatures near 30 °C promote primary root elongation but inhibit formation of the first vegetative leaf (25, 45). Pollination method affects seed quality as well as seed number. Hand pollination produced 96 % pod set, pods averaging 8.4 cm with 74 seeds per pod, 54 % germination and a vigour index of 1252, exceeding values obtained under other pollination methods (46). This is a consequential result for conservation practice: it indicates that the reproductive deficit under ambient pollination is not confined to how many seeds are produced but extends to the quality of those seeds, so that pollen limitation propagates into the



recruitment stage. The implications for supplemental pollination are developed below. Several determinants of regeneration remain unquantified. No published data address the minimum population size required for sustainable seed production, the effect of extreme drought years on seed viability, or differences in seed quality between forest-interior and edge populations.

Genetic consequences of pollination failure and population fragmentation

Genetic diversity, population structure and gene flow

Genetic diversity and population structure in *G. superba* have been assessed principally using dominant molecular markers, particularly inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD). An ISSR analyses of *G. superba* accessions from Tamil Nadu detected high levels of polymorphism, with 86.27 % polymorphic bands reported among five ecotypes (47) and 83.56 % among 16 accessions (19). The RAPD analysis of 58 primers reported 88 % polymorphism among *G. superba* accessions (48). An ISSR-based assessment of 50 *G. superba* accessions collected from several locations in the Western Ghats of Maharashtra distributed the accessions into three distinct clusters and reported high polymorphism, although genetic-diversity indices were low (49). The available studies indicate substantial genetic differentiation among the sampled accessions and ecotypes. In the five-ecotype ISSR study, coefficient of genetic differentiation (G_{ST}) values ranged from 0.4418–0.8436 and gene flow (number of migrants per generation) (N_m) from 0.2179–0.6387, with within-population genetic diversity (H_s) values as low as 0.0924 (47). All N_m estimates were below one, the classical one-migrant-per-generation benchmark, suggesting restricted gene flow among the sampled populations rather than complete genetic isolation (47). Two qualifications attach to these estimates. Both marker systems are dominant, so heterozygotes cannot be resolved directly and within-population diversity statistics rest on assumptions about Hardy–Weinberg equilibrium. The available studies also derive from limited geographic sampling of five ecotypes and 16 accessions from Tamil Nadu (19, 47) and 50 accessions from the Western Ghats of Maharashtra (49), so the reported values should not be extrapolated across the species' full range.

Genetic differentiation, restricted gene flow and conservation genetics

The combination of pronounced differentiation among the sampled accessions and low estimated gene flow creates conditions under which inbreeding and progressive loss of genetic variation could develop, particularly in small or isolated stands. The low within-population diversity recorded in some accessions (47) is consistent with this expectation, although neither inbreeding coefficients nor temporal change in diversity has been measured directly in this species. The low N_m estimates are consistent with restricted pollen-mediated gene flow, but they cannot be attributed specifically to the pollen-delivery limitation discussed above because

the two processes have not been measured concurrently in the same populations; restricted seed dispersal and historical population structure could also contribute. Intraspecific hybridisation offers a route to genetic rescue that does not require overcoming reproductive isolation between the populations concerned. Experimental crosses between genetically distinct ecotypes achieved pod set exceeding 90 % (50) and first filial generation (F1) progeny showed higher mean values than the parental mean for most measured growth and establishment traits, with mean germination of 18.28 %, a vigour index of 314.03 and mean seedling height of 11.01 cm (50). The relatively low F1 germination reported in that hybridisation study should not be compared directly with the 54 % germination reported for hand-pollinated seed (46), because the two studies evaluated different genetic materials and experimental seed lots. These results indicate that assisted gene flow may be capable of restoring variation and vigour in declining wild populations (19, 50); its application is considered below. Conservation genetic management for *G. superba* requires identification and maintenance of distinct genotypes. Individual accessions such as GSU-16 have been resolved as genetically distinct (19) and marker polymorphism between 0.710 and 0.958 is sufficient to discriminate such genetic resources (19). Genomic approaches remain little applied: transcriptome assembly and expressed sequence tag-simple sequence repeat (EST-SSR) marker mining have been reported for this species (51), but restriction site-associated DNA sequencing (RADseq) or whole-genome sequencing for fine-scale population structure analysis has not been undertaken.

Mitigating pollination bottlenecks: Intervention and management

Direct intervention at the pollination stage

The preceding sections established that reproductive output in *G. superba* is constrained by pollen delivery rather than by resource availability and that the deficit extends to seed quality as well as seed number. Supplemental pollination therefore addresses the limiting step directly and is the intervention for which species-specific experimental evidence is strongest. Hand pollination produced reported 96 % pod set, pods averaging 8.4 cm with 74 seeds per pod, 54 % germination and a vigour index of 1252, exceeding values obtained under alternative pollination methods (46). Trials at Nauni, Himachal Pradesh, likewise found hand pollination to give the highest fruit set and pod length among the treatments compared (18). Mechanical pollen transfer has also been tested directly in this species. Five pollination systems were compared at Nauni and blower-assisted pollination raised fruit set to 80.94 %, compared with 87.31 % under hand pollination and 59.55 % under open pollination (Fig. 4) (13). These results provide direct evidence that mechanically assisted pollen transfer can improve reproductive performance in *G. superba*, rather than requiring extrapolation from other crops. The published methods do not, however, report blower specifications, pollen preparation, application rate, working distance or

treatment duration. The available evidence therefore supports the feasibility of blower-assisted pollination in *G. superba* but does not yet constitute a validated recommendation for a particular device, air-flow setting or application schedule. Establishing such a protocol requires experimental optimisation of these parameters in this species rather than the transfer of protocols developed for other crops. Any intervention must operate within the reproductive timetable established above. Stigmas attain receptivity before anther dehiscence and maximum seed set follows pollination one day in advance of dehiscence (18); effective transfer is concentrated between 07:00 and 11:00 (18); and pollen viability is maximal within 4 hr of dehiscence, declining to approximately 50 % by the second day (13, 20). These constraints narrow the daily window in which fresh pollen can be applied. Cryopreservation offers a means of decoupling pollen collection from application: a protocol has been published for *G. superba* pollen specifically (20), which would permit stored pollen of known provenance to be applied at the optimal moment and, in combination with the ecotype crosses discussed below, to be selected for genetic complementarity.

Restoring and protecting the pollinator base

Because cross-pollination in *G. superba* depends on biotic vectors, measures directed at pollinator communities are integral to any conservation plan for the species (11). Priority taxa differ regionally. In South African populations, large-winged butterflies, principally *E. cleodora* and *P. demodocus*, are the principal effective vectors, while *C. chalybeus* has also been observed visiting flowers (11). In Indian populations, recent studies have documented a diverse insect assemblage dominated by hymenopteran visitors, including *A. cerana*, *A. mellifera* and *B. haemorrhoidalis*, with experimental evidence that honeybee pollination can contribute to reproductive output (13, 18). Habitat management should therefore be targeted regionally rather than applied generically. Three measures follow from the pressures identified above. First, provision of alternative floral resources: agroforestry systems maintaining flowering diversity across the season have been proposed as a means of sustaining pollinator populations in agricultural landscapes (52), an approach applicable where *G. superba* occurs at field margins and in cultivated stands. Second, restriction of agrochemical exposure in the vicinity of populations, given documented effects of neonicotinoids on foraging success, colony growth and pollination service delivery (5, 40–42). Third, management of artificial light at night near populations supporting nocturnal visitors, since artificial illumination reduces nocturnal pollinator visitation, with consequences propagating to diurnal visitors (43). Community-level restoration can be effective: removal of invasive plants in a degraded island ecosystem strengthened pollination network resilience and improved reproductive function in native species (35). Evidence from comparable fragmented systems indicates that reproductive output improves where pollinator conservation and *in situ* plant protection are pursued together, given the pollen limitation

characteristic of such landscapes (28, 32). No equivalent intervention has been trialled in *G. superba* populations.

Assisted gene flow and genetic rescue

The pronounced genetic differentiation and low gene flow estimates reported above identify small, isolated populations as candidates for genetic rescue. Intraspecific hybridisation provides a practical route. Controlled crosses between genetically distinct *G. superba* ecotypes achieved pod set exceeding 90 % in both direct and reciprocal combinations, indicating that differentiated accessions remain readily cross-compatible (50). The F1 progeny showed higher mean values than the parental mean for most measured growth and establishment traits (50), consistent with recovery of vigour following the introduction of new genetic material. Two qualifications bear on applying these results. The reported performance is that of F1 progeny only; where outbreeding depression occurs it characteristically appears in later generations, so F2 and backcross performance would require evaluation before assisted gene flow is applied at scale to wild populations. The crosses were also conducted between accessions under controlled conditions and their outcome does not establish that translocated material would establish and reproduce *in situ*. No translocation trial has been reported for this species. Implementation would require prior genetic characterisation of donor and recipient populations, for which the marker systems described above are adequate (19, 47), together with pollen storage to permit crosses between populations flowering at different times (20).

Reducing harvest pressure through cultivation substitution

Wild harvest and pollination failure act on the same populations and the interaction between them is the reason neither can be addressed in isolation. Approximately 75 % of the raw material demand for *G. superba* has been reported to be met through wild collection (9) and harvesting practices across range states including India and Bangladesh remain largely unregulated, having driven wild stands close to extirpation in parts of the species' Indian range (9). The reproductive significance of this pressure is specific rather than general. Harvest removes established tubers and therefore flowering individuals from stands that are already small and scattered, directly reducing the pool of plants capable of contributing to subsequent seed production. It also lowers local flowering density, the variable implicated in pollinator-mediated Allee effects in comparable systems. Harvest pressure and pollen limitation are therefore compounding rather than independent threats and regulation of harvest alone would not restore reproductive function to populations whose pollinator service has already been reduced. Substitution of cultivated for wild-collected material addresses the harvest component directly and cultivated stands offer an additional advantage: they are the setting in which the supplemental and mechanically assisted pollination described above could realistically

be applied, since dispersed wild populations are poorly suited to such intervention.

Ex situ conservation and propagation

Ex situ measures serve two functions for *G. superba*: securing genetic material against further decline in wild populations and supplying planting stock for cultivation and restoration. Seed storage is constrained by the species' seed biology. Seeds remain viable for approximately 2 years at ambient temperature, though physiological change and salt accumulation occur during extended storage (45). Dormancy and germination requirements are described above. Pollen storage is better resolved: a cryopreservation protocol has been published specifically for *G. superba* pollen (20), which addresses the rapid post-dehiscence viability decline documented above and underpins the supplemental pollination approaches described earlier. *In vitro* propagation provides an alternative to seed-based multiplication where natural seed set is low. Callus induction from corm-bud explants reached 98.30 ± 0.84 % under an optimised Murashige and Skoog (MS) medium containing 2,4-D and indole-3-acetic acid (IAA), followed by multiple-shoot regeneration and rooting (53). More recently, callus induction and indirect organogenesis from non-dormant corm explants were optimised through media priming, with subsequent rooting and *ex vitro* acclimatisation (54). A survival rate of 90 % was recorded following mist-house acclimatisation and greenhouse hardening (53), whereas 69 % survival was reported after seven weeks of acclimatisation under direct sunlight following an initial staged hardening period (54). Embryoidogenesis and plant regeneration from leaf tissue have also been demonstrated, providing an additional route for multiplication independent of tuber supply (55). Documented germplasm collections remain limited. Fifty wild-sourced accessions collected from several locations in the Western Ghats region of Maharashtra, with recorded collection localities, were established at the Council of Scientific and Industrial Research (CSIR)-National Chemical Laboratory, Pune (49) and the accessions used in the genetic diversity studies discussed above derive from separate collections in Tamil Nadu (19, 47, 48, 50). No consolidated national inventory of *G. superba* germplasm holdings has been published and accession-level documentation across institutions is not integrated. These figures describe performance under controlled propagation conditions and should not be interpreted as evidence of successful establishment or reproductive performance in restored natural populations. No study has yet followed *ex situ* propagated *G. superba* material through establishment, flowering and reproduction in natural or restored habitat.

Policy, research gaps and future priorities

Policy recommendations: Three regulatory measures follow from the evidence assembled above. First, although *G. superba* is assessed globally as LC under the IUCN Red List (8), this classification may mask substantial regional variation in population status. The assessment itself notes local depletion in India resulting

from over-collection and recommends that harvesting levels be monitored where populations might become locally threatened (8). It was last conducted in 2008 and published in 2013 and therefore predates the regional field and genetic studies reviewed here. Reported declines in parts of India and other range states (10, 18) accordingly justify systematic population surveys to determine current trends, to inform regional conservation measures and to establish whether the evidence would support reassessment. Second, existing international trade instruments require strengthened implementation: streamlining listing procedures and strengthening collaboration among member states have been identified as necessary to improve the effectiveness of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in protecting threatened plant resources (56). Third, any harvest quota must be tied to a stated monitoring metric. Because tubers permit clonal persistence after reproductive failure, counts of standing plants may overestimate population health; quotas should instead be indexed to flowering-plant density and per-plant seed set, the variables most directly related to recruitment. Socio-economic measures are equally necessary. Approximately 75 % of raw material demand is reported to be met through wild collection (9), so regulation without a viable cultivated supply could displace rather than eliminate harvesting pressure. Quantifying harvesting pressure across the supply and value chains and improving market information on cultivated material should therefore form part of an effective regulatory strategy. The wider policy framework for pollinator conservation provides an international context for these measures, given the documented contribution of animal pollination to crop production and the economic importance of pollination services (57–59).

Research gaps: The evidence base for *G. superba* has four principal deficiencies, each identified in the sections above. Geographic coverage is bimodal. Empirical data cluster in South Africa and in two Indian regions, with almost nothing from the species' extensive intervening range in East and West Africa, peninsular India or Southeast Asia. Consequently, no claim about the species' pollination system can currently be generalised across its distribution. Pollinator effectiveness has been assessed unevenly. South African populations have been studied using exclusion treatments and pollen deposition analysis, whereas Indian studies have characterised visitor assemblages and compared pollination treatments; equivalent single-visit pollen deposition data are not available for both regions. The foraging behaviour of non-lepidopteran visitors and its relationship to pollination efficiency remains poorly resolved, particularly in the Northwestern Himalaya (18). Several mechanisms are inferred rather than demonstrated. No MVP size or pollination density threshold has been established; no isolation-distance threshold has been determined; no pollen limitation index has been calculated across populations differing in size or isolation; no VOC profile has been published; and the relative contribution of trampling, resource



collection and roadside pollution to reproductive failure is unquantified. Pollination biology and conservation genetics have not been integrated. Gene flow estimates and pollen delivery have not been measured concurrently in the same populations and no consolidated inventory of germplasm holdings exists.

Future priorities: Five studies would materially advance the evidence base and each is specified in the section where the gap arises. A density-gradient study measuring local flowering-plant density, pollinator visitation, pollen deposition and per-plant seed set across a range of population sizes within one region would establish whether a pollination-mediated Allee effect operates. Paired supplemental and open pollination treatments applied to the same plants across multiple populations would quantify pollen limitation and its relationship to isolation. Direct characterisation of floral volatiles across the diurnal cycle and across geographically distinct populations would determine

whether scent contributes to visitor attraction and whether volatile profiles differ between regional assemblages. Experimental optimisation of blower-assisted pollination - device specification, pollen preparation, application rate, working distance and timing - would convert a demonstrated feasibility into a deployable protocol. Finally, evaluation of F2 and backcross performance from inter-ecotype crosses would establish whether assisted gene flow is safe to apply at scale. Beyond these, synthetic seed development for restoration and standardised somatic embryogenesis protocols have been proposed as responses to low natural seed set (55) and genomic resources adequate for fine-scale population structure analysis remain to be developed (51). The principal threats to *G. superba*, the corresponding knowledge gaps and the conservation actions indicated are summarised in Table 2.

Table 2. Principal constraints on reproduction in *Gloriosa superba*, corresponding evidence gaps and indicated conservation interventions

Constraint	Evidence in <i>G. superba</i>	Evidence gap	Indicated intervention	References
Pollen limitation	○ Fruit set 59.55 % open vs 87.31 % hand ○ Blower-assisted 80.94 % ○ Hand-pollinated seed: 54 % germination, vigour index 1252	○ No pollen limitation index across populations differing in size or isolation	○ Supplemental hand pollination ○ Standardisation of blower protocol ○ Pollen cryopreservation to widen the transfer window	(13, 20, 46)
Narrow transfer window	○ Stigma receptive 3–4 days ○ Pollen viable hours; 49.85 % by day 2 ○ Transfer 07:00–11:00	○ Effect of storage duration on stored-pollen efficacy untested	○ Application timed to the receptive window ○ Use of cryopreserved pollen of known provenance	(13, 18, 20)
Pollinator decline	○ Regional bee declines documented ○ No range-wide decline demonstrated for this species	○ Effectiveness assessed by different methods in the two best-studied regions	○ Agrochemical buffer zones ○ Floral resource provision ○ Artificial light management	(11, 13, 18)
Habitat fragmentation and low density	○ Wild stands small and scattered	○ No isolation-distance threshold ○ No minimum viable population size	○ Density-gradient study to establish a pollination threshold ○ Habitat connectivity measures	(11, 18)
Restricted gene flow	○ G_{ST} 0.4418–0.8436 ○ $N_m < 1$ among accessions	○ Gene flow and pollen delivery not measured concurrently ○ F2 performance unevaluated	○ Assisted gene flow between characterised ecotypes ○ Prior genetic screening of donor and recipient stands	(50)
Wild harvest pressure	○ 75 % of raw material demand met from wild collection ○ Global assessment last conducted 2008	○ Harvesting pressure across supply chains unquantified	○ Cultivation substitution ○ Quotas indexed to flowering density and per-plant seed set ○ Systematic regional population survey	(8, 9)

4. Conclusion

Applying a pollination bottleneck framework to *Gloriosa superba* identifies pollen delivery, rather than stigma availability, as the binding constraint on seed output: stigmas remain receptive for 3–4 days, while viable pollen persists for only hours and supplemental pollination raises both seed number and seed quality above ambient levels. Hand pollination and blower-assisted pollination are the most effective methods so far demonstrated in this species and a published pollen cryopreservation protocol makes their application feasible beyond the narrow daily transfer window. Priority actions are therefore the standardisation of assisted-pollination protocols, targeted regional population surveys and evaluation of F2 performance before assisted gene flow is applied at scale. Critically, the mechanisms most often invoked in conservation planning for this species - density thresholds, isolation effects and phenological mismatch - remain inferred from comparable taxa rather than demonstrated here. Securing pollination is the process on which the regional persistence of *G. superba* depends.

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Authors' contributions

AJPD conceived the review topic, conducted the literature survey, compiled and synthesised the scientific information, prepared the figures and tables and drafted the manuscript. ST supervised the work, provided conceptual guidance, critically reviewed the manuscript and contributed to its intellectual content. VM contributed to literature evaluation, interpretation of scientific findings and manuscript revision. SVP assisted in data compilation, critical review and improvement of the manuscript. SN contributed to literature collection, manuscript editing and final review. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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