Title: Novel consequences of bird pollination for plant mating

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Trends

- Recent molecular studies of mating patterns in flowering plants are providing important insights into the ecological genetic consequences of bird pollination.
- These studies show that bird-pollinated plants are typically sired by nearly twice as many mates as are insect-pollinated plants.
- High mobility, limited grooming, and intra- and inter-species aggression are likely to be key behavioural characteristics of birds that facilitate pollen carryover and promote high mate diversity in the plants they pollinate.

Abstract

Pollinator behaviour has profound effects on plant mating. Pollinators are predicted to minimise energetic costs during foraging bouts by moving between nearby flowers. However, a review of plant mating system studies reveals a mismatch between behavioural predictions and pollen-mediated gene dispersal in bird-pollinated plants. Paternal diversity of these plants is twice that of plants pollinated solely by insects. Comparison with the behavior of other pollinator groups suggest that birds may promote pollen dispersal through a combination of high mobility, limited grooming, and intra- and inter-species aggression. Future opportunities to test these predictions include seed paternity assignment following pollinator exclusion experiments, single pollen grain genotyping, new tracking technologies for small pollinators, and motion-triggered cameras and ethological experimentation for quantifying pollinator behavior.

Plants and pollinators

Mating is a key microevolutionary process, influencing the movement of genes in space and their transmission through time. An understanding of mating patterns is fundamental to evolutionary biology [1] and increasingly critical for the conservation and management of populations and species impacted by environmental change [2,3,4]. Plant mating systems are influenced by intrinsic factors such as floral morphology and self-compatibility and extrinsic factors such as pollinator mobility and foraging behaviour [5,6].

Almost 90% of the world's 400,000 flowering plant species rely on animals for pollination [7], the behaviour of which has profound effects on plant mating [8,9]. Food-foraging pollinators are predicted to conform to optimal foraging theory, whereby energetic costs during foraging bouts are minimised by moving preferentially between nearby flowers, resulting in leptokurtic pollen dispersal and correlated paternity [10,11]. However, from a

plant's perspective, longer-distance pollen dispersal decreases the likelihood of inbreeding, meaning that pollinators that move greater distances, skip more intervening flowers or show departures from optimal foraging that may result in higher offspring fitness.

Recent studies have highlighted novel mechanisms of pollinator attraction, and led to the discovery of increased diversity of animal taxa known to transport pollen, especially in the Southern Hemisphere [12,13,14,15]. The diversity of pollination strategies among plants is likely to be reflected in a similarly diverse range of patterns of pollen dispersal. Resolving the consequences of different pollination strategies for pollen dispersal across multiple spatial scales is important for understanding the fitness advantages, and thus evolution, of particular strategies. Here we focus on bird pollination, a strategy that has been at the forefront of investigations to understand floral adaptation to pollinators and mechanisms underpinning transitions between pollination strategies [16,17,18,19]

Bird pollination: what birds, what plants, and where

Although most flowering plants are insect pollinated, more than 900 bird species pollinate members of ca. 500 of the 13,500 vascular plant genera [19,20,21,22], making bird-pollination one of the most taxonomically widespread pollination strategies. The four main lineages of pollinating birds are hummingbirds (ca. 340 spp; Trochilidae), sunbirds (ca. 132 spp; Nectariniidae), honeyeaters (ca. 180 spp; Meliphagidae) and lorikeets (ca. 55 spp; tribe Loriini of the Psittaculidae), though there are also specialist nectarivores which make locally important contributions to pollination in the asities (Philepittidae), sugarbirds (Promeropidae), Hawaiian honeycreepers (Fringillidae), white-eyes (Zosteropidae) and Platycercini parrots (Fig. 1) [22, 23, 24]. In some regions there are also guilds of plants with readily accessible rewards that are pollinated primarily by short-billed birds such as weavers, bulbuls and starlings that are only occasionally nectarivorous [22, 25].

All of the major families of nectarivorous birds reach their highest diversity in the tropics and Southern Hemisphere, in particular the Andes and central American mountains (hummingbirds), African and Asian tropics (sunbirds), and eastern Australia and New Guinea (honeyeaters & lorikeets) (Fig. 2). Interestingly, the diversity of bird-pollinated plants appears not to correlate strongly with nectarivorous bird diversity. For example, both the Southwest Australian Floristic Region and the Greater Cape of South Africa have diverse bird-pollinated floras, but a relatively low diversity of nectarivores compared to other regions on their respective continents [23,24,26,27,28].

While some plants are pollinated by one or a few bird species, the majority of nectarivorous birds, including those with highly exaggerated bill morphology, feed on a range of plant species [24,29]. Plants that are primarily pollinated by birds often have red flowers, elongated floral tubes and copious amounts of dilute nectar [19,24]. However, a range of other colours (e.g. typically orange and yellow, but also white, violet, green and black) and morphologies are exhibited by a lesser diversity of bird-pollinated species [19,24,30]. In many cases bird-pollinated flowers are also visited by other pollinator groups, particularly bees and non-flying mammals [22,31,32], though the morphological adaptations to bird pollination often mean that insect visitors are comparatively ineffective at removing and depositing pollen [33]. This has been demonstrated by the sharp decline in seed production that is typically observed when birds, but not insects, are experimentally excluded from both bird-adapted flowers [33,34] and, remarkably, in some seemingly non-bird adapted flowers [22]. Zygomorphy and tubular flowers appear to be pre-adaptations that favour the evolution of bird pollination [19], with bird pollination more commonly evolving from bee pollination than it does from other strategies [35].

The diversity of bird species involved in pollination means that there are often multiple avian pollination niches to which plants can adapt. For example, in Australia it is common to have plants with long tubular flowers pollinated by long-beaked honeyeaters co-occurring with *Eucalyptus*, which has open-faced flowers pollinated by a range of honeyeaters and lorikeets [36]. Studies in

South America and Africa suggest that more than one bird pollination system may occur in a particular community, and that further niche partitioning may occur along a flowering time axis [37]. Consequently, mating outcomes for plants may vary according to the type of birds involved as well as interactions among plant species [37,38].

Bird pollinator behaviour

Given the variation in foraging and social behaviours between different groups of nectarivorous birds, there is expected to be substantial variation in the ecological and evolutionary consequences of bird pollination. Among hummingbirds, there is an apparent dichotomy between those that defend small territories within which focal nectar sources are repeatedly visited, and trap-line foragers (typically hermit hummingbirds) that move long distances to focal plants [39,40]. Sunbirds and honeyeaters that feed extensively on nectar-producing flowers show similar territoriality, but traplining behaviour has not been demonstrated in these groups. While the foraging behavior of nectarivorous parrots has received surprisingly little attention in the context of pollination, lorikeets appear to forage very differently than other bird groups. Lorikeets visit large numbers of flowers per plant, such as when feeding on *Eucalyptus* (Myrtaceae), and tolerate the presence of large numbers of other lorikeets [23]. They are strong, fast-flying birds, often moving vast distances in flocks seeking out patchily distributed flowering trees [23].

Aggression directed at conspecifics or other nectarivorous bird species is an important aspect of the behavior of nectar-feeding birds, as it provides a disruption from the foraging behavior that typically contributes to frequent self-pollination and leptokurtic pollen dispersal. For example, many Australian plant communities are dominated by large-bodied and social nectarivorous birds. These species are often highly mobile generalists and locally abundant in areas of copious nectar production [4,41], leading to frequent aggressive interactions within and among species [13,42]. As such, the aggressive behaviour of these nectar-feeding birds suggests greater pollen carryover from a wider diversity of pollen donors for the plants they pollinate compared with insects and flightless mammals, which display much less aggressive behaviour.

Previous work has highlighted the effects of different functional groups of pollinators on female reproductive success, yet there has been surprisingly little empirical data exploring how different pollinator classes influence pollen-mediated gene dispersal. While the seminal review of bird pollination by Stiles [39] predicted that differences between territorial and traplining birds will have important consequences for plant mating systems and gene flow, almost four decades later there remain surprisingly few genetic tests of these predictions. The potential novel consequences of bird pollination were hinted at by a rare early study where the hummingbird pollinated plant *Ipomopsis aggregata* was shown to have both high multiple paternity within fruits and extensive pollen carryover [43]. This finding is consistent with a recent theoretical model which predicts that the effective number of mates will be positively correlated with the extent of pollen carryover [44].

Multiple paternity in plants

Multiple paternity is pervasive in flowering plants [45], and occurs when the pollen from different potential sires is distributed among the flowers of a single individual, or when pollen from more than one donor is deposited on the same stigma. For a single flower, multiple paternity may arise when pollen from different donors is deposited separately during sequential visits by different pollinators, or when different donors are deposited simultaneously by a single vector carrying pollen from several plants (pollen carryover) [46]. Multiple mating promotes pollen competition, allows plants to choose among diverse male gametophytes, and can have fitness benefits through the production of more genetically variable offspring [45,47,48]. Within fruit estimates of paternal diversity reflect pollen carryover, while estimates combining data across fruits characterize genetic diversity at the plant level. To advance our understanding of the mechanisms and consequences of paternal diversity in plants, assessments of the relative costs and benefits of multiple paternity under field conditions are required.

The incidence of multiple mating in plants can be estimated through marker-based paternity inference [45]. Such studies characterise multiple paternity directly through paternity assignment

[46,49], or indirectly using coefficients of the probability of paternal identity (PPI; 50], and from these, estimates of the effective number of sires (N_{ep}) within and among fruit (Box 1). Direct paternity assignment procedures typically include complete exclusion of non-sires or maximum likelihood procedures [51]. Despite the availability of several methodological approaches to estimate mate diversity, in general data are surprisingly limited. Nonetheless, a few trends in relation to life-history characteristics have been deduced, including lower paternal diversity in herbs than in tree species, and for trees, higher paternal diversity for wind-pollinated than animal-pollinated species [51,52]. However, there is a paucity of parentage studies for most pollination strategies, including vertebrate pollinated species [53]. Thus, while multiple paternity is known to be ubiquitous - perhaps inevitable - in outcrossing land plants, a preliminary search for patterns among paternal diversity data did not reveal a specific feature that might allow us to predict the factors influencing effective number of sires [45,53]. This, at least in part, is due to the small number of relevant studies to date, and the noise associated with confounding effects (e.g. taxonomic group, geography, population parameters) across relatively few studies.

A predictive model for pollen movement

Though many groups of pollinators have been well-studied in terms of their foraging behavior and home ranges, a series of testable predictions related to pollen movement and mating systems in plants has yet to be developed. Based on the animal behavioural literature (see Table 1 for citations), here we predict the extent of pollen movement at varying spatial scales and the likely contributing processes for different groups of pollinators (Table 1, and references therein). We have undertaken this at two levels; firstly between the major behavioural types in specialist nectar-feeding birds (territorial birds, trap-lining hummingbirds and lorikeets) and between bird pollination and other systems (bees, bats, moths, non-flying mammals) common in genera that contain bird-pollinated plants.

We predict that pollen dispersal within- and among-stands is likely to be more extensive for plants pollinated by traplining birds than for plants pollinated by territorial birds. There is evidence that

plants pollinated by trap-lining hermit hummingbirds are more likely to be self-compatible than those pollinated by non-hermits and other pollinators, suggesting that traplining behaviour could ameliorate the risks of pollinator-mediated selfing in these self-compatible species [38]. For plants pollinated by territorial birds, the extent of pollen-mediated gene dispersal will also depend on the magnitude of aggressive interactions by avian visitors to focal plants. These aggressive interactions can be either interspecific or intraspecific and are affected by the amount of nectar available relative to bird density. For example, aggressive interactions may increase in frequency in larger plant populations with greater nectar crops that support high numbers of behaviourally dominant bird species [13]. Lorikeets show a different pattern of foraging, where they forage on large numbers of flowers per plant, but can move long distances in search of nectar sources, suggesting high levels of within plant pollen transfer but also the potential for long-distance dispersal [19].

Bats, non-flying mammals and, in some cases, bees also engage in aggression during nectar feeding, but are less likely to generate significant disturbances to foraging behavior, suggesting that bird pollination may lead to comparatively greater pollen dispersal within stands of flowering plants. However, like territorial birds, the bees and flightless mammals involved in pollination generally move within a home range of less than 5ha, which establishes a predicted upper limit for most pollen movement. Alternatively, trap-lining hummingbirds move considerable distances, and bats and lorikeets have large home ranges that can lead to pollen movement between distant stands. At the landscape scale, with the exception of bees and flightless mammals, some members of all groups undertake regional movements, either being nomadic, migratory, or undertaking regular movements to distant food resources. As such, we predict that most of these groups of pollinators have a capacity to effect some level of long-distance pollen dispersal.

Given that the transition from bee to bird pollination (not including parrots) is perhaps the most common evolutionary pathway to bird pollination [17,18], it is worth focusing in more detail on the difference between these two pollinator functional groups. At small spatial scales, bees are likely to have comparatively more frequent within-plant movements and shorter pollen distance dispersal

due to a combination of optimal foraging behaviour, comparatively short flight distances and regular grooming leading to less pollen carryover. Although there are known movements of at least 6km in the bee genera *Apis* and *Bombus* and the tribes Euglosini and Xylocopini, most bees and most nectarivorous territorial birds have home ranges of less than 3 ha. However, in territorial birds, because of pollen carryover and frequent interruptions to foraging behaviour through aggression, we predict that despite similar home ranges to most bees, birds will more frequently disperse pollen from a larger number of individuals more widely within stands. At larger scales, birds that undertake seasonal or dispersive movements in pursuit of patchy nectar resources are more capable of mediating pollen dispersal distances of many kilometres.

Consequences of bird-pollination for plant mating: testing the predictions

Detailed mating studies for bird-pollinated plants are in their infancy. For example, in a recent review [54], only 5 of 92 paternity studies of trees were species that included birds as pollinators, demonstrating an overwhelming focus to date on insect/wind pollination systems from the Northern Hemisphere. However, recent studies on plants that include honeyeaters as pollinators are revealing high levels of multiple mating and a significant departure from near-neighbour mating that typifies plants pollinated by insects (Table 2). Drawing on the global literature, we reviewed 16 studies that have applied genetic markers to measure multiple paternity (N_{ep} , the mean effective number of sires per plant) for plants pollinated by birds, and contrasted to an equivalent number of plant species pollinated by insects. Multiple paternity was estimated either from paternity assignment data using the method of [50] or from the inverse of the estimate of correlated paternity (r_p) as calculated in MLTR [55,56] (Box 2). The mean effective number of sires per plant for bird-pollinated plants ($N_{ep} = 12.37$; $N = 16$) is significantly greater ($F_{(1,32)} = 7.37$, $P = 0.012$) and almost twice that of plants pollinated solely by insects ($N_{ep} = 6.86$; $N = 18$).

From this summary we draw four main conclusions: (i) there is evidence of higher paternal diversity for plants that include birds as pollinators compared to those pollinated only by insects; (ii) there are clear exceptions to this general conclusion, with very low N_{ep} for some bird-pollinated plants

and very high N_{ep} for some insect-pollinated plants; (iii) almost all bird-pollinated plants studied were also visited by insects and mammals, thus obscuring the influence of bird pollinators alone; and (iv) there are few mating system studies of bird-pollinated plants outside of Australia. Based on pollinator behaviour, we suggest that insect and/or flightless mammal contributions to pollination are likely to reduce the paternal diversity through lower mobility and/or lower pollen carryover than that associated with bird pollination alone. To tease apart these contributions to pollination, whilst avoiding the potentially confounding factors associated with variation in plant and population properties that impact paternal diversity, ideally requires pollinator exclusion experiments on plants pollinated by all three pollinator groups (see future directions/opportunities). Nonetheless, these data suggest that bird pollination may be a strong predictor of multiple paternity in plants, with significant evolutionary and conservation consequences.

Mating systems and the evolutionary consequences of bird pollination

This new insight into multiple mating in these plants has important implications for understanding the evolutionary consequences of bird pollination [19]. Multiple mating is of evolutionary significance because it impacts the genetic diversity and relatedness among maternal sibs within and among fruits, and hence initially the response to selection when competing for maternal resources during seed maturation. Under limited seed dispersal, multiple mating may lessen the intensity of competition among siblings, potentially increasing maternal fitness through greater numbers of genetically diverse offspring [57]. While increased seedling survival was associated with multiple paternity in *Eucalyptus gracilis* [47], experimental evidence is needed from other systems to test the hypothesis that multiple paternity increases offspring fitness via genetic gains.

The advantage of increased pollen dispersal and higher multiple paternity from bird versus insect visitors is compatible with the idea that some floral traits often seen as adaptations to bird pollination, may actually be mechanisms to reduce insect visitation. For example, while red floral colouration is readily detectable by birds [58], it is less readily detected by nectar-foraging hymenoptera than shorter wavelengths of light [59]. Further, floral traits such as long floral tubes, which increase the efficiency of birds at removing and depositing pollen, may also play a

secondary role in minimizing visits by less effective insect pollinators [19]. Similarly, the nectar of some bird-pollinated plants has been shown to be distasteful to insects [60], and the typically low sugar concentration of bird-pollinated plants may be comparatively sub-optimal for bees [19], thereby acting as a deterrent [61]. Flowering in winter or at high altitudes may also discourage insects [4].

The evolution of traits associated with bird pollination may also have important consequences for ongoing floral evolution. For example, bird-pollinated flowers act as a pre-adaptation to the evolution of hawkmoth pollination in *Aquilligea* following an increase in nectar spur length [62]. In some systems, bird-pollination constrains the evolutionary potential for future switches in pollination strategy, such as through the loss of the ability to produce pigments needed for attraction of bees [63]. Further, loss of particular components of the floral odour involved in pollinator attraction may limit potential pollinator shifts. The ecology of nectarivorous birds could also potentially affect rates of speciation and extinction in the plants that they pollinate. Recent phylogenetic research in some genera of Australian Fabaceae showed that bird-pollinated lineages are on average less diverse than bee-pollinated lineages [17]. Similarly, comparative phylogenetic research suggests that once hummingbird pollination evolves within clades with bee pollination, further speciation is rare [18]. Based on these findings it has been hypothesized that extensive long-distance movement of pollen by birds reduces allopatric speciation and diversification rates, and bird-pollination *per se* may not be a diversifying factor.

Conservation and restoration consequences of bird pollination

Pollinator decline in anthropogenically modified landscapes has been identified as a global threat to ecosystem services in wild plant populations, impacting thousands of plant species [64,65,66]. For example, markedly reduced fruit set leading to population decline was associated with the loss of avian pollinators in New Zealand [67]. Increasingly, reinstating pollinator services is recognised as critical for self-sustainability of restored plant communities [68,69,70]. Further, for threatened species recovery programs, reintroductions of plants with ecologically specialized pollination

systems are usually unsuccessful in the longer term unless the distribution and habitat preference of the pollinator is incorporated into the management plan prior to plant reintroduction [71].

While the primary focus of the literature relating to declining pollinator populations has been on demographic issues for plants, genetic consequences are also important. Lower multiple paternity can have negative conservation consequences for conservation through reduced heterozygosity, genetically effective population size [48], and fitness [58]. In some regions, invasive pollinator species such as honeybees and bumblebees, are having a major impact on many bird-pollinated plants by reducing fecundity, outcrossing, and mate diversity [72,73]. Alternatively, the high mobility of many nectarivorous birds suggests that the impact of disturbance and fragmentation to plant mating patterns can be mitigated through frequent pollinator-mediated gene flow [74,75]. For example, polyandry increased with increasing geographical isolation of isolated paddock trees for the primarily bird-pollinated *Eucalyptus leucoxylon*, but not for the primarily insect pollinated *E. camaldulensis* [76]. Similarly, highly mobile bird pollinators can rapidly restore genetic connectivity and pollinator services in rehabilitated plant communities - a critical requirement for self-sustainability [68,70,77]. However, in Australia, the increased prevalence of highly aggressive “competitive despot” bird species (e.g. the Noisy Miner *Manorina melanocephala*) in fragmented remnant vegetation is excluding smaller species, with potentially detrimental impacts on pollination, seed set and plant persistence [78]. From a plant’s perspective, it remains to be established if these dominant birds are as effective pollinators as the original honeyeater community.

Concluding remarks and future perspectives

We advocate testing our predictions by combining cutting-edge molecular analyses of paternity with elegant field experiments that quantify siring success by different classes of pollinators on the same plant species. This approach avoids potentially confounding phylogenetic and ecological effects, such as floral traits, associated with comparisons across species. Similarly, experimental manipulation of plant population size and density will be critical in testing the prediction that bird pollinators can ameliorate the genetic effects of small population size. An important component in

both of these avenues of investigation will be to combine estimates of pollen dispersal distances with fitness estimates (e.g. fecundity, seed viability and offspring vigour) to formally test the consequences of different patterns of pollen dispersal.

Genotyping of individual pollen grains recovered from pollinators will allow powerful tests of pollen carryover. For example, paternity analyses based on microsatellite genotyping of individual pollen grains found on insect pollinators (bees, flies, beetles) visiting Japanese chestnut (*Castanea crenata*) trees showed that all insect groups carried outcross pollen grains for long distances via pollen carryover, suggesting that a wide range of insects are potential outcross-pollen vectors for this self-incompatible species [79]. This study also highlights that, in plants visited by a diverse assemblage of pollinators, single pollen grain genotyping will make it possible to readily compare the extent of pollen carryover by different pollinator species. Recent developments in the use of Next Generation Sequencing for DNA metabarcoding of pollen [80,81] provide new opportunities to assess pollen carryover. While currently limited to species-level identification, the potential exists to extend these methods to genetic diversity in mixed-pollen samples from pollinators, or even individual identification.

One of the major challenges in understanding how different pollinator groups influence mating patterns is determining the underlying behavioural mechanisms responsible for pollen movement. In particular, among territorial birds there can be pronounced seasonal, population level and interspecific variation in foraging behavior and the extent of aggression [82]. Traditional methods such as direct observation of colour-banded individuals are useful for determining home ranges and recognizing behaviourally dominant individuals. These can be complemented by ethological experimentation and the tracking of animals for detailed analysis of foraging behaviour, particularly for more cryptic species, and enable the detection of rarer long distance movements that are likely to be critical for plant gene flow. For example, radio-telemetry studies have demonstrated that pollinators move longer distances than expected based on direct observation and, in many cases, are likely to have the capacity to link spatially segregated plant populations [83]. While the tracking of animals has previously been limited to large-bodied species, recent technological advances on

two levels will generate unprecedented insight into the movements of all vertebrate pollinators and even large insects [84,85]. Firstly, reductions in transmitter size, primarily through advances in the technology of batteries, mean that almost all nectarivorous vertebrates, and even larger-bodied insects, can now be tracked [86]. Further, transmitters with GPS are accurate and allow constant recording of the location of the animal, without a human observer [85]. Radio-transmitters with GPS are not yet small enough for most pollinators but soon will be [85]. Harmonic radar and RFID are potentially very powerful techniques with minute transmitters – but cost and restriction to a small spatial range have limited their use.

Increasingly sophisticated heat- and movement-triggered motion-sensor cameras with a capacity for filming use infrared illumination is an exciting recent technological development for pollination biology studies [87,88]. This technology offers opportunities for new insight into the natural behaviour of bird pollinators and consequences for the plants they visit. This technology can aid in quantifying visitation rates to individual flowers and inflorescences, and enable continuous monitoring 24hrs/day to dramatically increase both the total observation time, and the detection of rare and/or night-time visits. For example, motion-triggered camera footage was used to confirm that the critically endangered orchid *Satyrium rhodanthum* was pollinated solely by a single species of sunbird, despite the presence of other potential pollinators [89]. Further, deploying numerous cameras for concurrent observations with a precise and co-ordinated recording of time and duration of visitation enables the accurate tracking of pollinator movements within and among plants.

Integrating use of these new technologies with traditional field-based observations will allow unprecedented insight into the foraging behaviour of birds and the testing of predictions for pollen movement and plant mating. Given the taxonomic and behavioural diversity of avian pollinators, and the diversity of plants involved, much remains to be learnt about bird pollination and its evolutionary ecological consequences for plant mating.

Outstanding Questions:

- Is multiple paternity within fruits influenced primarily by pollen carryover in single pollinator visits or by sequential visits to a flower?
- Does a decline or loss of avian pollinators impact fitness of plants adapted for bird-pollination, and how much do insects contribute to pollination of bird-adapted flowers?
- Are frequent nectarivores more or less effective at cross-pollination than occasional nectarivores?
- How do behavioural differences associated with different groups of birds influence paternal diversity in the plants they pollinate?

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Figure 1. Examples of nectarivorous birds visiting flowers and effecting pollination. (A) New Holland honeyeater (*Phylidonyris novaehollandiae*) on *Anigozanthos flavidus* (Western Australia); (B) Tawny-bellied hermit (*Phaethornis syrmatorhynchus*) visiting *Fuchsia* spp. (Ecuador) (C) Copper-rumped Hummingbird (*Amazilia tobaci*) on *Calliandra californica* (Mexico) (D) Eastern spinebill (*Acanthorhynchus tenuirostris*) on *Telopea speciosissima* x *T. mungaensis* (Eastern Australia) (E) Dark-capped bulbul (*Pycnonotus tricolor*) on *Aloe vryheidensis* (South Africa) (F) Female amethyst sunbird (*Chalcomitra amethystina*) on *Aloe maculata* (South Africa) (G) Rainbow lorikeet (*Trichoglossus haematodus*) on *Corymbia maculata* (Western Australia). Photo credits (A) Myles Menz; (B & C) Herbert Stärker; (D) Suzi Bond; (E and F) Steve Johnson; (G) Steve Hopper

Figure 2. Global distribution and diversity of the four major bird pollinator groups. Maps have been generated at a spatial resolution of 10x10km and use the Eckert IV equal-area projection. Maps represent native, extant species only. Maps were derived by Clinton Jenkins from digital distribution maps for all the world's birds in Birdlife International and NatureServe (2013) (<http://www.birdlife.org/datazone/info/spcdownload>). (A) Honeyeaters (family Meliphagidae): ca. 180 spp, .ca. half are native to Australia, many of the remainder are native to New Guinea, but some can also be found in New Zealand, remote Pacific Islands, Wallacea, and one species in Bali. (B) Sunbirds (family Nectariniidae): ca. 132 spp, with an Old World distribution that spans from Africa to Australia, across Madagascar, Egypt, Iran, Yemen, Southern China, Indian subcontinent, Indochinese peninsulas, SE Asia, nearby Pacific Islands, and northern Australia. Species diversity is greatest in equatorial and tropical areas. (C) Hummingbirds (family Trochilidae): ca. 340 spp, 2 subfamilies, the hermits (subfamily Phaethornithinae) and the typical hummingbirds (subfamily Trochilinae). Species are restricted to the Americas, from South Central Alaska to Tierra del Fuego. Species richness is greatest in humid tropical and subtropical forests of the northern Andes and adjacent foothills. (D) Loris and Lorikeets (tribe Loriini within the parrot family Psittacidae): ca. 55 spp with australasian distribution, highest diversity in New Guinea, but absent from New Zealand and arid Australia.

Table 1. Predictive model for pollen movement based on behaviour of territorial birds, traplining hummingbirds, lorikeets, bees, moths, flightless mammals and bats.

	PHOTO 1 HERE	PHOTO 2 HERE	PHOTO 3 HERE	PHOTO 4 HERE	PHOTO 5 HERE	PHOTO 6 HERE	PHOTO 7 HERE
Functional group	Territorial bird	Trap-lining bird	Lorini parrots	Bee	Lepidoptera (Moths and Butterflies)	Flightless mammal	Bat
Examples	Honeyeaters, hummingbirds, sunbirds	Hermit hummingbirds , swordbill hummingbirds	Australian lorikeets	Honeybees, Bumblebees, numerous solitary bees	Hawkmoths	Possums, rodents	Blossom bats
<i>Contributing processes</i>							
Pollen carryover	High, flatter curve than insects [90]	High, indirect evidence, more than territorial [91]	Not studied	Moderate, steeper curve than birds ([16]), reduced by grooming [92, 93]	Not studied	Low, steep curve due to frequent grooming [94]	High, less steep curve than birds [90]
Foraging strategy	Forages within nectar rich stand, moves to near neighbor plants [13,95]	Moves to relatively small, dispersed resources [13]	Numerous flowers per plant, can move to dispersed resources [23,96]	Move to near neighbors of preferred plants [11]	Move to near neighbors of preferred plants [97,98]	Not studied	Moves to dispersed resources [99,100]
Home range impacts on axial variance	Moderate, typically less than 3 ha, often less than 1 ha [23,101,102]	High, can move over 1km from lek [95,103]	High	Small bees less than 3 ha, larger bees up to 700 ha, [104,105,106,107]	Unknown	0.1 – 300 ha, typically less than 5 ha, [82,108,109,110]	High; 12 -1800 ha, [99,111,112,113]
Long-distance movements (> 5 km)	Some species, seasonal movements, nomads and migrants [27,114]	Some species, seasonal movements, nomads and migrants [114]	High, follow patchy nectar resources	No, some exceptions e.g. <i>Apis</i> , euglossine [93,115]	Rarely, but possible in migrants [116,117,118]	Low	High, forage over broad areas from roost [111,118,119]
Aggression	High, disrupts foraging [13,27,121,122]	Moderate [95,122]	Moderate, but not usually sufficient to greatly disrupt foraging [23,96]	Rarely [123,124,125]	Absent	Rarely [126]	Moderate, in some species, disrupts foraging [127]
<i>Predicted Pollen movement</i>							
Relative frequency of within vs among plant movements	Moderate	Low	High	Moderate - High	Low - High	High	High
Distance within stands	Moderate	High	Moderate	Low – Moderate (euglossine likely exception)	Low-Moderate	Low	Moderate
Between stands (> 500 m)	Some cases yes, dispersed resources, outside of breeding season	Yes	Yes	No, some exceptions e.g. <i>Bombus</i> , <i>Apis</i> , euglossine	Typically no, occasional longer movements	No	Yes
Long distance dispersal via migrants/nomads/seasonal movement (> 5 km)	Yes	Yes	Yes	No, some exceptions e.g. <i>Apis</i> , euglossine)	Yes	No	Yes

Photo credits: 1. Eastern spinebill (*Acanthorhynchus tenuirostris*) (Benjamin Twist); 2. Swordbill hummingbird (*Ensifera ensifera*) (Herbert Stärker); 3. Australian lorikeet (*Trichoglossus haematodus*) (Fir0002/Flagstaffotos); 4. African honeybee (*Apis mellifera scutellata*) (Steve Johnson); 5. Convolvulus hawkmoth (*Agrius convolvuli*) (Steve Johnson); 6. Honeypossum (*Tarsipes rostratus*) (Jiri Lochman); 7. Lesser long-nosed bat (*Leptonycteris curasoae*) (MerlinTuttle.org).

Table 2: Estimates of mean effective number of sires per plant (N_{ep}) for plants pollinated by birds (dark shaded rows) and plants pollinated by insects (light shaded rows). * N_{ep} measured by paternity (P) or MLTR (M) – see box 1 for explanation. In “pollinator” column, “bird/mammal/insect” indicates birds are the main visitors and likely to be the most effective pollinators.

Species	Family	Pollinator	N_{ep}	P/M*	Location	Self-incompatible?
<i>Banksia heisteria</i>	Proteaceae	bird / insect / mammal	25.6	P	Western Australia	Y
<i>Eucalyptus caesia</i>	Myrtaceae	bird / insect / mammal	24	P	Western Australia	N
<i>Erigeron angustifolius</i>	Asteraceae	bee / butterfly / bee	23.8	P	North America	Y
<i>Eucalyptus globulus</i>	Myrtaceae	bird / insect / mammal	22.2	M	Eastern Australia	N
<i>Arctostaphylos uva-ursi</i>	Ericaceae	insect	21	M	Europe	Y
<i>Banksia attenuata</i>	Proteaceae	bird / insect / mammal	17.3	P	Western Australia	Y
<i>Ipomopsis aggregata</i>	Polemoniaceae	bird	17.1	M	North America	Y
<i>Banksia oligantha</i>	Proteaceae	bird / insect / mammal	12.5	M	Western Australia	N
<i>Calothamnus quadrifidus</i>	Proteaceae	bird / insect / mammal	12.2	M	Western Australia	N
<i>Banksia littoralis</i>	Proteaceae	bird / insect / mammal	11.7	P	Western Australia	N
<i>Eucalyptus ramiflora</i>	Myrtaceae	bird / insect / mammal	11.1	M	Western Australia	N
<i>Eucalyptus gracilis</i>	Myrtaceae	bird / insect / mammal	10.3	M	South Australia	N
<i>Asclepias tuberosa</i>	Mimosaceae	insect	10	M	Western Australia	N
<i>Carduus arvensis</i>	Asteraceae	insect	8.8	M	Europe	N
<i>Lambertia orbifolia</i>	Proteaceae	bird	8.5	M	Western Australia	N
<i>Banksia cuneata</i>	Proteaceae	bird / insect / mammal	8.3	M	Western Australia	N
<i>Sorbus domestica</i>	Rosaceae	insect	7.1	M	Europe	N
<i>Eucalyptus incrassata</i>	Myrtaceae	bird / insect / mammal	5.9	M	South Australia	N
<i>Carduus arvensis</i>	Asteraceae	insect	5.1	M	Europe	Y
<i>Calluna myrsinoides</i>	Ericaceae	bee	5	M	Eastern Australia	N
<i>Eucalyptus paniculata</i>	Myrtaceae	insect / bird / mammal	5	M	Eastern Australia	N
<i>Banksia sphaerocarpa</i>	Proteaceae	bird / insect / mammal	4.8	P	Western Australia	N
<i>Verticordia racemosa</i>	Myrtaceae	insect	4.8	M	Western Australia	N
<i>Euphorbia corollata</i>	Sapindaceae	insect	4.8	M	Asia	dioecous
<i>Mimulus guttatus</i>	Phrymaceae	bee	4.7	M	North America	N
<i>Eriosema pinnatifidum</i>	Penstemonaceae	bee	4.2	M	South America	dioecous
<i>Asclepias tuberosa</i>	Mimosaceae	insect	4	M	Western Australia	N
<i>Calluna myrsinoides</i>	Ericaceae	bee	4	M	Eastern Australia	N
<i>Mimulus lewisii</i>	Phrymaceae	bee	4	M	North America	N
<i>Calothamnus quadrifidus</i>	Myrtaceae	bird / insect / mammal	3.2	M	Western Australia	N
<i>Persea mollis</i>	Proteaceae	bee	3.1	P	Eastern Australia	N
<i>Abutilon trifidum</i>	Malvaceae	insect	2.9	M	Asia	Y
<i>Grevillea laspleura</i>	Proteaceae	bird / insect	2.4	M	Eastern Australia	N
<i>Grevillea angustata</i>	Proteaceae	insect	1.8	M	Eastern Australia	N

GLOSSARY:

Mating system: Describes patterns of syngamy between and within individuals, or equivalently, who shares parentage with whom in a population. Measuring it ideally takes the form of a complete enumeration of matings.

Outcrossing rate: Proportion of seeds that are the product of mating between individual plants. Estimated using molecular markers.

Multiple paternity: Offspring being sired by more than one male pollen donor.

Correlated paternity: The degree to which seed from the same fruit or plant shares the same male parent. The inverse provides an estimate of the effective number of sires.

Self-incompatibility: Inability of a hermaphroditic plant to produce seeds following self-pollination due to a physiological barrier acting before fertilization.

Geitonogamy: Transfer of pollen from one flower to another on the same plant

Pollination: The reception by a stigma of a pollen-grain contributed either from the same plant (self-pollination) or from a different plant (cross-pollination).

Pollen vector: An external force that transfers pollen (e.g. wind, water or an animal).

Optimal foraging: Describes the foraging behaviour exhibited by animals that minimises time and energy expenditure in the search for food.

Pollen carryover: The proportion of pollen removed from a focal flower that is deposited on each successive flower in a visitation sequence.

Pollen grooming: The removal of pollen by a pollinator from its body, for which the timing and frequency will impact on pollen carryover.

Traplining: the travelling by animals between small, scattered nectar resources, which are not defended against other floral visitors. These resources are visited by following a regular route and a particular sequence of plants.

Territoriality: where aggression is used by an animal to behaviourally dominate others in order to defend the resources associated with a particular area.

Box 1: Estimating the effective number of sires (N_{ep}).

The effective number of sires (N_{ep}), measured at the fruit or plant level, provides a fundamental parameter of ecological and evolutionary significance. As a summary parameter of paternal diversity, it allows a ready comparison among species and their biological circumstances.

Estimates of paternal diversity based on molecular marker variation employ either a direct approach through paternity analysis, or an indirect approach, one of which involves the estimation of mating system parameters such as outcrossing rate and correlated paternity. Direct and indirect approaches have been shown to be generally comparable [50].

The direct approach draws on accurate paternal inference to estimate the probability of paternal identity (PPI) of two offspring drawn at random from a single female, where $N_{ep} = (1/\text{PPI})$ [50,51]. In circumstances where all potential candidate sires are known, where molecular markers are sufficiently variable, and when the maternal genotype is also known, then paternity can potentially be assigned unambiguously through the total exclusion of non-sires based on Mendelian inheritance and allelic mismatches. Where this is not possible, then a paternal allocation approach can be used to choose among many candidate sires. This is the most common approach in paternity assignment studies. In categorical assignment, offspring are assigned to the candidate parent with the highest likelihood or posterior probability of being the true sire, and simulation-based methods for determining confidence in paternal assignment can be applied. Further detail and other approaches are described in [49].

In the studies that employed paternity assignment (Table 2), we applied the approach of [51] in using r_{gg} as an unbiased measure of PPI, and the inverse of r_{gg} as the estimate of N_{ep} at the plant and population level. Here, we used formula 3 in [51] to estimate PPI directly as a tally of the paternal matches, including selfs, from paternity assignment data supplied to us by the authors of the relevant studies.

One indirect approach draws on the correlated matings model [56] to estimate the level of correlated paternity (r_p). Correlated paternity is the degree to which seed from the same fruit or plant share the same sire, rather than are the result of a random sample of pollen from the entire population. The inverse of r_p (N_{ep}) provides an estimate of the effective number of sires. Multi-locus

estimation is conducted using the software MLTR [57]. These methods have been widely used for biologically meaningful insights into plant mating [2,3,45]. Other indirect approaches include TWOGENER and the use of relationship coefficients [51].