

Plant mating systems in a changing world

Christopher G. Eckert^{1*}, Susan Kalisz^{2*}, Monica A. Geber^{3*}, Risa Sargent^{4*}, Elizabeth Elle^{5*}, Pierre-Olivier Cheptou⁶, Carol Goodwillie⁷, Mark O. Johnston⁸, John K. Kelly⁹, David A. Moeller¹⁰, Emmanuelle Porcher¹¹, Richard H. Ree¹², Mario Vallejo-Marín¹³ and Alice A. Winn¹⁴

¹ Department of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada

² Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

³ Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA

⁴ Department of Biology, University of Ottawa, Ottawa, Ontario K1N 6N5, Canada

⁵ Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

⁶ UMR 5175 CEFE-CNRS, F-34293 Montpellier Cedex 05, France

⁷ Department of Biology, East Carolina University, Greenville, NC 27858, USA

⁸ Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

⁹ Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7534, USA

¹⁰ Department of Genetics, University of Georgia, Athens, GA 30602, USA

¹¹ UMR 7204 MNHN-UPMC-CNRS, F-75005 Paris, France

¹² Botany Department, Field Museum of Natural History, Chicago, IL 60605, USA

¹³ School of Biological and Environmental Science, University of Stirling, Stirling FK9 4LA, UK

¹⁴ Department of Biological Science, Florida State University, Tallahassee, FL 32306, USA

There is increasing evidence that human disturbance can negatively impact plant–pollinator interactions such as outcross pollination. We present a meta-analysis of 22 studies involving 27 plant species showing a significant reduction in the proportion of seeds outcrossed in response to anthropogenic habitat modifications. We discuss the evolutionary consequences of disturbance on plant mating systems, and in particular whether reproductive assurance through selfing effectively compensates for reduced outcrossing. The extent to which disturbance reduces pollinator versus mate availability could generate diverse selective forces on reproductive traits. Investigating how anthropogenic change influences plant mating will lead to new opportunities for better understanding of how mating systems evolve, as well as of the ecological and evolutionary consequences of human activities and how to mitigate them.

Human activities influence plant–pollinator interactions

Human activities have dramatically altered natural habitats in myriad ways with potentially significant consequences for plant and animal inhabitants. Although anthropogenic change has caused much concern regarding the fate of biodiversity, human perturbations have also provided opportunities for scientists to study ecology and evolution in action. In some cases, these unplanned experiments have been exploited to gain a better understanding of fundamental problems in ecology and evolution, which can ultimately yield improved strategies for conservation. In this review we discuss the consequences of human

impact on the ecology and evolution of plant mating systems.

Most plant species are hermaphroditic and many are self-compatible. Hence, a major axis of variation in plant mating systems involves the proportion of progeny produced through self-fertilization (selfing, s) versus cross-fertilization with unrelated conspecific individuals (outcrossing, $t = 1 - s$). The outcrossing rate (t) is estimated retrospectively by assaying seed progeny for polymorphic genetic markers [1] and is influenced by interactions between flowers and pollinators, which determine the relative amount of self versus outcross pollen arriving on stigmas and are followed by physiological processes that dictate the relative siring success of different pollen types (e.g. self-incompatibility). Because plants have little or no direct control over the environment in which they mate, t varies in response to an array of factors that affect outcross pollination, such as the density and dispersion of conspecific individuals and the abundance and foraging behavior of pollinators [2].

There has been much discussion on how plant–pollinator interactions are altered by a variety of human disturbances, including habitat fragmentation [3–7], invasion by nonindigenous competitors, pollinators and herbivores [8,9] and climate change [10,11], but this has primarily focused on how disturbance might affect pollinator populations and plant reproduction and demography, with little consideration of impacts on mating patterns [12]. However, anthropogenic influences on mating systems have significant ecological and evolutionary consequences. Because most plant species harbor a significant genetic load, the frequency of outcrossing directly affects progeny vigor and the demography of populations [13]. The mating system

Corresponding author: Eckert, C.G. (chris.eckert@queensu.ca)

* Authors indicated with an asterisk are listed in order of contribution; all others are listed alphabetically..

Glossary

Autofertility (AF): capacity of a plant to produce seed by spontaneous (autonomous) self-pollination when isolated from pollinators, usually measured by caging

Autogamy (a): transfer of self pollen between anthers and stigmas within the same flower

Autonomous autogamy: spontaneous self-pollination without pollinator activity caused by close proximity of dehiscent anthers and receptive stigmas

Competing or simultaneous selfing (c): occurs during the time when outcrossing also occurs

Delayed selfing (d): occurs after all opportunities for outcrossing have passed, often by contact between still-receptive stigmas and anthers containing still-viable pollen during flower senescence

Early-acting inbreeding depression: increased mortality of selfed zygotes relative to outcrossed zygotes expressed between fertilization and seed maturation

Facilitated autogamy (f): autogamous self-pollination caused by pollinator activity

Geitonogamy (g): transfer of pollen between anthers in one flower and stigmas in another flower on the same plant (geitonogamy is always facilitated, so it is not specially designated as such)

Inbreeding depression (ID): inferiority of inbred progeny compared to outbred progeny due primarily to the expression of deleterious recessive mutations in homozygous inbred progeny. When comparing selfed versus outcrossed progeny, ID is quantified as $\delta = 1 - \omega_s/\omega_x$, where ω_s and ω_x are the fitness of selfed and outcrossed progeny, respectively

Outcross fertilization (X): Fertilization with pollen from an unrelated individual (outcrossing)

Outcrossing rate (t): Proportion of seeds outcrossed, estimated using genetic markers

Pollen discounting (PD): a reduction in outcrossed siring success of an individual caused by self-pollination

Pollen limitation (PL): the extent to which the seed production of individual plants is limited by the availability of pollen.

Prior selfing (p): occurs before opportunities for outcrossing; often caused by dehiscence of anthers within an unopened bud

Reproductive assurance (RA): increase in seed production afforded by self-pollination when a scarcity of pollinators or mates limits outcross pollination

Seed discounting (SD): reduced production of outcrossed seeds caused by self-fertilization, either because selfing pre-empts ovules or because selfed seeds use resources that could otherwise have been used to produce outcrossed seed. Because SD involves resources, it can occur among ovules within ovaries, among flowers on the same plant or between reproductive seasons

Self-incompatibility (SI): inability of a hermaphrodite plant to produce seeds following self-pollination due to physiological processes acting before fertilization.

Self-pollination (s): fertilization with pollen from the same individual (selfing)

also influences the effective population size and opportunities for gene flow and consequently the genetic diversity and differentiation of populations, metapopulation dynamics and possibly the rate and trajectory of evolution [14]. Changes to mating systems might influence the evolution of key floral traits over ecological time scales. Striking variations in reproductive traits among closely related plant species and among populations within species, in conjunction with substantial heritability for many plant traits that affect mating [15,16], suggest that mating systems might respond quickly to altered selective pressures. It is quite likely that significant and detectable evolutionary shifts in plant mating systems are happening all around us in human-altered habitats. A large body of theory explores how genetic and ecological selective pressures interact to cause mating system evolution [17,18], which can be used to predict the evolutionary responses to human disruption of pollination systems.

The effects of human disturbances on pollination systems are expected to be complex. Humans typically modify more than one biotic or abiotic variable simultaneously, creating novel or stressful environments [19]. These effects

together might influence pollination, plant reproductive success and the degree of inbreeding, which can feed back on plant and pollinator demography [4,11,20]. We focus on whether disturbance affects the quantity of pollen transferred to stigmas or the composition of that pollen, be it self pollen, outcross pollen or pollen from other species that might interfere with conspecific fertilization and zygote fitness [21]. A recent meta-analysis indicates that, in general, anthropogenic habitat fragmentation decreases opportunities for outcross pollination [3]. For other human disturbances, such as competition for pollinators by invasive plants, introduction of invasive pollinators into pollination webs and climate change, there are not enough studies to make general predictions, but anticipation of intensified outcross pollen limitation seems a reasonable starting point. Here we review evidence that human disturbances influence the frequency of outcrossing. We then present the concepts and techniques required to anticipate and test for the effects of an anthropogenic increase in pollen limitation on the trajectory of mating system evolution. Specifically, we define important pollination parameters, describe how to measure them in natural populations and discuss potential scenarios for evolutionary change spurred by human-induced pollen limitation.

Evidence that human disturbance reduces outcrossing in plant populations

We exhaustively searched the literature for studies that compare the proportion of seeds outcrossed (t), as estimated from marker gene analysis, between populations in disturbed and relatively undisturbed habitats. We found 22 studies representing 27 species and 11 families (Table 1, Figure 1). Overall, t is significantly lower in disturbed habitats (paired t -test, $P < 0.0001$) and sometimes substantially lower [22,23]. We would expect the effect of disturbance on t to be more pronounced among the 17 species in this sample that the authors claimed were self-compatible than the 10 thought to be self-incompatible. With no physiological barrier to self-fertilization, the mating system should be more sensitive to changes in pollination that alter the ratio of outcross to self pollen on stigmas. Repeated-measures analysis of variance with species as subject, self-incompatibility status as a between-subject effect and disturbance as a within-subject effect revealed the expected significant interaction between self-incompatibility and disturbance ($P = 0.035$), supporting this expectation. However, a significant effect of disturbance still occurred among the 10 species reputed to be self-incompatible and thus physiologically incapable of selfing regardless of the relative amount of self versus outcross pollination (paired t -test, $P = 0.048$). For example, the putatively self-incompatible dipterocarp tree *Shorea curtisii* exhibited near-complete outcrossing in undisturbed forest compared to only 50% outcrossing in selectively logged forests [24]. The extent to which substantial apparent selfing in these self-incompatible populations results from mating between close relatives (biparental inbreeding) rather than true self-fertilization caused by weakened self-incompatibility combined with increased self-pollination is not clear.

Table 1. Comparison of mating systems between populations in human-disturbed and relatively undisturbed habitat for 27 species of seed plants involving 141 populations.

Family	Species	Life form	SI?	Location of study	Disturbance type	n_{pops} (D/U)	Mean outcrossing (t_m)		Source
							D	U	
Asteraceae	<i>Crepis sancta</i>	A	SI	France	HF	2/9	0.766	0.911	[25]
Bombacaceae	<i>Ceiba aesculifolia</i>	T	SI	Mexico	HF	1/1	0.969	0.962	[29]
	<i>Ceiba grandiflora</i>	T	SI	Mexico	HF	1/1	0.913	0.896	[29]
	<i>Pachira quinata</i>	T	SI	Costa Rica	HF	1/1	0.777	0.915	[27]
Caryocaraceae	<i>Caryocar brasiliense</i>	T	SI	Brazil	HF	1/3	1.000	1.000	[71]
Dipterocarpaceae	<i>Dryobalanops aromatica</i> ^a	T	SC	Malaysia	SL	1/1	0.766	0.923	[72]
	<i>Shorea curtisii</i>	T	SC	Malaysia	SL	1/1	0.522	0.963	[24]
	<i>Shorea megistophylla</i>	T	SC	Sri Lanka	SL	1/1	0.713	0.866	[73]
Fabaceae	<i>Dinizia excelsa</i>	T	SC	Brazil	HF	1/1	0.848	0.875	[74]
	<i>Enterolobium cyclocarpum</i>	T	SI	Costa Rica	HF	1/1	0.999	1.000	[26]
	<i>Samanea saman</i>	T	SI	Costa Rica	HF	1/1	0.912	0.990	[28]
Meliaceae	<i>Carapa guianensis</i>	T	SI	Costa Rica	SL	1/1	0.986	0.967	[75]
	<i>Carapa procera</i>	T	SC	French Guiana	SL	1/1	0.630	0.850	[76]
Myrtaceae	<i>Calothamnus quadrifidus</i>	S	SC	Australia	HF	2/7	0.580	0.756	[30]
	<i>Eucalyptus benthamii</i>	T	SC	Australia	HF	2/2	0.495	0.690	[77]
	<i>Eucalyptus marginata</i> ^b	T	SC	Australia	DD	2/2	0.755	0.865	[78]
	<i>Verticordia fimbriolepis</i> ssp. <i>fimbriolepis</i>	S	SC	Australia	HF	2/2	0.650	0.625	[30]
Pinaceae	<i>Picea glauca</i> ^c	T	SC	Canada	HF	20/3	0.905	0.920	[32]
Poaceae	<i>Calamagrostis canadensis</i>	P	SC	Canada	SL	2/1	0.894	0.998	[79]
	<i>Deschampsia cespitosa</i>	P	SI	Canada	MC	1/1	0.870	0.970	[80]
	<i>Spartina alterniflora</i>	P	SC	USA	WC	2/1	0.778	0.790	[81]
Proteaceae	<i>Banksia cuneata</i>	S	SC	Australia	HF	1/3	0.670	0.893	[30]
	<i>Banksia oligantha</i>	S	SC	Australia	HF	3/1	0.923	1.030	[30]
	<i>Embotrium coccineum</i>	T	SI	Chile	HF	4/4	0.875	0.920	[82]
	<i>Hakea carinata</i> ^d	S	SC	Australia	HF	8/19	0.103	0.114	[31]
Rubiaceae	<i>Lambertia orbifolia</i>	S	SC	Australia	HF	1/3	0.410	0.650	[30]
	<i>Psychotria tenuinervis</i>	S	SC	Brazil	SL	1/1	0.370	0.500	[83]

Life forms are denoted as: A, annual herb; P, perennial herb; S, woody shrub; and T, tree. SI? denotes whether the species is reported as self-incompatible (SI) or self-compatible (SC). Disturbance types, as described by the authors of the studies, are denoted as: HF, habitat fragmentation; SL, selective logging; DD, disturbance and disease; MC, metal contamination of soils; and WC, wetland creation. The number of populations (n_{pops}) sampled in disturbed (D) and undisturbed (U) habitat is presented, along with the proportion of seeds outcrossed estimated from all loci assayed (t_m) in each habitat type. Populations were classified as disturbed and relatively undisturbed by the authors of each study, except for the following cases denoted by superscripts.

^aSeed orchard populations were not included in this analysis.

^bWe classified populations as disturbed and relatively undisturbed based on the authors' description of the study sites provided in the methods.

^cWe classified populations as being in fragmented versus contiguous undisturbed habitats based on the information in Table 1 in the paper.

^dWe classified the eight roadside populations as disturbed; the 19 other were relatively undisturbed.

Our results are consistent with a previous meta-analysis of the effect of habitat fragmentation on population genetic parameters [12]. For instance, the inbreeding coefficient of progeny tends to be higher in fragmented than unfragmented populations, as would be expected under higher frequencies of selfing [12], although this might also result from a reduction in effective population size caused by disturbance. However, it is important to point out that in our analysis, the sample of species for which data are available is far from representative of plants in general. Sixteen of the 27 species are trees, 15 of which occur in tropical forests. Seven species are woody shrubs and only four are herbaceous plants, all but one long-lived clonal grasses (Table 1). Most studies focused on the effects of habitat fragmentation (17 studies) or selective logging ($n = 6$) and a disproportionate number were in Australia ($n = 8$), with surprisingly few in northern hemisphere temperate regions ($n = 5$).

Sampling issues notwithstanding, these results suggest that human disturbance often alters mating patterns in plant populations. However, the ecological factors that underlie these effects have usually not been identified. A lower level of outcrossing in disturbed habitats is usually attributed to a reduction in the amount of outcrossed

pollen deposited on stigmas rather than increased self-pollination. Reduced outcross pollen delivery might be due to lower pollinator abundance, smaller and sparser plant populations that attract fewer pollinators and contain fewer potential mates or some interaction between these two direct consequences of disturbance. For example, the 50% reduction in outcrossing associated with selective logging in *S. curtisii* might be due to reduced density of reproductive individuals because this species is thought to be pollinated by thrips and/or small beetles with limited mobility, but there is no direct evidence to evaluate this explanation [24].

In fact, 17 of the 22 studies examining the effect of human disturbance on mating systems are population genetic in nature and do not include comparisons of pollinators, pollination or reproductive output in disturbed versus undisturbed habitats. Some of the studies that did examine pollination fulfill our expectations. For instance, pollinator visitation and seed production in highly fragmented urban populations of *Crepis sancta* increased significantly with plant density [25–27]. In some cases, reduced outcrossing was associated with reductions in pollination and progeny fitness [28]. In other cases, disturbance was associated with antagonistic effects on

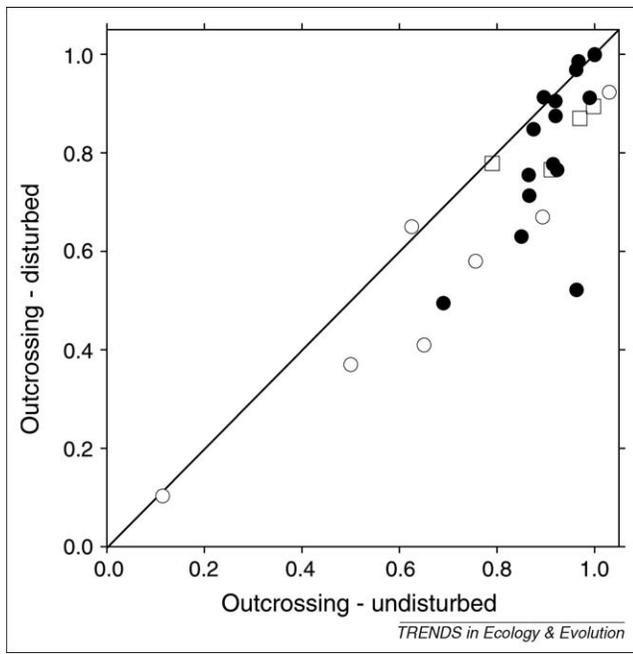


Figure 1. Consequences of human mediated habitat disturbance for plant mating systems. Each point is the mean proportion of seeds outcrossed (t) in disturbed versus relatively undisturbed habitat for 27 plant species represented by a total of 141 populations. The life form of each species is indicated by different symbols (closed circles for trees, open circles for woody shrubs and open squares for herbaceous species). The diagonal line indicates no difference in outcrossing between habitat types. Points lying below the diagonal indicate lower outcrossing in disturbed habitats. There are two species for which near-complete outcrossing ($t \approx 1.0$) occurred in both habitat types (indicated as a single closed circle). Mean outcrossing values for each species and habitat type are listed in Table 1.

different components of reproductive success. For example, visitation to two bat-pollinated *Ceiba* spp. was higher or lower in fragmented than continuous forest, depending on the bat species involved. Trees in forest fragments produced more flowers but the effect on seed production differed between tree species and neither exhibited reduced outcrossing [29].

Human disturbance is likely to simultaneously alter multiple ecological factors, with potentially conflicting effects on different components of fitness. Because large-scale experimental manipulations are rarely possible, isolating the effects of important ecological factors is a challenge. Extensive replication of populations across ecological gradients with both disturbed and undisturbed habitats can be used to approximate an experimental manipulation [30] but the replication involved in the studies to date is generally very low. For almost half the species studied in our meta-analysis, a single disturbed site was compared to a single undisturbed site in a single year (Table 1) [31,32].

Anticipating mating system evolution in human-disturbed habitats

The reductions in outcrossing in disturbed habitat illustrated in Figure 1 most likely represent short-term responses by populations to changes in pollination environment rather than evolved changes in the mating system. However, chronic outcross pollen limitation in disturbed habitats selects for selfing as a means of reproductive assurance [33–35] but the evolutionary consequences of anthropogenic effects on plant mating systems has rarely

been considered. Outcross pollen limitation (PL_x) has been quantified in hundreds of species by comparing the seed production of flowers (F) experiencing natural pollination (F_{IN}) with that of flowers hand-pollinated with abundant outcross pollen (see Box 1 for pollination treatments and [36–38] for discussion of this technique and its assumptions). This large body of experimental work indicates that PL_x is a significant constraint on seed production in a wide range of species [39–41] but there are relatively few direct tests of the hypothesis that PL_x is stronger in disturbed than undisturbed habitats [41]. Moreover, the ubiquity of PL_x might be due, in part, to the pervasive effects of human disturbance, even in populations not identified as disturbed.

For strictly self-incompatible species, PL_x caused by human disturbance should simply reduce seed production. Self-compatible species might exhibit more diverse responses [42]. Those with a capacity for autonomous self-pollination (autofertility, Box 1) could produce a full complement of seed but a greater proportion of seed will be self-fertilized. Self-fertilization (without the intervention of pollinators) through floral mechanisms that place self pollen in contact with receptive stigmas (autonomous autogamy) provides reproductive assurance (RA) by compensating for a shortage of outcross pollen. Hence, the ubiquity of PL_x has widely been viewed as supporting the important role of RA in the evolution of selfing from outcrossing. However, experimental tests of the RA hypothesis are few [18]. The role that selfing plays in mitigating pollen limitation in self-compatible populations is rarely evaluated and the assay of PL_x commonly used does not adequately quantify the potential fecundity advantage of RA via selfing.

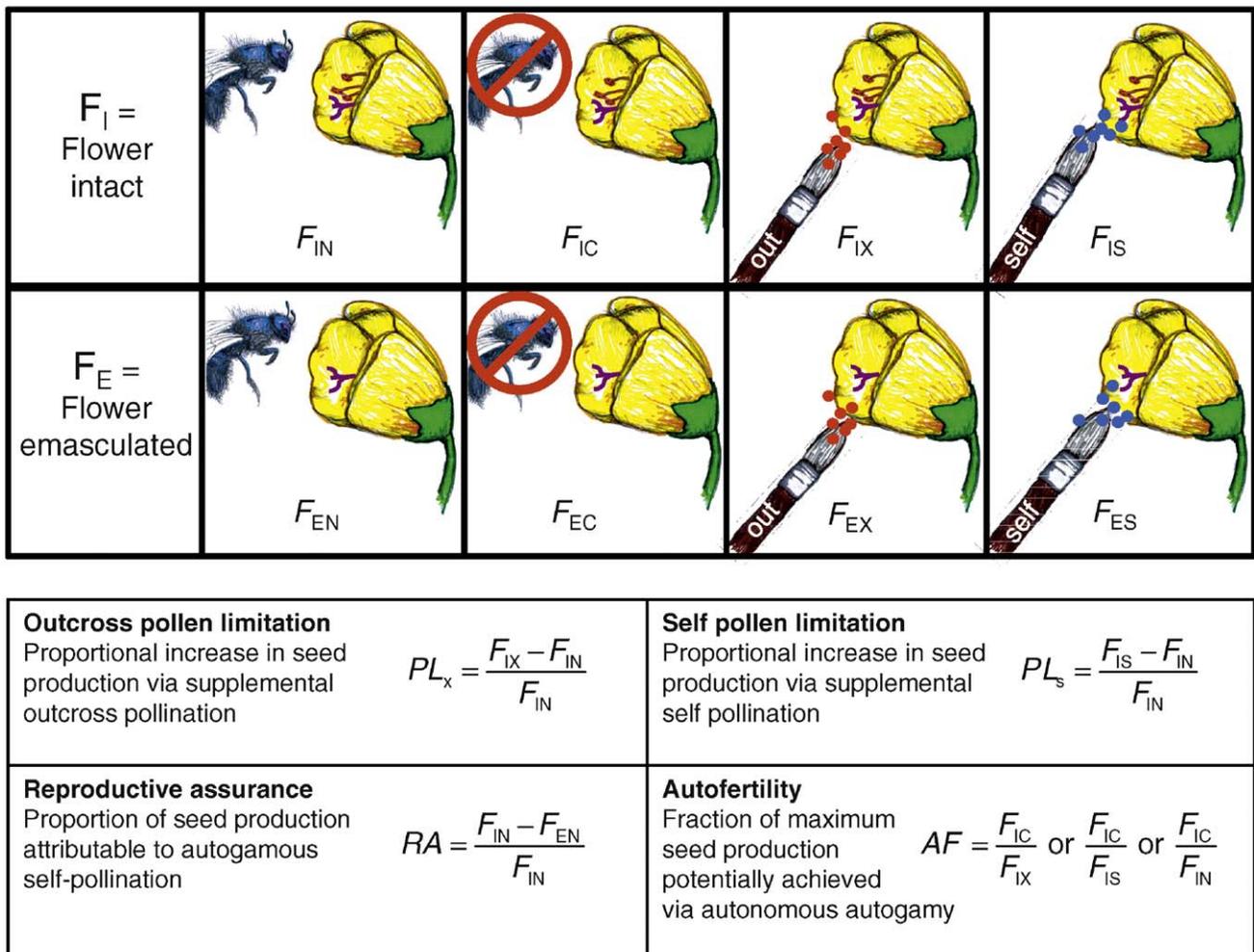
Consider a simple study in which estimates of PL_x are found to be higher in disturbed than natural habitats. It might be proposed that selection in a fragmented habitat favors floral traits that cause autonomous selfing as a mechanism of RA. The extent to which autonomous selfing can increase seed production also depends on the survival of selfed versus outcrossed embryos to seed maturation. For many outcrossing species, self-fertilized embryos do not survive to the seed stage as often as outcrossed embryos [43]. This early-acting inbreeding depression causes the standard measure of PL based on supplemental outcross pollination to overestimate the benefits from autonomous deposition of similar amounts of self pollen. This bias can be substantial. Studies of the RA hypothesis should use an alternative measure of PL based on supplemental self-pollination (PL_s , Box 1) but the potential increase in seed production via self-pollination is almost never measured [38].

An evolutionary response to selection of selfing as a mechanism of RA also requires that populations possess genetic variation for floral traits that enhance the deposition of self pollen on receptive stigmas in the absence of pollinators and allow self pollen to fertilize ovules [44,45]. This autofertility (AF) is estimated by comparing the seed production of flowers that have been excluded from pollinators (caged) to those that have been hand-pollinated (Figure 2). However, the extent to which AF compensates for a lack of outcross pollination in natural populations

Box 1. Experimental manipulations for estimating pollination parameters

Simple floral manipulations can be used to estimate pollination parameters important for understanding the influence of human disturbance on plant mating systems (Figure 1). We define F_{AB} as the seed production by individual flowers given floral manipulation (I = intact, E = emasculated) and the pollinator environment (N = natural pollination, C = caged to exclude pollinators, X = hand outcrossed, S = hand selfed). Pollen limitation (PL) is the proportional difference in seed production of naturally pollinated flowers (F_{IN}) and those for which pollen loads on stigmas have been supplemented by hand pollination. In almost all experimental studies of PL, supplemental pollination involves outcross pollen from another individual (F_{IX}), which we define as PL_x . Significant PL might result in selection for self-pollination as a mechanism of reproductive assurance (RA), which is measured as the proportional difference in seed production between flowers potentially capable of autonomous autogamous self-pollination (F_{IN}) and those for which that capacity has been eliminated by removing anthers before they shed pollen (emasculated, F_{EN}). However, the extent to which autonomous self-fertilization can increase seed production, thereby alleviating PL_x , will be reduced by inbreeding depression acting

before seed maturation. This early-acting inbreeding depression causes PL_x to overestimate the fertility benefits of autonomous selfing. Hence, we define a more relevant measure of pollen limitation (PL_s), which is the proportional difference in seed production between naturally pollinated flowers (F_{IN}) and those supplemented with self pollen (F_{IS}). Autofertility (AF) is the potential for RA via autonomous autogamous self-fertilization and is estimated by comparing spontaneous seed production of plants caged to exclude pollinators (F_{IC}) to seed production after hand outcrossing (F_{IX}) or hand selfing (F_{IS}). When these data are unavailable, a crude estimate can be obtained using seed production after natural pollination (F_{IN}). It can be verified that AF is caused by autonomous autogamy and not some form of apomixis or ineffective caging by showing that emasculated flowers fail to set seed when cages exclude pollinators ($F_{EC} = 0$). Note that RA can also be caused by facilitated autogamy, which is accounted for in the measurement of RA but not AF. These techniques assume that (1) caging alone does not reduce seed production and (2) emasculatation does not reduce natural outcross pollination or outcross seed production by damaging flowers or making them less attractive to pollinators.



TRENDS in Ecology & Evolution

Figure 1. Simple experimental manipulations required for estimation of the two forms of pollen limitation (PL_x and PL_s), reproductive assurance (RA) and autofertility (AF).

must be evaluated by directly estimating RA as the excess seed production of intact, naturally pollinated flowers compared to flowers for which autogamous selfing is prevented by experimental anther removal [46]. RA has been

measured in this way for several plant species and does not generally correlate with AF, emphasizing that AF only indicates the potential for RA [17]. For populations in relatively undisturbed habitats, the selective benefit of

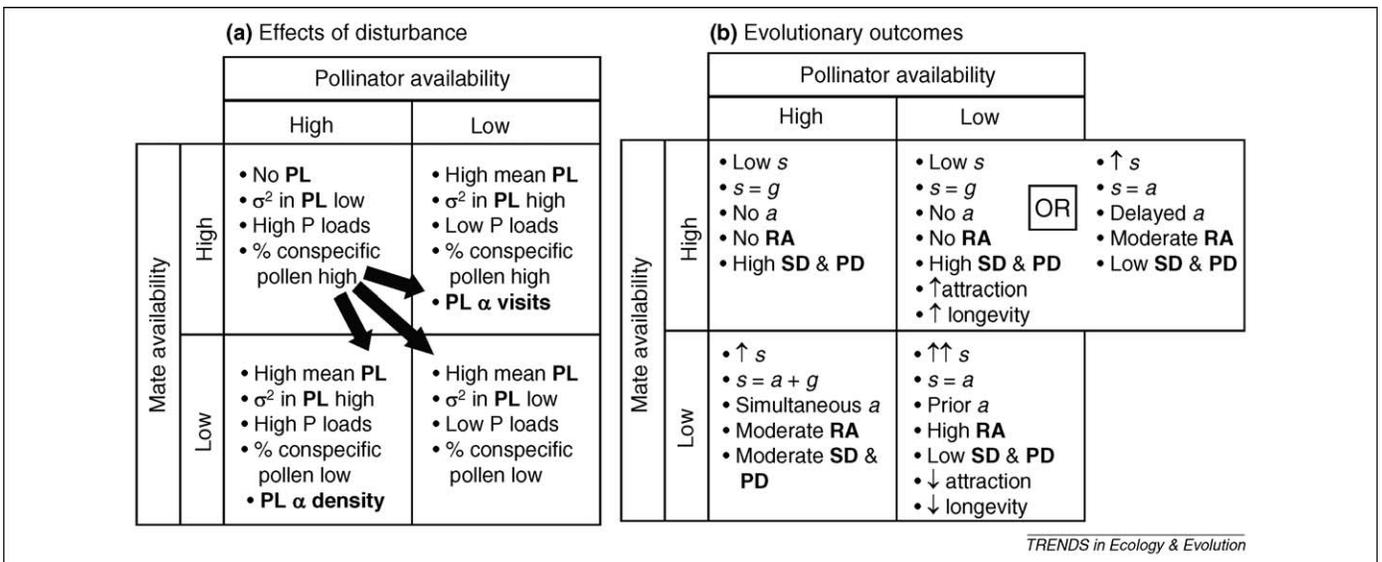


Figure 2. Consequences of reduced pollinator and/or mate availability caused by human disturbance on (a) selective pressures on the mating system and (b) the evolutionary response to these selective pressures. The combinations of high and low pollinator availability with high and low mate availability represent extremes in a continuum of pollination environments. Arrows indicate the effects of human disturbance, from conditions represented by the top left cell (high pollinator and mate abundance) to combinations of reduced pollinator and/or mate abundance. Important pollination parameters that affect the magnitude and direction of selection on the mating system include the mean and variance (σ^2) for pollen limitation (PL) among individuals, the size of pollen loads on stigmas (P), the proportion of total pollen from conspecifics (% conspecific) and, where relevant, the dependence of PL on pollinator visitation and mate density (α denotes proportional to). Evolutionary outcomes are described in terms of the amount of self-fertilization (s), selfing modes ($a =$ autogamy, $g =$ geitonogamy) and timing (prior, simultaneous, delayed), reproductive assurance (RA), seed discounting (SD), pollen discounting (PD), changes in floral structure that attract pollinators (attraction), and changes in floral longevity.

RA has been evaluated in light of the costs of selfing, such as inbreeding depression [47] and trade-offs between producing selfed seed and outcrossed seed (seed discounting [48]) or siring seeds on other plants (pollen discounting [49]). However, there is a need for studies that combine comparison of outcrossing (t) in disturbed versus undisturbed habitats with empirical measures of PL_s or RA.

Anthropogenic pollen limitation might yield diverse outcomes for mating system evolution

The evolutionary response of plant populations to anthropogenic PL is likely to depend on the extent to which PL is caused by reduced pollinator visitation versus reduced number or density of conspecific pollen donors (mates). These two situations can occur independently [50–52] but will often co-occur because pollinators are attracted to higher flowering plant density [25,53] and habitat perturbation can diminish both flowering plant density and pollinator populations. The contribution of each of these factors to PL has rarely been evaluated in natural populations [54,55] and the implications for plant mating system evolution have not been considered in much detail [38,56–59].

In Figure 2 we illustrate the diversity of evolutionary responses of the mating system to human-induced PL by considering how a self-compatible but largely outcrossing plant might evolve in response to reduced pollinator visitation, reduced mate availability and the combination of the two. We consider a case in which, in undisturbed habitat, plants experienced high visitation from generalist pollinators and high mate availability (top left cell of Figure 2a). Under these conditions, outcrossing is maintained by strong inbreeding depression. What little selfing (s) occurs is due primarily to geitonogamy ($s = g$), which provides no reproductive assurance (RA). If human

disturbance causes severe chronic PL due to a scarcity of both pollinators and mates (Figure 2a, bottom right), there should be little variance in PL among individuals. Floral mechanisms that promote autonomous selfing and provide substantial RA are favored. Because of the very limited opportunities for outcrossing, there is no trade-off between self-fertilized seeds and either outcrossed seeds (seed discounting, SD) or seeds sired on other plants (pollen discounting, PD). Prior selfing (p) is favored as a mechanism of RA because it also reduces the resource costs of maintaining long-lived flowers [60]. As autonomous selfing evolves, there will be secondary selection for reduced investment in attractive structures and pollinator rewards (e.g. nectar) and shorter flower life span and, eventually, a suite of reduced floral traits associated with the selfing syndrome will evolve (Figure 2b, bottom right).

When PL is not as severe and is caused by either a scarcity of mates or pollinators, but not both (bottom left and top right of Figure 2a, respectively), facultative modes of selfing (delayed autonomous and simultaneous autogamy) can be selected and mixed mating systems are more likely. However, the relative importance of the two causes of PL can determine the nature of selection on the mating system and might differ among various types of human disturbance. For example, climate change and habitat invasion by co-flowering nonindigenous plants can strongly reduce pollinator visitation with little effect on mate availability. By contrast, some forms of habitat fragmentation can reduce plant density and hence mate availability rather than reducing pollinator abundance. When PL is caused primarily by low mate availability, there should be high PL variance among individuals that correlates most strongly with the local density of conspecifics. Pollinator visitation is frequent and pollen loads on stigmas are large but most pollen deposited comes from other species, which

might interfere with fertilization of ovules by conspecific pollen [21]. Under these conditions, selfing results in moderate SD and PD because there are opportunities for outcrossing. As a result, we expect simultaneous autogamy to be most effective at reducing interspecific pollen contamination while maintaining opportunities for outcrossing (Figure 2b, bottom left).

PL caused by low pollinator visitation when potential mates are abundant can generate divergent evolutionary responses (Figure 2b, top right). In this case, PL variance among individuals correlates most strongly with pollinator visitation to individual plants. One outcome is the maintenance of predominant outcrossing reinforced by selection for increased attractive structures and floral life span because these traits improve success in intraspecific competition for pollinator service. Thus, s might decrease as more attractive phenotypes increase in the population. Although there is a cost for floral maintenance, pollinators, when they do visit, deposit primarily conspecific pollen on stigmas, although few flowers will probably be pollinated. An alternative outcome is the evolution of higher s (mixed mating or complete selfing) through delayed autonomous autogamy to provide RA. Delayed autogamy can increase female fitness with little SD or PD.

The thought experiment described above suggests that it is likely that different causes of PL will lead to different evolutionary outcomes in terms of the extent to which the mating system shifts from outcrossing to selfing, the mode and timing of selfing and concomitant changes to floral biology. Much depends on whether the human disturbance generates variance in PL among individuals and the extent to which individual PL correlates with local mate density versus pollinator visitation (something that is rarely measured). Although PL might often select for selfing as a mechanism of RA, the particular mode of selfing will depend on the causes of PL and, in some instances, populations might evolve greater investment in pollinator attraction, reward and outcrossing. Which trajectory a population takes (e.g. Figure 2b, top right) can depend on the strength of inbreeding depression and the relative evolvability (standing additive genetic variance) of attractive traits versus those that promote autogamy. In general, it might be expected that a high density of conspecifics intensifies the expression of inbreeding depression, but empirical studies have shown that this effect is inconsistent [61]. However, human disturbance can complicate matters because types of disturbance that reduce habitat quality can simultaneously reduce population density but increase the expression of inbreeding depression. The net result will depend on the extent to which inbreeding depression varies with biotic stress (density dependence) versus abiotic stress, which is largely unknown for plants.

At present, there is limited information available to evaluate the assumptions of and predictions from this thought experiment. Investigations of the ecological factors causing PL [54,55,62,63] rarely include analyses of RA or phenotypic selection for floral traits generated specifically by PL [58,64,65]. However, it is clear from comparative and experimental studies that selfing evolves in response to diverse ecological scenarios and that selfing as a mechanism of RA is not the only evolutionary response

to PL. For instance, the evolution of self-fertilization in *Eichhornia paniculata* [66] and *Clarkia xantiana* [58] seems to have been caused by reduced visitation by specialist pollinators, whereas in *Arenaria uniflora* it probably arose as a response to heterospecific pollination in spite of frequent pollinator visitation [67]. In arctic and alpine species, PL occurs as a result of adverse environmental conditions and is often associated with increased flower size rather than facultative selfing, although studies of plant mating systems in these habitats are few [59].

Conclusions

We have argued that many of the ways in which humans disturb natural habitats are likely to disrupt plant–pollinator interactions, with consequent outcross pollen limitation, and that the ecological context of anthropogenic PL can generate diverse selective forces on plant mating systems. In many cases, we expect an evolutionary shift towards higher frequencies of self-fertilization, although the selective forces and traits involved are likely to vary with the nature of the disturbance. There is a clear need for studies that quantify how human disturbance alters pollination systems with regard to selective forces impinging on the mating system. Recent studies have used experimental manipulations of plants in natural populations combined with marker gene analysis to evaluate selection on the mating system [49,64,68,69]. These approaches could be profitably applied to test for contrasting selection regimes in disturbed versus undisturbed habitats. Simple comparisons of the floral traits that affect mating between disturbed and undisturbed populations could be used to test for evolutionary responses in short-lived species. However, the effect of disturbance on the mating system of short-lived species has rarely been studied (Figure 2) and trait comparisons between disturbed and undisturbed populations have rarely been made [25,70]. It will be particularly important to determine whether human disturbance is qualitatively different from natural factors that cause PL and whether plant populations have the demographic and genetic capacity for evolutionary responses adequate to ensure persistence in changing environments. However, change presents opportunities as well as challenges. Although human disturbance of pollination systems is a major conservation problem, it provides new opportunities to gain a better understanding of how and why plant mating systems evolve.

Acknowledgments

We thank the National Evolutionary Synthesis Center (NESCent) for supporting the “Understanding the Paradox of Mixed Mating Systems” working group from which this paper emerged; Jon Auman, Karen Henry, Hilmar Lapp and Jeff Sturkey at NESCent for logistic support; Paula Jean Tonsor for the pollination treatment artwork (Box 1); Joe Hereford for enjoyable discussions; Mark van Kleunen for comments on the manuscript; the United States National Science Foundation for grants to SK NSF DEB 0324764; and the Natural Sciences and Engineering Council of Canada for Discovery Grants to MOJ, RS, EE and CGE.

References

- Ritland, K. (2002) Extensions of models for the estimation of mating systems using n independent loci. *Heredity* 88, 221–228
- Holsinger, K.E. (1996) Pollination biology and the evolution of mating systems in flowering plants. *Evol. Biol.* 29, 107–149

- 3 Aguilar, R. *et al.* (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecol. Lett.* 9, 968–980
- 4 Aizen, M.A. and Vázquez, D.P. (2006) Flower performance in human-altered habitats. In *The Ecology and Evolution of Flowers* (Harder, L.D. and Barrett, S.C.H., eds), pp. 159–179, Oxford University Press
- 5 Cane, J.H. *et al.* (2006) Complex responses within a desert bee guild (Hymenoptera: Apiformes) to urban habitat fragmentation. *Ecol. Appl.* 16, 632–644
- 6 Brosi, B.J. *et al.* (2008) The effects of forest fragmentation on bee communities in tropical countryside. *J. Appl. Ecol.* 45, 773–783
- 7 Steffan-Dewenter, I. and Westphal, C. (2008) The interplay of pollinator diversity, pollination services and landscape change. *J. Appl. Ecol.* 45, 737–741
- 8 Traveset, A. and Richardson, D.M. (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol. Evol.* 21, 208–216
- 9 Bjerknes, A.L. *et al.* (2007) Do alien plant invasions really affect pollination success in native plant species? *Biol. Conserv.* 138, 1–12
- 10 Memmott, J. *et al.* (2007) Global warming and the disruption of plant–pollinator interactions. *Ecol. Lett.* 10, 710–717
- 11 Hegland, S.J. *et al.* (2009) How does climate warming affect plant–pollinator interactions? *Ecol. Lett.* 12, 184–195
- 12 Aguilar, R. *et al.* (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. *Mol. Ecol.* 17, 5177–5188
- 13 Cheptou, P.O. (2004) Allee effect and self-fertilization in hermaphrodites: reproductive assurance in demographically stable populations. *Evolution* 58, 2613–2621
- 14 Takebayashi, N. and Morrell, P. (2001) Is self-fertilization a dead end? Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. *Am. J. Bot.* 88, 1143–1150
- 15 Ashman, T.L. and Majetic, C.J. (2006) Genetic constraints on oral evolution: a review and evaluation of patterns. *Heredity* 96, 343–352
- 16 Herlihy, C.R. and Eckert, C.G. (2007) Evolutionary analysis of a key floral trait in *Aquilegia canadensis* (Ranunculaceae): genetic variation in herkogamy and its effect on the mating system. *Evolution* 61, 1661–1674
- 17 Goodwillie, C. *et al.* (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* 36, 47–79
- 18 Eckert, C.G. *et al.* (2006) Reproductive assurance and the evolution of uniparental reproduction in flowering plants. In *The Ecology and Evolution of Flowers* (Harder, L.D. and Barrett, S.C.H., eds), pp. 183–203, Oxford University Press
- 19 Didham, R.K. *et al.* (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends Ecol. Evol.* 22, 489–496
- 20 Biesmeijer, J.C. *et al.* (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* 313, 351–354
- 21 Morales, C.L. and Traveset, A. (2008) Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Crit. Rev. Plant Sci.* 27, 221–238
- 22 Lowe, A.J. *et al.* (2005) Genetic resource impacts of habitat loss and degradation: reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* 95, 255–273
- 23 Ward, M. *et al.* (2005) To self, or not to self. . . A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95, 246–254
- 24 Obayashi, K. *et al.* (2002) Genetic diversity and outcrossing rate between undisturbed and selectively logged forests of *Shorea curtisii* (Dipterocarpaceae) using microsatellite DNA analysis. *Int. J. Plant Sci.* 163, 151–158
- 25 Cheptou, P.O. and Avendaño, L.G. (2006) Pollination processes and the Allee effect in highly fragmented populations: consequences for the mating system in urban environments. *New Phytol.* 172, 774–783
- 26 Rocha, O.J. and Aguilar, G. (2001) Reproductive biology of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. *Am. J. Bot.* 88, 1607–1614
- 27 Fuchs, E.J. *et al.* (2003) Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns of the tropical dry forest tree *Pachira quinata*. *Conserv. Biol.* 17, 149–157
- 28 Cascante, A. *et al.* (2002) Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conserv. Biol.* 16, 137–147
- 29 Quesada, M. *et al.* (2004) Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat-pollinated bombacaceous trees. *Biotropica* 36, 131–138
- 30 Coates, D.J. *et al.* (2007) Plant mating systems and assessing population persistence in fragmented landscapes. *Aust. J. Bot.* 55, 239–249
- 31 Starr, G.J. and Carthew, S.M. (1998) Genetic differentiation in isolated populations of *Hakea carinata* (Proteaceae). *Aust. J. Bot.* 46, 671–682
- 32 O'Connell, L.M. *et al.* (2006) Impacts of forest fragmentation on the mating system and genetic diversity of white spruce (*Picea glauca*) at the landscape level. *Heredity* 97, 418–426
- 33 Lloyd, D.G. (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. *Int. J. Plant Sci.* 153, 370–380
- 34 Morgan, M.T. and Wilson, W.G. (2005) Self-fertilization and the escape from pollen limitation in variable pollination environments. *Evolution* 59, 1143–1148
- 35 Morgan, M.T. *et al.* (2005) Plant population dynamics, pollinator foraging, and the selection of self-fertilization. *Am. Nat.* 166, 169–183
- 36 Knight, T.M. *et al.* (2006) A quantitative synthesis of pollen reallocation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. *Am. J. Bot.* 93, 271–277
- 37 Wesselingh, R.A. (2007) Pollen limitation meets resource allocation: towards a comprehensive methodology. *New Phytol.* 174, 26–37
- 38 Aizen, M.A. and Harder, L.D. (2007) Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. *Ecology* 88, 271–281
- 39 Burd, M. (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot. Rev.* 60, 83–139
- 40 Ashman, T.L. *et al.* (2004) Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85, 2408–2421
- 41 Knight, T.M. *et al.* (2005) Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Evol. Syst.* 36, 467–497
- 42 Larson, B.M.H. and Barrett, S.C.H. (2000) A comparative analysis of pollen limitation in flowering plants. *Biol. J. Linn. Soc.* 69, 503–520
- 43 Husband, B.C. and Schemske, D.W. (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50, 54–70
- 44 Kalisz, S. *et al.* (1999) The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *Am. J. Bot.* 86, 1239–1247
- 45 Runions, C.J. and Geber, M.A. (2000) Evolution of the self-pollinating flower in *Clarkia xantiana* (Onagraceae). I. Size and development of floral organs. *Am. J. Bot.* 87, 1439–1451
- 46 Schoen, D.J. and Lloyd, D.G. (1992) Self- and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *Int. J. Plant Sci.* 153, 381–393
- 47 Kalisz, S. *et al.* (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* 430, 884–887
- 48 Herlihy, C.R. and Eckert, C.G. (2002) Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* 416, 320–323
- 49 van Kleunen, M. and Ritland, K. (2004) Predicting evolution of floral traits associated with mating system in a natural plant population. *J. Evol. Biol.* 17, 1389–1399
- 50 Steffan-Dewenter, I. and Tschardtke, T. (1999) Effects of habitat isolation on pollinator communities and seed set. *Oecologia* 121, 432–440
- 51 Forsyth, S.A. (2003) Density-dependent seed set in the Haleakala silversword: evidence for an Allee effect. *Oecologia* 136, 551–557
- 52 Munoz, A.A. and Arroyo, M.T.K. (2004) Negative impacts of a vertebrate predator on insect pollinator visitation and seed output in *Chuquiraga oppositifolia*, a high Andean shrub. *Oecologia* 138, 66–73
- 53 Groom, M.J. (1998) Allee effects limit population viability of an annual plant. *Am. Nat.* 151, 487–496
- 54 Moeller, D.A. (2004) Facilitative interactions among plants via shared pollinators. *Ecology* 85, 3289–3301
- 55 Campbell, L.G. and Husband, B.C. (2007) Small populations are mate-poor but pollinator-rich in a rare, self-incompatible plant, *Hymenoxys herbacea* (Asteraceae). *New Phytol.* 174, 915–925
- 56 Harder, L.D. and Barrett, S.C.H. (1996) Pollen dispersal and mating patterns in animal pollinated plants. In *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants* (Lloyd, D.G. and Barrett, S.C.H., eds), pp. 140–190, Chapman & Hall

- 57 Kalisz, S. and Vogler, D.W. (2003) Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* 84, 2928–2942
- 58 Moeller, D.A. and Geber, M.A. (2005) Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution* 59, 786–799
- 59 Arroyo, M.T.K. *et al.* (2006) Erratic pollination, high selfing levels and their correlates and consequences in an altitudinally widespread above-tree-line species in the high Andes of Chile. *Acta Oecol.* 30, 248–257
- 60 Ashman, T.L. and Schoen, D.J. (1997) The cost of floral longevity in *Clarkia tembloriensis*: an experimental investigation. *Evol. Ecol.* 11, 289–300
- 61 Willi, Y. *et al.* (2007) Inter-specific competitive stress does not affect the magnitude of inbreeding depression. *Evol. Ecol. Res.* 9, 959–974
- 62 Knight, T.M. (2003) Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. *Oecologia* 137, 557–563
- 63 Waites, A.R. and Ågren, J. (2004) Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *J. Ecol.* 92, 512–526
- 64 Fishman, L. and Willis, J.H. (2008) Pollen limitation and natural selection on floral characters in the yellow monkeyflower, *Mimulus guttatus*. *New Phytol.* 177, 802–810
- 65 Ashman, T.L. and Diefenderfer, C. (2001) Sex ratio represents a unique context for selection on attractive traits: consequences for the evolution of sexual dimorphism. *Am. Nat.* 157, 334–347
- 66 Barrett, S.C.H. *et al.* (1989) The dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata*. *Evolution* 43, 1398–1416
- 67 Fishman, L. and Wyatt, R. (1999) Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). *Evolution* 53, 1723–1733
- 68 Eckert, C.G. and Herlihy, C.R. (2004) Using a cost-benefit approach to understanding the evolution of self-fertilization in plants: the perplexing case of *Aquilegia canadensis* (Ranunculaceae). *Plant Species Biol.* 19, 159–173
- 69 Kennedy, B.F. and Elle, E. (2008) The reproductive assurance benefit of selfing: importance of flower size and population size. *Oecologia* 155, 469–477
- 70 Devoto, M. and Medan, D. (2004) Effects of grazing disturbance on the reproduction of a perennial herb *Cypella herbertii* (Lindl.) Herb. (Iridaceae). *Plant. Syst. Evol.* 243, 165–173
- 71 Collevatti, R.G. *et al.* (2001) High resolution microsatellite based analysis of the mating system allows the detection of significant biparental inbreeding in *Caryocar brasiliense*, an endangered tropical tree species. *Heredity* 86, 60–67
- 72 Lee, S.L. (2000) Mating system parameters of *Dryobalanops aromatica* Gaertn. f. (Dipterocarpaceae) in three different forest types and a seed orchard. *Heredity* 85, 338–345
- 73 Murawski, D.A. *et al.* (1994) Outcrossing rates of two endemic *Shorea* species from Sri Lankan tropical rain forests. *Biotropica* 26, 23–29
- 74 Dick, C.W. *et al.* (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Mol. Ecol.* 12, 753–764
- 75 Hall, P. *et al.* (1994) Genetic diversity and mating system in a tropical tree, *Carapa guianensis* (Meliaceae). *Am. J. Bot.* 81, 1104–1111
- 76 Doligez, A. and Joly, H.I. (1997) Mating system of *Carapa procera* (Meliaceae) in the French Guiana tropical forest. *Am. J. Bot.* 84, 461–470
- 77 Butcher, P.A. *et al.* (2005) Increased inbreeding and inter-species gene flow in remnant populations of the rare *Eucalyptus benthamii*. *Conserv. Genet.* 6, 213–226
- 78 Millar, M.A. *et al.* (2000) Mating system studies in jarrah, *Eucalyptus marginata* (Myrtaceae). *Aust. J. Bot.* 48, 475–479
- 79 MacDonald, S.E. and Loeffers, V.J. (1991) Population variation, outcrossing, and colonization of disturbed areas by *Calamagrostis canadensis*: evidence from allozyme analysis. *Am. J. Bot.* 78, 1123–1129
- 80 Bush, E.J. and Barrett, S.C.H. (1993) Genetics of mine invasions by *Deschampsia cespitosa* (Poaceae). *Can. J. Bot.* 71, 1336–1348
- 81 Travis, S.E. *et al.* (2004) Population structure and inbreeding vary with successional stage in created *Spartina alterniflora* marshes. *Ecol. Appl.* 14, 1189–1202
- 82 Mathiasen, P. *et al.* (2007) Genetic structure and early effects of inbreeding in fragmented temperate forests of a self-incompatible tree, *Embothrium coccineum*. *Conserv. Biol.* 21, 232–240
- 83 Ramos, F.N. *et al.* (2008) Mating systems of *Psychotria tenuinervis* (Rubiaceae): distance from anthropogenic and natural edges of Atlantic forest fragment. *Biochem. Genet.* 46, 88–100