# Effects of Inbreeding, Outbreeding, and Supplemental Pollen on the Reproduction of a Hummingbird-pollinated Clonal Amazonian Herb

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## ABSTRACT

Understory herbs are an essential part of tropical rain forests, but little is known about factors limiting their reproduction. Many of these herbs are clonal, patchily distributed, and produce large floral displays of nectar-rich 1-d flowers to attract hummingbird pollinators that may transport pollen over long distances. The aim of this study was to investigate the effects of clonality, cross-proximity, and patchy distribution on the reproduction of the hummingbird-pollinated Amazonian herb *Heliconia metallica*. We experimentally pollinated flowers within populations with self-pollen and with pollen of different diversity, crossed flowers between populations, and added supplemental pollen to ramets growing solitarily or in conspecific patches. Only flowers pollinated early in the morning produced seeds. Selfed flowers produced seeds, but seed number and mass were strongly reduced, suggesting partial sterility and inbreeding depression after selfing. Because of pollen competition, flowers produced more seeds after crosses with several than with single donor plants. Crosses between populations mostly resulted in lower seed production than those within populations, suggesting outbreeding depression. Ramets in patches produced fewer seeds than solitary ramets and were more pollen-limited, possibly due to geitonogamy and biparental inbreeding in patches. We conclude that high rates of geitonogamy due to clonality and pollen limitation due to the short receptivity of flowers and patchy distribution constrain the reproduction of this clonal herb. Even in unfragmented rain forests with highly mobile pollinators, outbreeding depression may be a widespread phenomenon in plant reproduction.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

*Key words*: clonality; geitonogamy; Heliconiaceae; hummingbird pollination; Peru; pollen limitation; pollination experiment; rain forest understory.

UNDERSTORY HERBS ARE AN ESSENTIAL PART OF TROPICAL RAIN FORESTS (Gentry & Emmons 1987), but little is known about factors limiting their reproduction (but see Horvitz & Schemske 1988, Bruna *et al.* 2004). Many Neotropical understory herbs are clonal and produce large floral displays and ample amounts of nectar to attract hummingbird pollinators (Stiles 1979, Kay & Schemske 2003). Genets of these herbs are usually patchily distributed (Kay & Schemske 2003, Schleuning *et al.* 2008), and patches occur at low densities in the forest understory (*e.g.*, Bruna *et al.* 2004). Their scattered inflorescences are visited by trap-lining hummingbirds along foraging routes of up to 1 km length (Linhart 1973, Stiles & Wolf 1979). Although plant–pollinator interactions in the forest understory are an interesting model system for tropical ecology, few studies have experimentally investigated the reproduction of

hummingbird-pollinated herbs in Neotropical forests (but see Horvitz & Schemske 1988, Bruna *et al.* 2004).

Plant reproduction can be reduced due to limitations in the quantity or quality of the deposited pollen (Aizen & Harder 2007); both are strongly influenced by pollinator behavior (Ghazoul 2005). Low visitation rates of pollinators may limit pollen quantity, reduce stigmatic pollen competition (Snow & Spira 1993), and subsequently plant reproduction (quantity limitation; Knight *et al.* 2005), while selfing and pollen transfer between closely related plants can reduce plant reproduction due to mechanisms of self-incompatibility or inbreeding depression (quality limitation; Burd 1994, Herlihy & Eckert 2002). In clonal plant species, pollen limitation as a consequence of low pollen quality may be particularly strong (Honnay *et al.* 2006, Scobie & Wilcock 2009). High degrees of geitonogamy in populations of clonal plants can substantially reduce reproduction (Araki *et al.* 2007, Honnay & Jacquemyn 2008). Although the clonal life form is widespread among rain forest understory herbs (Villegas 2001, Schleuning *et al.* 2008), the costs of clonality for the reproduction of these herbs is not known.

The spatial scale of pollen flow provided by pollinators strongly affects patterns of gene flow (Ellstrand 1992). Pollen flow among populations increases genetic exchange and thus reduces inbreeding (Dudash & Fenster 2000). Pollen flow between widely separated individuals, however, may also have deleterious effects due to the disruption of local adaptation or allelic coadaptation, resulting in outbreeding depression (Waser & Price 1989, Dudash & Fenster 2000). Most evidence of outbreeding depression derives from crosses between widely separated populations (Fischer & Matthies 1997, Stacy 2001), but outbreeding depression may also occur at scales of only tens to hundreds of meters in spatially structured populations (Waser *et al.* 2000). Outbreeding depression may be less likely in systems with highly mobile pollinators that connect plant patches over several hundred meters and thus reduce genetic subdivision of populations (Hamrick & Loveless 1989). The consequences of the trap-lining behavior of hummingbird pollinators for plant reproduction in tropical forests, however, are hardly known.

Patterns of pollen deposition are also strongly influenced by the spatial distribution of plants within populations (Mustajärvi *et al.* 2001, Knight *et al.* 2005). Plants growing in dense floral neighborhoods of conspecifics are often more attractive for pollinators than isolated plants and are visited more frequently (Feinsinger *et al.* 1991, Kunin 1993). This density dependent foraging behavior can translate into increased deposition of conspecific pollen and increased reproduction in dense patches (Ågren 1996, Roll *et al.* 1997). In dense patches, however, reproduction may also be reduced because of higher competition for resources or pollinators and higher biparental inbreeding (Waser & Price 1993, Ghazoul 2005), but there is yet little empirical evidence for negative density dependence of plant reproduction due to pollen limitation (see Ghazoul 2005). Overall, the mechanisms linking plant density and reproduction are manifold and the importance of limitations of pollen quantity and quality can vary with species, environment, and time (Burd 1994, Bosch & Waser 2001). In a previous study from Amazonia, the conspecific density of flowers in the forest understory did not affect the reproduction of a tropical herb (Bruna *et al.* 2004), but further studies are required to test whether this is a general phenomenon.

The aim of this study was to assess factors limiting reproduction of a tropical understory herb. We experimentally tested the effects of clonality, cross-proximity, and patchy distribution on reproduction. We chose the widespread clonal herb *Heliconia metallica* Planchon et Linden ex Hooker (Heliconiaceae) as our model system, because it is the most abundant, hummingbird-pollinated understory herb in the Peruvian Amazon. Like most of its 200 Neotropical congeners (Berry & Kress 1991), *H. metallica* produces nectar-rich and brightly colored flowers that are mostly visited and pollinated by hermit hummingbirds (Linhart 1973, Stiles 1979). We asked the following questions: (1) Do selfing and low pollen diversity within populations reduce reproduction? (2) Does reproductive success benefit from between-population crosses? (3) Does supplemental pollen increase reproduction and, if so, is the effect of pollen addition stronger in solitary ramets than in patches? To address these questions, we pollinated flowers of *H. metallica* with self-pollen and with pollen of different diversity within a population, conducted between-population crosses, and applied supplemental pollen to ramets in conspecific patches and to solitary ramets.

## METHODS

STUDY SITE AND SYSTEM.—Field studies were carried out from October 2005 to December 2006 in *ca* 2 km$^2$ of a floodplain forest of the Madre de Dios River in south-eastern Peru (12°21′20″ S, 70°42′44″ W; 260 m asl). Annual precipitation is *ca* 2500 mm with a pronounced rainy season from October to April ( > 80% of annual precipitation).

*Heliconia metallica* is a perennial understory herb distributed from Honduras to Bolivia (Berry & Kress 1991). Like other *Heliconia* spp., it is widespread in the understory of Neotropical forests. In the floodplain forests of south-eastern Peru, *H. metallica* is common, produces flowers throughout the whole year and thus is a crucial nectar resource for its hummingbird pollinators. The species is clonal and forms lateral rhizomes that produce erect aboveground shoots (ramets) that can reach a height of up to 3.5 m and produce three to nine broad, banana-like leaves. Clones of *H. metallica* are patchily distributed and can reach a size of at least 200 m$^2$ consisting of several hundred shoots, but most patches are multi-clonal (M. Schleuning, G. P. Vadillo, & W. Durka, unpubl. data). In small canopy gaps, ramets may develop a single-terminal inflorescence with three to six bracts each subtending about ten hermaphroditic 1-d flowers (each pink flower *ca* 4 cm long; Schleuning *et al.* 2008) that are abscised in the early afternoon. Over a period of 2–4 mo, each ramet produces one or two new flowers *ca* every 3 d. Ramets eventually die after reproduction, but new shoots are formed from their rhizomes. The nectar-rich flowers are visited by various species of hummingbirds, in particular by the trap-lining hermit hummingbirds *Phaethornis philippii* and *Phaethornis hispidus*, and the territorial woodnymph *Thalurania furcata* (M. Schleuning, pers. obs.). After fertilization, it takes *ca* 4 wk for a fertilized flower to develop into a mature fruit. Each fruit produces a maximum of three seeds (mean = 2.3, SD = 0.92, *N* = 939 fruits) each weighing 25–118 mg (mean = 80.8 mg, SD = 13.7 mg, *N* = 1528 seeds). The outcrossing rate, based on the analysis of 79 seeds of open-pollinated flowers from 21 fruiting ramets, was close to one (M. Schleuning, G. P. Vadillo, & W. Durka, unpubl. data) suggesting that selfed seeds have a low viability.

EFFECTS OF SELF-POLLINATION AND POLLEN DIVERSITY.—We selected a population of *H. metallica* in the floodplain forest ('Floodplain' population) to examine the effects of self-pollen and pollen diversity on the reproduction of this species. In October 2005, 50 flowering ramets of *H. metallica* were marked. The inflorescences of these ramets were enclosed in nylon mesh bags (30 × 13 × 10 cm, mesh size ≈ 2 × 2 mm) to exclude pollinators, seed dispersers, and predators, and were fixed to wooden poles to stabilize the ramet. For the experiment, pollen was collected early in the morning at 0530–0600 h shortly after anthesis. Two types of pollen were collected: (1) pollen from two flowers of a single donor ramet; and (2) a mixture of pollen from six different donor ramets growing in three different patches separated from each other by at least 70 m. Pollen was scraped from the anthers into plastic Petri dishes with

toothpicks. Freshly opened flowers of bagged inflorescences randomly received one of the following treatments: (1) no pollination ($N = 33$); (2) pollination with self-pollen collected from the same flower before emasculation ($N = 28$); (3) pollination with pollen from a single donor ramet ($N = 39$); or (4) pollination with pollen from six donor ramets ($N = 44$). Flowers from treatments (2)–(4) were emasculated with tweezers, and stigma and perianth were cleaned with cotton buds for treatments (3) and (4). All hand pollinations were carried out early in the morning between 0600 h and 0730 h. For the pollinations, we ran a toothpick three times through a pollen sample and applied the pollen to the stigma. Afterwards, the flower was marked with colored twine to indicate the type of treatment. Toothpicks and cotton buds were discarded after each flower. Four weeks after the pollinations, ripe fruits were collected from all ramets, except for ten ramets that had died. Seeds were extracted and bathed in a 0.5 percent solution of sodium hypochlorite for 5 min to inhibit fungal pathogens. Seeds were dried with paper tissue and stored in paper bags filled with dried peat moss until the seeds were weighed to the nearest 0.002 mg in the laboratory.

CROSSES WITHIN AND BETWEEN POPULATIONS.—To investigate the effects of pollen origin on the reproduction of *H. metallica*, we selected two populations ('River' and 'Floodplain'), which were separated from each other by *ca* 300 m of dense forest; in between the populations only solitary, mostly nonflowering ramets occurred. Each population contained several patches of *H. metallica* separated from each other by at least 70 m. In the River population, we selected a single patch in a riverine bamboo forest, whereas in the Floodplain population, we selected two patches that were situated in a 'Backwater' (*i.e.*, at low elevation) and a 'Levee' habitat (*i.e.*, at high elevation), respectively. In September 2006, we randomly marked 20 flowering ramets in each patch and enclosed their inflorescences. During the 2-wk experiment, pollen from four open flowers of spatially separated ramets (minimum distance between ramets: 10 m) was collected in each patch early every morning. Pollen collection in the three patches was carried out synchronously. After pollen collection, all bagged ramets were visited in a random order to assure that on average plants in different patches received pollen at the same time and that the storage time for pollen from different sources was similar. Each ramet received pollen from all three patches, but each open flower only received pollen from one of the three patches in a random order. Ramets that produced at least six flowers after enclosure received pollen from the three patches twice (*i.e.*, six pollinated flowers). Hence, in this reciprocal design, each ramet in the River population received pollen from the single River patch and from the two Floodplain patches, while ramets in the Floodplain population received pollen from the two Floodplain patches and from the single River patch. Hand pollinations followed the protocol described above, and the time of pollination was recorded. Four weeks later, ripe fruits were collected, seeds extracted, counted, and weighed as described above.

EFFECTS OF SUPPLEMENTAL POLLINATION.—To assess whether the reproduction of *H. metallica* was pollen limited, supplemental pollen was applied to flowers in the River and the Floodplain population.

In September 2006, we selected five patches in each population with a high density of flowering ramets of *H. metallica* (mean density: 8.6 flowering ramets in a 5-m radius) and marked five flowering ramets per patch at random. In addition, 24 flowering solitary ramets were selected that were isolated from the next flowering ramet by > 15 m. All solitary ramets were situated in the Floodplain population, because only very few occurred in the River population. For 2 wk, mixed probes of pollen from five ramets growing in different patches were collected early every morning in each population. Afterwards, we visited all marked ramets in random order. Open flowers randomly received one of the pollination treatments: (1) no additional pollen (*i.e.*, control); (2) additional pollen from the home population; and (3) additional pollen from the other population. The flowers were marked with colored twine to indicate treatment type, and the time of pollination was recorded. Each ramet received all treatments. When the last treatment had been applied to a ramet, the respective inflorescence was enclosed in a nylon mesh bag to protect developing fruits from predation. Four weeks later, ripe fruits were collected, their seeds extracted, counted, and weighed as described above.

STATISTICAL ANALYSIS.—We carried out analyses of deviance with a logit-link and a binomial error distribution (Crawley 2007) to investigate the effects of pollen origin and supplemental pollination on fruit set. Effects on seed set and seed mass were analyzed by analysis of variance. Seed set was angular transformed to improve normality of residuals. In the experiment of self-pollination and pollen diversity, the effects of pollen source were partitioned into two orthogonal contrasts to assess the effect of self-pollen vs. cross-pollen and of one vs. six donor ramets. Ramet identity was not considered because treatments were randomly distributed over ramets. In the analysis of crosses within and between populations, we tested the effects of site against the variation among ramets. We partitioned the effect of site (*i.e.*, recipient patch) into two orthogonal contrasts: (1) River vs. Floodplain population and (2) Backwater vs. Levee patch (within Floodplain). We also partitioned the effect of pollen origin into two orthogonal contrasts: (1) home vs. away population and (2) home vs. away patch (within Floodplain). In the analysis of supplemental pollination, data from the five ramets in each of the ten patches were pooled, and the effects of population and floral neighborhood type (solitary vs. patch) were tested against the variation among patches of ramets, and all other effects against the residual. The effect of the pollen treatments was partitioned into two orthogonal contrasts: (1) control vs. additional pollen and (2) additional home vs. additional distant pollen.

## RESULTS

EFFECTS OF SELF-POLLEN AND POLLEN DIVERSITY.—Bagged flowers that were not hand pollinated did not produce fruits, indicating that flowers do not self-fertilize. Selfed flowers produced fruits and seeds, but fruit set, seed set, and mean individual seed mass were significantly lower than in outcrossed flowers (Table S1; Fig. 1). Fruit set and seed mass of flowers that had been pollinated with pollen from one or six donor plants did not differ, but seed set was
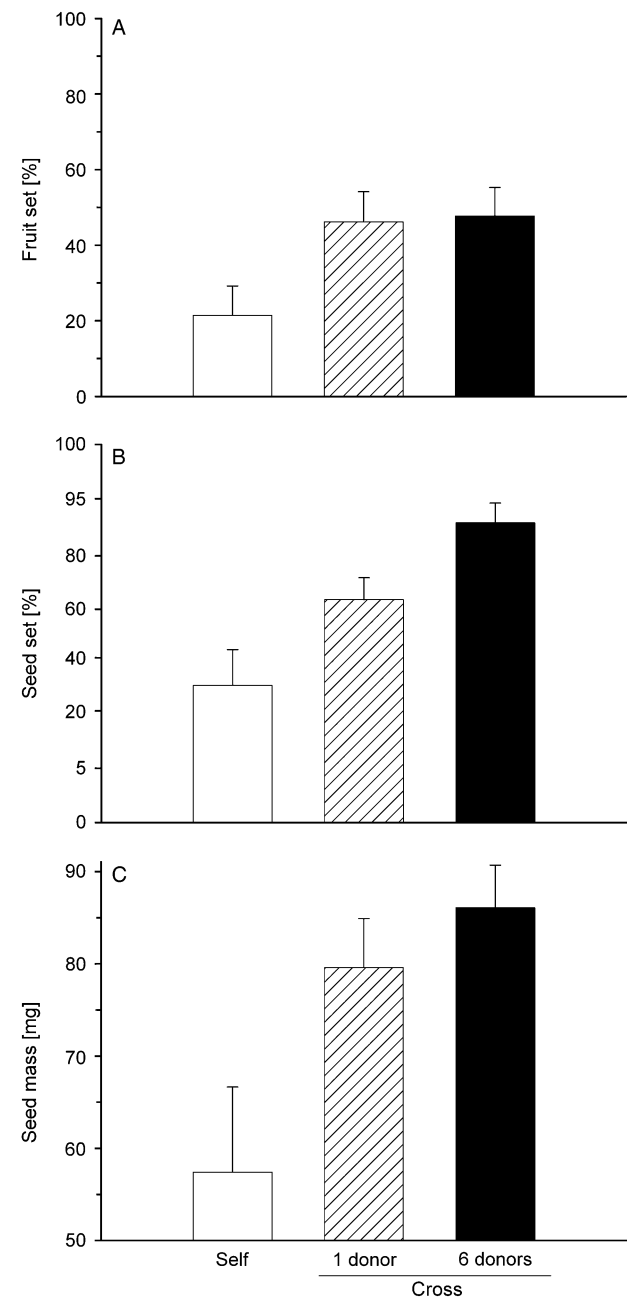
FIGURE 1. The effects of pollination with self-pollen and cross-pollen from one or six donor ramets on (A) fruit set, (B) seed set, and (C) seed mass in a population of *Heliconia metallica*. Means+SE are given; note angular scale for seed set.
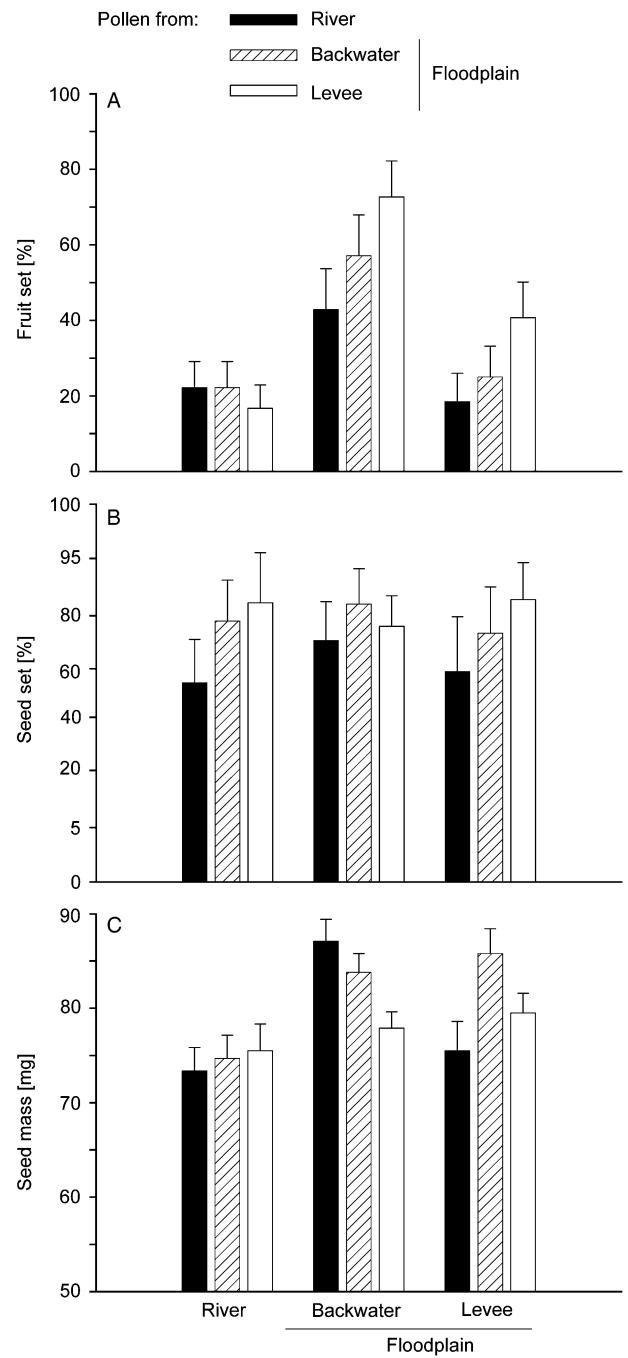


FIGURE 2. The effects of within- and between-population crosses on (A) fruit set, (B) seed set, and (C) seed mass in three patches of *Heliconia metallica* situated in the River and Floodplain populations. Pollinations were carried out in 20 ramets of *H. metallica* in each patch; each ramet received pollen from all three patches. Means+SE are given; note angular scale for seed set.

higher in flowers pollinated with mixed pollen than with pollen from a single donor (Table S1; Fig. 1).

CROSSES WITHIN AND BETWEEN POPULATIONS.—The time of hand pollination strongly affected fruit set (Table S2A). Pollinations conducted later than 0820 h were not successful. Ramets of *H. metallica* in the River population produced fewer fruits than ramets in the Floodplain population, while ramets in the Backwater patch

produced more fruits than those in the Levee patch (Table S2A; Fig. 2A). The effects of pollen origin were less strong than the effects of the recipient patch. Nevertheless, flowers pollinated with pollen from the home population produced more fruits than those pollinated with pollen from the distant population (Table S2A;
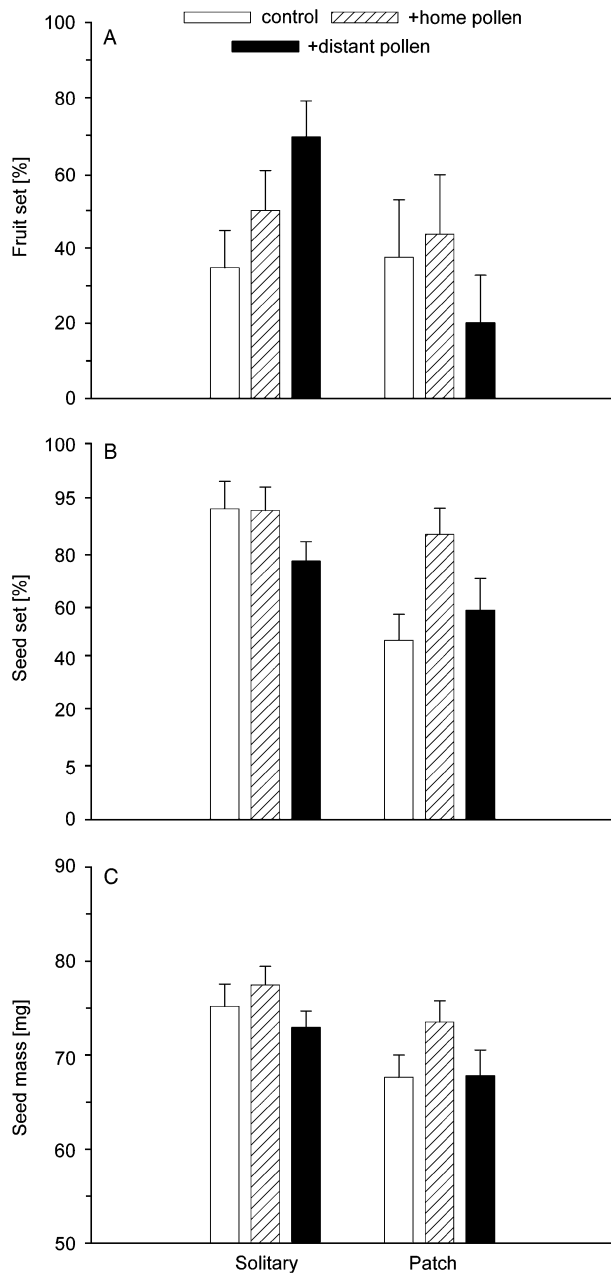
FIGURE 3. The effects of supplemental pollen from the home and a distant population on (A) fruit set, (B) seed set, and (C) seed mass in solitary ramets and in ramets in patches of *Heliconia metallica*. Solitary ramets flowered isolated from other conspecifics by > 15 m ($N = 24$ ramets), whereas ramets in patches flowered close to other conspecifics ($N = 10$ patches). Means + SE are given; note angular scale for seed set.

Fig. 2A). In contrast to fruit set, seed set was not affected by the recipient population. In the Floodplain population, flowers produced more seeds per fruit with home than with distant pollen, but the opposite effect was found in the River population (Table S2B; Fig. 2B). Seed mass was strongly influenced by ramet identity

(Table S2C), and tended to be lower in ramets growing in the River than in the Floodplain population, but was not affected by pollen origin (Fig. 2C).

EFFECTS OF SUPPLEMENTAL POLLINATION.—Natural fruit set was similarly low in solitary and patch ramets. Supplemental pollination increased fruit set of *H. metallica*, but only in solitary ramets (Table S3A; Fig. 3A). The origin of the supplemental pollen affected the fruit set of ramets in patches and solitary ramets differently. Fruit set of solitary ramets increased after addition of pollen, in particular of pollen from the distant population, whereas addition of distant pollen decreased fruit set of ramets in patches (Fig. 3A). Seed set was higher in solitary ramets than in patches. Supplemental pollination increased seed set, but only in ramets in patches (Table S3B; Fig. 3B). Mean seed mass strongly varied among ramets (32.2–91.7 mg; Table S3C). Supplemental pollen increased seed mass, but only if pollen originated from the home population (Fig. 3C).

## DISCUSSION

EFFECTS OF SELF-POLLEN AND POLLEN DIVERSITY.—Like most of its congeners, *H. metallica* was not able to self-pollinate, but was at least partially self-compatible, because it produced fruits after hand self-pollination (Kress 1983, but see Bruna *et al*. 2004). Genetic analyses of seeds from open-pollinated flowers revealed that seeds of *H. metallica* had mostly been formed by outcrossing, indicating efficient pollen flow between clones. The initial outcrossing rate of ovules, however, might have been much lower than the outcrossing rate at the seed stage, because seed production was much lower in selfed than outcrossed flowers (0.19 vs. 1.09 seeds/flower, reduction in seed production: 83%). Fruit set, seed set, and seed mass were all reduced after selfing. Partial sterility after selfing is a widespread phenomenon in plants and can be due to maternal control of fertilization and seed maturation (Waser 1993) or to early inbreeding depression at the seed stage (Husband & Schemske 1996). In a meta-analysis of 30 outcrossing angiosperm species, however, the mean reduction of seed production after selfing was only 19 percent (Husband & Schemske 1996). Similarly, in tropical understory herbs of the genus *Costus*, the reduction of seed production after selfing (12–33%; Schemske 1983) was much lower than in *H. metallica*. In contrast, high costs of selfing have been reported for other herbaceous plants with a high potential for geitonogamy (Herlihy & Eckert 2002, Michalski & Durka 2007). The benefit of outcrossing was higher when flowers of *H. metallica* were pollinated with pollen from six than from single donor ramets (1.29 vs. 0.88 seeds/flower, increase in seed production: 47%). The increase in reproduction with pollen diversity could be a sampling effect due to a higher probability of fertilization with superior pollen in combination with pollen competition (Paschke *et al*. 2002), but could also involve maternal control over pollen tube choice (Aizen & Searcy 1998). Our results indicate that selfing reduces reproduction of *H. metallica* more strongly than that of most other outcrossing plants so that the species can be considered partially self-incompatible and thus benefits from an increase in pollen diversity.

EFFECTS OF CROSSES BETWEEN POPULATIONS.—In *H. metallica*, fruit set was lower after crosses between than within populations, suggesting outbreeding depression on a relatively small spatial scale. In the two Floodplain patches, seed production was much lower after crosses with distant pollen (0.59 seeds/flower) than with home pollen (1.15 seeds/flower, reduction: 48.7%). Thus, the optimum reproductive success is achieved after crosses within populations, but among patches of *H. metallica*. Intermediate crossing distances also provided highest seed production in another Neotropical herb (Schemske & Pautler 1984). The results from the pollen addition experiment also suggest outbreeding depression, because seed production was lower with distant than with close supplemental pollen, in particular in ramets in patches (reduction: 68.6%). Outbreeding depression of similar magnitudes has been reported for several other species (Fischer & Matthies 1997, Dudash & Fenster 2000, Stacy 2001). In *H. metallica*, however, outbreeding depression depended on the specific combination of pollen donor and recipient population. In contrast to the Floodplain population, seed set in the River population was higher after crosses between populations (increase in seed production: 30.1%). This is consistent with earlier findings that outbreeding depression may vary strongly among populations or even genotypes (see Waser *et al.* 2000, Becker *et al.* 2006).

Previous studies found outbreeding depression mostly at large distances between populations (Fenster & Galloway 2000, Stacy 2001, Pélabon *et al.* 2005, but see Waser *et al.* 2000); however, genetic distances between populations might be more important than geographic distances (Becker *et al.* 2006) and can be pronounced on small spatial scales due to spatially restricted dispersal of seeds and pollen (Price & Waser 1979, Fenster 1991). In *H. metallica*, genetic distances between populations are high (M. Schleuning, G. P. Vadillo, & W. Durka, unpubl. data), even if they are only separated by a few hundred meters. Lack of seedling recruitment in the long-lived populations of *H. metallica* (Schleuning *et al.* 2008, 2009) and genetic drift may strongly contribute to genetic differentiation among populations. Pollen flow between populations of *H. metallica* is likely to occur, because hermit hummingbirds visit flowers in the forest understory along traplines of up to 1 km length (Stiles & Wolf 1979). It is unclear how many pollen grains are actually moved from one population to another, because visits to heterospecific flowers can drastically reduce pollen loads of hummingbirds (Murcia & Feinsinger 1996). Nevertheless, experimentally isolated plants of a hummingbird- and bumblebee-pollinated herb received substantial pollen transfer from source populations up to 400 m apart (Schulke & Waser 2001), but other studies suggest that the transfer of conspecific pollen decreases more drastically with isolation (Richards *et al.* 1999, Duncan *et al.* 2004). For future studies, the rain forest understory provides an interesting model system to examine the spatial scale of pollen movement by traplining pollinators and its consequences for plant reproduction. Such studies might strengthen the notion that outbreeding depression also occurs in plants with mobile pollinators that frequently move pollen between populations.

POLLINATOR LIMITATION AND RECEPTIVITY.—Fruit set of open-pollinated flowers of *H. metallica* was only about 35 percent, which is similar to the mean fruit set recorded for *Heliconia acuminata* (Bruna *et al.* 2004). Previous studies of hummingbird-pollinated understory herbs found that flowering plants received less than one pollinator visit per hour (Kay & Schemske 2003, Bruna *et al.* 2004). Visitation frequencies of *H. metallica* were also very low (M. Templin, pers. obs.). Therefore, standardized pollinator observations in our study populations were not feasible.

Overall, supplemental pollen increased seed production of *H. metallica* (mean increase with supplemental home pollen: 69%), strongly suggesting pollinator limitation. Reproduction of many plant species, in particular if they are outcrossing (Ghazoul 2005), is limited by the visitation rates of their pollinators (Horvitz & Schemske 1988, Knight *et al.* 2005). In *H. metallica*, short receptivity may strongly contribute to pollinator limitation of reproduction. The 1-d flowers of *H. metallica* only set fruit when pollinated between 0530 h and 0830 h. The time of receptivity corresponds to the early-morning activity of hermit hummingbirds (Stiles & Wolf 1979), and nectar production of *Heliconia* spp. is also particularly high early in the morning (Stiles 1975). The short receptivity may be due to the short lifespan of a flower, because the pollen tubes have to grow and reach the ovary before the flower is abscised at *ca* 1500 h.

POLLINATOR LIMITATION AND FLORAL NEIGHBORHOOD.—Natural seed production of ramets of *H. metallica* in patches was lower (0.52 seeds/flower) than that of solitary ramets (0.97 seeds/flower). In solitary ramets, intraspecific competition is lower and thus more resources are potentially available. Resource limitation can strongly affect plant reproduction in dense populations (Mustajärvi *et al.* 2001). However, in *H. metallica* ramet size (*i.e.*, number of leaves and bracts) did not differ between solitary and patch ramets, suggesting that resource availability was similar. Moreover, the increase in seed production with supplemental home pollen was actually stronger in ramets in patches (1.13 vs. 0.52 seeds/flower, increase: 117%) than in solitary ramets (1.39 vs. 0.97 seeds/flower, increase: 43%).

Hence, a more likely explanation for the negative density dependence of reproduction in *H. metallica* is competition for pollinators, in particular for visitors providing high-quality pollen (Ghazoul 2005). The results from pollen addition experiments suggest that low pollen quality rather than insufficient quantity caused negative density dependence. Supplemental pollen in patch ramets increased seed set but not fruit set, indicating that supplemental pollen often failed to fertilize any ovule due to prezygotic barriers. Previous studies have shown that germination of outcross pollen can be reduced, if large amounts of self-pollen clog the stigma (Galen & Stanton 1989, Waser & Price 1991). In *H. metallica*, distant supplemental pollen even reduced fruit set, suggesting negative interactions between pollen from close and distant donors. Several previous studies found inconsistent effects of pollen augmentation on seed production and quality due to interactions of pollen diversity with recipient genotypes (Snow & Spira 1993, Colling *et al.* 2004, Kron & Husband 2006). Our results suggest that inconsistent effects of supplemental pollen could also result from the genotypes involved in pollen–pollen interactions. Allelopathic effects of foreign pollen are known from interspecific

pollen interactions (Roshchina *et al.* 2009) but may also occur among genotypes of a single species. If supplemental pollen fertilized ovules despite these prezygotic barriers, seed production of *H. metallica* increased, suggesting a limitation of postzygotic performance by pollen quality. Low quality of pollen in patches may have been caused by pollen transfer between neighboring ramets, *e.g.*, by territorial males of *T. furcata* (Linhart 1973), resulting in geitonogamy and biparental inbreeding. The notion that ramets in patches were mostly limited by pollen quality is consistent with earlier studies which found that pollen limitation is often due to the quality rather than the quantity of deposited pollen (Aizen & Harder 2007, Chacoff *et. al.* 2008).

In contrast to ramets in patches, we found that fruit set but not seed set of solitary ramets was pollen-limited. This suggests that many control flowers of solitary ramets did not receive pollen at all, and is consistent with other studies showing that low densities of flowering plants attract fewer pollinators (Jennersten & Nilsson 1993, Burd 1994). In *H. metallica*, control flowers of solitary ramets that actually had been pollinated produced many seeds. Thus, their postzygotic performance was not limited by pollen quality, probably because hermit hummingbirds transfer pollen between spatially isolated ramets (Stiles & Wolf 1979). The results from the pollen addition experiment suggest that solitary ramets of *H. metallica* were limited by pollen quantity, whereas ramets in patches were mostly limited by pollen quality.

## CONCLUSIONS

The clonal life form and patchy distribution of the hummingbird-pollinated understory herb *H. metallica* impose several constraints on its reproduction. Selfing reduced reproduction of *H. metallica* stronger than that of most other species, indicating high costs of geitonogamy for reproduction. Crosses between populations only 300 m apart, however, also resulted in lower seed production, but outbreeding depression differed between recipient populations. Strong genetic differentiation among populations of clonal plants may affect reproduction on a small spatial scale, even in unfragmented rain forests with highly mobile hummingbird pollinators. In contrast to most previous pollination studies, reproduction in patches of *H. metallica* was low and strongly pollen-limited, possibly due to geitonogamy and biparental inbreeding. In *H. metallica*, the patchy distribution increased pollen limitation of reproduction. We conclude that pollen limitation may be a widespread phenomenon in clonal rain forest understory plants due to the low visitation rates of pollinators, short duration of receptivity of flowers, and the consequences of both inbreeding and outbreeding depression.

## ACKNOWLEDGMENTS

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

TABLE S1. *The effects of pollination with self-pollen and cross-pollen of different diversity (one or six donor ramets) on fruit set, seed set, and seed mass of* H. metallica.

TABLE S2. *The effects of crosses within and between populations on fruit set, seed set, and seed mass of ramets of* H. metallica *in a patch in the River population, and in two patches in the Floodplain population.*

TABLE S3. *The effects of supplemental pollen from the home and from a distant population on fruit set, seed set, and seed mass of solitary and patch ramets of* H. metallica.

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