ELSEVIER

GfÖ
GfÖ Ecological Society of Germany,
Austria and Switzerland

Basic and Applied Ecology 13 (2012) 277–285

Basic and
Applied Ecology

www.elsevier.com/locate/baae

Status of pollinators and their efficiency in coffee fruit set in a fragmented landscape mosaic in South India

Smitha Krishnan[a,*], Cheppudira. G. Kushalappa[b], R. Uma Shaanker[c,d], Jaboury Ghazoul[a]

[a] *Institute of Terrestrial Ecosystems, Department of Environmental Sciences, ETH Zürich, 8092 Zurich, Switzerland*
[b] *College of Forestry, University of Agricultural Sciences, Bangalore 560064, India*
[c] *Ashoka Trust for Research in Ecology and the Environment, Bangalore 560064, India*
[d] *Department of Crop Physiology, University of Agricultural Sciences, GKVK Campus, Bangalore 560065, India*

Received 19 April 2011; accepted 8 March 2012

Abstract

The region of Kodagu, South India, comprises a fragmented landscape with a high density of remnant forest patches dispersed within a mosaic dominated by shaded coffee agro-forests. We evaluated the role of self, wind and insect pollination to *Coffea canephora* production in this landscape. The giant Asian honeybee, *Apis dorsata*, which nests in remnant forests, was the main pollinator of coffee (accounting for 58% of the floral visitors). The proportion of flowers that developed into fruits was highest when hand cross-pollinated (44%), followed by open- (insect and wind combined; 33%) and wind- (22.1%) pollination treatments. Pollination by bees therefore increases fruit production by 50% over that achieved by wind. Self-pollination (1.7%) and no pollination (1%) treatments produced very low fruit set, emphasizing the importance of cross-pollination in *C. canephora*. Unlike measures of pollination success, initial fruit set (five weeks after flowering) proved an unreliable proxy for final fruit set. Size of adjoining forest fragments (mostly 0.3–20 ha, with a few exceeding 200 ha) positively influenced pollinator visitation to coffee flowers, but distance to such fragments had no influence on pollination. This study demonstrates the importance of cross-pollination for crop production in *C. canephora*, the important contribution that pollinating insects make to coffee production, and the benefits of relatively large forest fragments within the landscape mosaic to support insect pollinators of coffee. A comparison of pollinator composition to that of 100 years ago indicated that coffee pollination services remained intact despite changes in pollinator community composition.

Zusammenfassung

In der Region Kodagu im Süden Indiens findet sich eine fragmentierte Landschaft mit einer hohen Dichte noch vorhandener Waldreste, die mosaikartig in einer Landschaft verteilt sind, die von beschatteten Kaffee-Waldplantagen dominiert wird. In dieser Landschaft verglichen wir die Rollen der Selbst-, Wind- und Insektenbestäubung für die Coffea canephora-Produktion. Die asiatische Riesenhonigbiene, Apis dorsata, die in den verbliebenen Wäldern ihre Nester hat, war die Hauptbestäuberin für Kaffee (insgesamt 58%der Blütenbesucher). Der Anteil der Blüten, die sich zu Früchten entwickelten, war am höchsten bei kreuzweiser Handbestäubung (44%), gefolgt von offenen Blüten (Insekten und Wind kombiniert, 33%) und nur Wind (22,1%). Die Bestäubung durch die Bienen erhöhte die Fruchtproduktion demnach um 50% in Verhältnis zu der, die allein durch den Wind erreicht wurde. Die Selbstbestäubung (1,7%) und fehlende Bestäubung (1%) produzierten beide einen sehr geringen Fruchtansatz und betonen die Wichtigkeit der kreuzweisen Bestäubung bei C. canephora. Anders als bei der Messung

---

[*] Corresponding author. Tel.: +91 9686530776; fax: +41 44 632 15 75.
E-mail addresses: smithakrishnan@gmail.com, smitha.krishnan@env.ethz.ch (S. Krishnan).

http://dx.doi.org/10.1016/j.baae.2012.03.007

des Bestäubungserfolgs zeigte sich der initiale Fruchtansatz (fünf Wochen nach der Blüte) als ein unzuverlässiges Maß für den endgültigen Fruchtansatz. Die Größe der benachbarten Waldfragmente (meistens 0.3 bis 20 ha, wenige über 200 ha) war mit den Bestäuberbesuchen auf Kaffeeblüten positiv korreliert, während der Abstand zu den Fragmenten keinen Einfluss auf die Bestäubung hat. Diese Untersuchung demonstriert die Wichtigkeit der kreuzweisen Bestäubung für die Ernteproduktion bei C. canephora, den wichtigen Beitrag, den bestäubende Insekten zur Kaffeeproduktion leisten, und die Vorteile von relativ großen Waldfragmenten im Landschaftsmosaik, um die Bestäuberinsekten für Kaffee zu unterstützen. Ein Vergleich mit der Bestäuberzusammensetzung heute und vor 100 Jahren wies darauf hin, dass die Bestäubungsleistung beim Kaffee trotz der Veränderungen in der Zusammensetzung der Bestäubergemeinschaft intakt geblieben ist.

Keywords: Bees; *Coffea canephora*; Landscape mosaic; Pollen tube; Pollination services; Pollination success; Seed set

Introduction

Conversion of diverse natural habitats into less diverse agro-ecosystems has caused biodiversity losses and affected ecosystem services across the world (MEA, 2005). Habitat loss could directly impact wild pollinators in agricultural landscapes by reducing forage and nesting resources (Kevan and Wojcik, 2007), which could cause a decline in pollination services to many agricultural crops (Kearns, Inouye, & Waser 1998; Klein et al., 2007). Several studies have shown that insect pollination increases fruit production in a number of important agricultural crops (Ricketts et al., 2008; and references therein). It is less clear whether natural habitat loss actually translates into loss of agricultural production by undermining pollination services (Ghazoul, 2005; Ghazoul and Koh, 2010). Nevertheless, the potential loss of pollinating insects and associated effects on pollinator-dependent agricultural crops is an issue of wide concern.

In this context, the extent to which coffee crops are dependent on insect pollinators is critical information for over 25 million coffee farming families (ICO, 2010), who collectively produce about 8.26 million tonnes of coffee annually (FAOSTAT, 2010). Many of these producers are smallholder farmers who produce coffee within diverse agro-forestry systems (DaMatta, Ronchi, Maestri, & Barros 2007; ICO, 2010). Despite the importance of all *Coffea* species to global coffee production, most studies on pollination services to coffee have focused on *C. arabica*, and many of these have quantified the contribution of pollinators to immature fruits within a few weeks of flowering rather than final fruit set about 10 months after flowering (but see studies on *Coffea arabica* by Ricketts, Daily, Ehrlich, & Michener 2004; Roubik, 2002b). Furthermore, *C. arabica* is self-compatible and out-crossing is not required for fertilisation, while other coffee species such as *Coffea canephora* (‘robusta’ variety) are self-incompatible, but also thought to be wind-pollinated (Ferwerda, 1948; Le Pelley, 1973).

C. canephora accounts for 41.3% of worldwide coffee production (ICO, 2011), though in India it comprises 68% of coffee production (Coffee Board India, 2010). Although most studies on pollination of coffee have been conducted on *C. arabica*, it is likely that *C. canephora* is perhaps even more vulnerable to pollinator loss owing to self-incompatibility and consequent dependency on gene exchange between individuals. The importance of insects relative to wind for pollination and fruit production (final fruit set) in *C. canephora* is, however, not known.

While concerns about declining pollinators are widespread, information about actual changes in pollinator composition and abundance is limited. Yet in South India, such concerns of loss of wild bees as pollinators of coffee date back to 1911 (Fletcher, 1915). Although these studies are limited by low sampling intensity and data being largely qualitative rather than quantitative, the information they provide allows a crude assessment of changing pollinator communities across this time interval during which coffee agro-forests have been intensified and expanded at the expense of forest.

In this study, we seek to determine the main pollinators of *C. canephora* in Kodagu, the main coffee growing region in India, and the extent to which pollination success and coffee production are affected by insect- vs. wind-pollination. We further assess the degree to which early fruit maturation can be used as a proxy for final fruit set in *C. canephora*. Finally, we evaluate the extent to which distance to and size of the adjoining forest fragments affect the pollination service to coffee. A clear understanding of the role of wild and domesticated pollinators for coffee production will provide the basis for an ecosystem service approach to land and agro-forest management which could benefit conservation and sustainable production.

Methods

Study area

The district of Kodagu, also known as Coorg, in the state of Karnataka is one of the main coffee growing areas in India. Coffee agro-forests in Kodagu were first established in the 1850s, and about two percent of the total geographical area (4106 km^2) comprised coffee agro-forests by the late 1860s, the rest being mainly forest (Bidie, 1869). Since the 1970s, there has been dramatic expansion in the area of coffee agro-forests, coupled with increasing intensification as native shade trees are removed and replaced with fast growing exotic *Grevillea robusta* trees. By 2007, coffee agro-forests (the large majority being *Coffea canephora*) covered 32.5% of Kodagu, while forests had been reduced from 68% in 1977 to 46% of land area (Garcia et al., 2010).

Our study was conducted in Virajpet taluk (12°00′ to 12°29′N and 75°39′ to 76°33′E) during 2007–2009. We selected 41 locations, 34 of which were located adjacent to forest fragments ranging in size from 0.3 to 20 ha, with the remaining seven locations adjacent to officially protected forest reserves (all ≥200 ha). Within each of these locations, agro-forests at distances of <10, 50, 100, 250 and 500 m from the forest edge were selected for this study. These coffee agro-forests were created several decades ago by clearing the understory of moist-deciduous to evergreen forest. The common native shade trees that persist in coffee agro-forests include *Aporusa lindleyana* (Wt.) Baill, *Artocarpus heterophyllus* Lam., *Syzygium cumini* (L.) Skeels., and *Dalbergia latifolia* Roxb. Over the past two decades native shade trees have been gradually replaced in many agro-forests by the exotic *Grevillea robusta* A. Cunn. ex R. Br. The mean density of all trees within agro-forests is 297 ± 90 ha^{-1} (range 120–543) and the density of *G. robusta* is 68 ± 52 ha^{-1} (range 0–215). Average shade cover is 43 ± 11% (range 17–84). The density of coffee is about 1500–2000 plants ha^{-1} and the stem girth ranged from 17 to 53 cm.

Study species

C. canephora inflorescences are borne in a sequence of discrete inflorescence clusters along the axils of the leaves. Flowers at the axils of two opposite leaves (at each node) are henceforth referred to as ‘clusters’. Previous studies have suggested that *C. canephora* is primarily wind-pollinated and fruit set might be enhanced by insect pollination (Fletcher, 1915; Free, 1993). The ovary is composed of two ovules, and two seeded fruits, referred to as ‘cherries’, are formed when both are fertilised. In some cases only one ovule develops to produce a single seeded fruit called a ‘pea-berry’. Flowering in *C. canephora* is initiated when the plants are subjected to a drought stress (about two to three months) followed either by rain or irrigation. Irrigation leads to flowering at a local (agro-forest) scale, while rain stimulates simultaneous flowering of many coffee agro-forests across the landscape corresponding to the rainfall-receiving area.

Floral visitors

In each of the 41 coffee agro-forests we selected five coffee plants at distances of <10, 50, 100, 250 and 500 m from the forest edge, giving 1025 observational plants in 205 sampling sites. We only included data from 141 sites (rain-fed = 69 sites and irrigated = 72 sites) in subsequent analyses, as we discarded data from sites that were subject to rain on the day of flowering (which potentially affects both pollinator activity and flower retention). On these plants, we randomly chose and marked five branches each with six flower clusters. The buds on each cluster were counted two days before flowering. Pollinator observations, noting identity of pollinators, were made during 15-min observation periods of the five selected branches. This was replicated at each of the five plants. Pollinators that could not be readily identified were collected for later identification by experts.

To evaluate long term changes in the coffee pollinator community in Kodagu we compared our pollinator observation results to those reported by Fletcher (1915). Fletcher’s study, also undertaken in Kodagu, comprised similar observations of flower visitors to coffee, made on two days between 8:00 AM and 4:00 PM and totalling 16 hours of observation.

Pollination experiments

Pollination experiments were conducted in a sub-set (28 of the 41) of the locations at 5 distances accounting to 140 agro-forests (28 × 5 = 140, see Appendix A). Data from 28 sites had to be discarded due to rain on the day of observation. The five branches on each of the five marked plants at all sites that were used for floral visitor observations were also used for open-pollination experiments. One branch (with six marked clusters) on each of the same marked plants was used for wind-pollination treatments where pollinators were excluded by covering the branch with a mesh bag with mesh size 0.8–1.2 mm before anthesis. The bags were left on the branch for three days after which flowers had begun to wilt and were no longer attractive to bees.

We also selected one branch on one plant at each site for cross-pollination treatment (140 sites, one branch/site), and self-pollination, and no-pollination treatments (95 sites, one branch/site/treatment). We used fresh flowers as pollen source for artificial pollinations, flowers being collected from the same plant for self-pollination, and from five to ten different plants within each agro-forest at random distances from the experimental plant for cross-pollination. Branches used for self-pollination and no-pollination treatments were covered with paper bags to exclude all pollinating agents; the bags were retained on the branches for 14 days, as stigmas remain receptive (if not pollinated) for over seven days (see Appendix B).

Pollen deposition and pollen tube abundance

To quantify pollination success we counted the number of pollen grains on stigmas and pollen tubes in the styles. We determined that pollen tube growth to the ovule required 26–49 h (see Appendix B) and therefore we sampled ten randomly selected flowers from ten open-pollinated plants three days after flowering from all sites. From the marked branches on which the pollination experiments were applied, 20 styles per plant were collected from the pollinator-excluded branches (112 useable sites), and 18 styles from artificially cross-pollinated (103 useable sites), self-pollinated and no-pollination treatments (58 and 59 usable sites). The styles were fixed in FAA (formalin:acetic acid:70% ethanol in 5:5:90 proportions, respectively). In the laboratory, the styles were washed thoroughly in water and softened in 8N NaOH for 18 h. The softened tissue was rinsed in water several times before being stained in decolourised aniline blue solution (0.1% solution of water soluble aniline blue dye in 0.1 M K_3PO_4) for 18 h. Pollen tubes, which fluoresce under ultra-violet light, can be easily enumerated using an epi-fluorescence microscope.

Pollen grains were counted using a fluorescence microscope at 100× magnification. The number of pollen tubes per style was recorded for 50 random samples from each site for open- and wind-pollinated treatments, and 18 samples from each site for cross-, self- and no-pollination treatment. The average number of pollen tubes per flower (pollen tube abundance) and the proportion of flowers with two or more pollen tubes that successfully reached the bottom of the style (henceforth referred to as 'pollination success') were scored.

Initial fruit set, final fruit set and seed set

Developing fruits were counted five weeks after flowering on the branches used for pollinator observations. The proportion of flowers that developed into fruits (at five weeks) is referred to as initial fruit set. Mature fruits on the same marked branches were counted just prior to harvesting 10 months after flowering. Cherries and pea-berries were counted separately to evaluate the final seed output. The proportion of flowers that developed into mature fruits is referred to as final fruit set and the proportion of ovules that developed into seeds is referred to as seed set.

Analysis

Data from plants were averaged across each site to avoid pseudo-replication, and thus the level of replication is at the site level. A negative binomial generalised linear model was used to test the effects of distance and size of the adjoining forest fragments on pollinator abundance, with rainfall/irrigation included as a categorical variable. Other agro-forest variables such as shade tree density, shade cover (%) and relative humidity (%) were included in the analysis. We arrived at the final model by step-wise elimination of the least significant variables (variables with p-values >0.05 were eliminated). The results of the most parsimonious model (also with the lowest Akaike Information Criteria-AIC) are discussed in this paper. The final model was checked for heteroscedasticity. The independent variables in the models with interactions were centred to reduce multi-collinearity (Quinn and Keough, 2004).

Since all the treatments were applied on the same plant replicates, the influence of other possible variables on the treatments is eliminated. Since the data were not normally distributed, we applied a Wilcoxon signed rank test to evaluate if there was a significant difference in abundance of pollen tubes, pollination success, initial fruit set, final fruit set, or seed set between treatments. No-pollination and artificial self-pollination treatments were not compared with other treatments since the microclimate within the paper bags used to enclose the treatment branches might have affected the pollination success, final fruit set and seed output. The statistical software 'R' (R Development Core Team, 2010) was used for all the statistical analyses.

Results

Pollinators of coffee

Bees comprised 95.7% of all 5915 floral visitors observed, of which 94.6% were social bees and 1.1% were solitary bees (Table 1). Other occasional visitors (4.3% of the total) included thysanopterans, dipterans, hemipterans, lepidopterans, blattarians, and one purple-rumped sunbird (*Leptocoma zeylonica*), but their role in pollination is likely to be insignificant due to infrequent visitation and because they very rarely touched the stigma or anthers. Therefore, they have been excluded from further analysis. Social bees were the most common visitors and among them, *Apis dorsata* (accounting for 58% of all social bee visits), *Apis cerana indica* (23.4%; henceforth *A. cerana*), and *Tetragonula iridipennis* (18%)

Table 1. List of bee species visiting coffee. The bees are listed in descending order of abundance. Species also recorded by Fletcher (1915) are given in bold. In addition to that, *Megachile umbripennis*, *Parevaspis cartonaria* and *Prosopis* spp. were recorded by Fletcher but not in the present study.

Bee species list	Social bees	Individuals observed	Percentage occurrences
Apis dorsata	√	3246	57.37
Apis cerana indica	√	1310	23.15
Tetragonula iridipennis[a]	√	1006	17.78
Apis florea	√	34	0.60
Braunsapis picitarsus		20	0.35
Ceratina hieroglyphica		15	0.27
Ceratina smaragdula		9	0.16
***Amegilla* spp.**[b]		7	0.12
Thyreus spp. [c]		4	0.07
Xylocopa aestuans		1	0.02
Xylocopa latipes		1	0.02
Nomia iridiscens		1	0.02
Megachile rotundata		1	0.02
Megachile bicolor		1	0.02
Lasioglosum spp.		1	0.02
Unknown		1	0.02
		5658	

[a] Fletcher refers to a species group *Melipona* which probably is *Tetragonula iridipennis*.

[b] *Amegilla confusa* and *A. zonata* not differentiated.

[c] *Thyreus histrio* and *T. massuri* not differentiated.

Table 2. Effects of landscape and agro-forest variables on abundance of bees at coffee flowers. Results of stepwise generalised linear model (negative binomial) of the effects of: Size of the forest (ha)[a], Distance from forest (m), No. of flowers observed, Shade cover (%), Density of shade trees per hectare and Relative air humidity (%). Since management is a categorical variable with two levels-rain and irrigation, the interaction of the management with the rest of the continuous variables were also checked for. All variables from the final models have been displayed. The flower visitation was observed for 75 min (15 min on 5 plants on 5 branches/plant).

Negative binomial GLM	Estimate	Std. error	z value	Pr(>|z|)
(Intercept)	3.715	0.1462	25.420	<0.001
Management(R)	−1.765	0.2174	−8.119	<0.001
Size of forest in ha[a]	1.327	0.2834	4.680	<0.001
Relative humidity (%)	0.001	0.0116	0.107	0.914
Management(R): size of forest in ha[a]	−1.751	0.3728	−4.697	<0.001
Management(R): relative humidity (%)	−0.085	0.0198	−4.288	<0.001

[a] Variable natural log transformed.

were the most abundant, with *Apis florea* (0.6%) being an occasional visitor.

Distance of coffee plants from the nearest forest edge had no effect on pollinator abundance. There was an interaction between the size of the forest and the management used to initiate flowering. The size of the adjoining forest did, however, increase pollinator abundance, but only in irrigated agro-forests while an increase in relative humidity had a negative influence on pollinator abundance but only in rain-fed agro-forests. The pollinator abundance in irrigated agro-forests was higher than in rain-fed agro-forests (see Table 2 and Appendix C).

Pollination success, pollen tube abundance and fruit/seed set

Open-pollinated flowers received 50–800 (231 ± 135) pollen grains per stigma, while wind-pollinated flowers (insects excluded) received 27–257 (106 ± 45). The cross-pollination treatment consistently produced higher pollination success, pollen tube abundance, initial fruit set, final fruit set and seed set compared to open-pollinated flowers (Fig. 1). Although pollination success, pollen tube abundance, final fruit set and seed set were significantly higher in open-pollinated treatments than wind-pollinated treatments, there was no significant difference in initial fruit set between the two treatments: open-pollination 81 ± 9.5%; wind-pollination 78.5 ± 18.5% (Fig. 1B). Final fruit set by wind-pollination (excluding insects) was significantly lower than in the open-pollination treatments, which in turn was significantly lower than artificial cross-pollination treatment (Fig. 1C). There was no significant difference in pollination success, initial fruit set, final fruit set or seed set between self-pollination and no-pollination treatments (Fig. 1), where in both cases values were close to zero.

Most open-pollinated mature fruits developed into two-seeded cherries (66 ± 13%), the rest being one-seeded pea-berries. The proportion of fruits that developed as cherries in wind-pollinated flowers was 51 ± 15%, and seed set was 16 ± 9%, both significantly lower than in open-pollinated flowers (Wilcoxon signed rank test: $N = 108$, $P < 0.001$ for cherries; $N = 106$, $P < 0.001$ for seed set) (see Appendix E: Table 1 and Appendix D: Fig. 1).

Discussion

Coffee pollinator species composition

Compared to other recent studies in coffee agro-forestry systems there is an apparent paucity of pollinating species despite Kodagu being a richly forested landscape. Eighteen species accounted for all pollinator visits to coffee in Kodagu, of which three bee species accounted for around 95% of visits. Studies on *Coffea* reported 40 bee species in Costa Rica (Ricketts, 2004), 33 in Sulawesi, Indonesia (Klein, 2009), 29 in Ecuador (Veddeler, Klein, & Tscharntke 2006), 22 in Panama (Roubik, 2002a), 7, 5 and 17 in Mexico (Jha and Vandermeer, 2009; Philpott, Perfecto, & Vandermeer 2006; Vergara and Badano, 2009). All studies (where abundance data are provided) report social bees, principally species of *Apis* and the Meliponinae subfamily, as the most common floral visitors, often accounting for over 50% of all visits (e.g., Klein, Steffan-Dewenter, & Tscharntke 2003; Ricketts, 2004).

To explore whether the relative paucity of pollinators at Kodagu (compared to elsewhere) is a relatively recent feature of landscape change, or whether coffee pollinators have always been mainly limited to three social bees, we compared our data to that of Fletcher (1915) who surveyed coffee pollinators at the same locality 100 years ago. Fletcher's non-quantitative study is limited, being based on approximately 16 h of observation (as compared to our 250 h over three years), but nevertheless provides some indication of bee diversity at coffee flowers in 1911. Fletcher (1915) reported eight bee species visiting coffee, of which *A. cerana* was the most common. In our study *A. cerana* was less abundant than *A. dorsata* (Table 1), possibly due to Thai-sac brood virus which decimated *A. cerana* colonies in the early 1990s (97% decline in colony numbers from 1990 to 1994 in Karnataka;

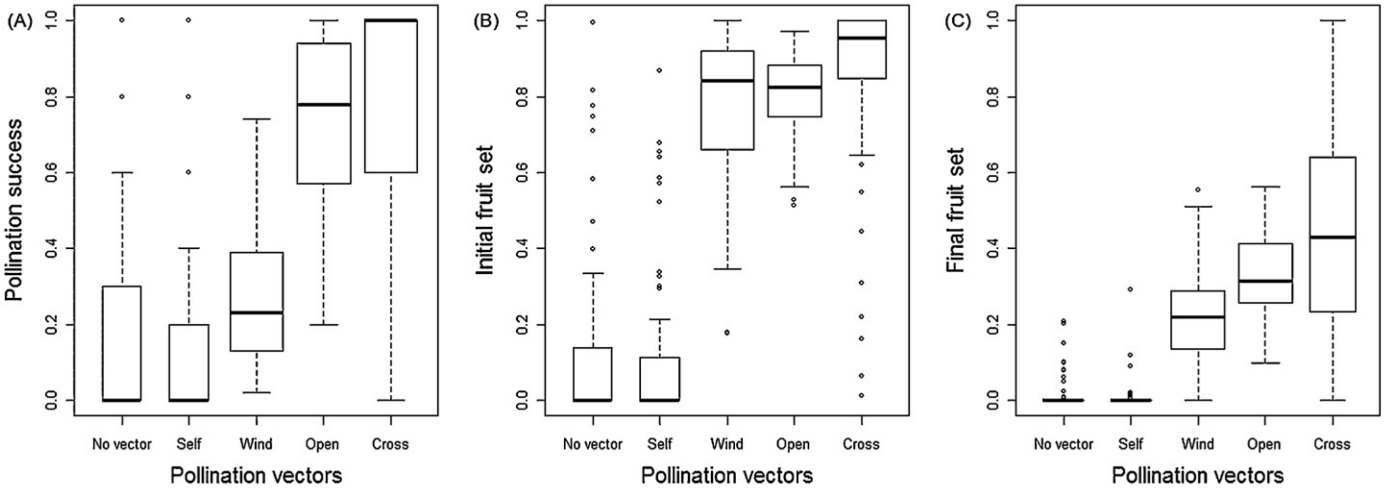

Fig. 1. Pollination success (proportion of flowers with two or more pollen tubes), initial fruit set (five weeks after flowering) and fruit production (final fruit set) for three pollination treatments with results of Wilcoxon's paired rank test. Median and interquartile ranges represented by bold line and box, whiskers represent 1.5 times the inter-quartile range, and outliers shown outside this range. (A) Pollination success: open- and wind- ($N = 108$, $P < 0.001$), cross- and wind- ($N = 103$, $P < 0.001$), cross- and open- ($N = 103$, $P = 0.144$) and no- and self-pollination ($N = 56$, $P = 0.792$). (B) Initial fruit set: open- and wind- ($N = 108$, $P = 0.495$), cross- and wind- ($N = 103$, $P < 0.001$), cross- and open- ($N = 103$, $P < 0.001$) no- and self-pollination ($N = 56$, $P = 0.368$). (C) Final fruit set: open- and wind- ($N = 106$, $P < 0.001$), cross- and wind- ($N = 81$, $P < 0.001$), cross- and open- ($N = 81$, $P = 0.013$) and no- and self-pollination ($N = 57$, $P = 0.433$) (See Appendix D: Fig. 1 for graphs of abundance of pollen tubes in pollinated flowers (PTA) and seed set).

Gatoria, Singh & Jhajj 1997), and from which populations are only now beginning to recover.

The other substantial difference is that *Amegilla* species, listed by Fletcher as the second most abundant visitor to coffee flowers, accounted for only 0.1% of all visits to coffee in our study. We frequently observed *Amegilla* bees foraging at flowers of the invasive alien *Lantana camara* in forest fragments adjoining coffee agro-forests, and the wide availability of this alternative foraging resource might explain the current low abundance of *Amegilla* on coffee plants. Changing resource distributions resulting from the spread of invasive species might, therefore, cause behavioural shifts among pollinators (see also Baskett, Emery, & Rudgers 2011; Ghazoul, 2004) though the impact on crop production remains uncertain.

Distance from and size of forest

Although several previous studies revealed a negative effect of distance from natural habitats on pollination service (Garibaldi et al., 2011; Ricketts et al., 2008), there are some exceptions where isolation did not affect certain species (see Greenleaf and Kremen, 2006) as in our own study. This is likely to be due to the foraging ranges of the main pollinators in the context of the Kodagu landscape. Despite extensive forest decline in recent decades, there remains a high density of forest fragments in Kodagu that are widely distributed across the landscape: more than 1200 forest fragments have been enumerated within 4106 km^2 (Garcia et al., 2010). Most coffee agro-forests are consequently located within 500 m of a forest fragment. The foraging range of *Apis dorsata* is up to several kilometres, and estimates for *A. cerana* and *Tetragonula* species range from 0.5 km to 2 km or more (Dyer and Seeley, 1991; Roubik, 1989), and so almost all coffee agro-forests lie within the foraging range of these bees even if we assume that bee colonies are restricted to forest fragments. *Tetragonula iridipennis* and *A. cerana* nest in coffee agro-forests as well as forest fragments, while *A. dorsata* colonies do appear to be largely restricted to forests due to a requirement for large trees (Paar, Oldroyd, & Kastberger 2000). Large foraging ranges and the availability of nesting sites within agro-forests coupled with a high forest fragment density, likely explain why distance effects on pollination at 500 m scale are not observed.

Size of the adjoining forest did have a significant positive effect on pollination, but only in agro-forests that flowered following irrigation. Irrigation stimulates flowering at a small (agro-forest) scale when few other agro-forests are flowering, and such agro-forests therefore represent a localised abundance of resources in an otherwise comparatively resource-poor landscape. The increase in pollinators at irrigated agro-forests likely reflects the attraction and concentration of pollinators at such sites. In contrast, rain-fed agro-forests flowered synchronously across the landscape following a rainfall event, which led to spread of pollinators across a larger area leading to a dilution of pollinators across the landscape (see Jha and Vandermeer, 2009; Veddeler et al., 2006). The very low pollinator abundance in rain-fed agro-forests likely confounds the detection of a forest size effect even if such an effect exists. This result emphasises that pollinator service benefits of natural habitat remnants can be mediated by management interventions (e.g. irrigation) and therefore ecosystem services need to be considered in the context of a variety of alternative management practices.

Contribution of bees to coffee pollination and production

The importance of insects in augmenting pollination success and seed set is evident when open-pollinated and wind-pollinated experiments are compared: open-pollinated flowers (combined wind and insect pollination) received more than twice the number of pollen grains as wind-pollinated flowers. One third of the flowers developed into fruits when open-pollinated (Fig. 1C), the contribution of bees being to increase fruit set by 50% over that attributed to wind pollination alone. Fruit set in Koadgu is higher than in Java, where final fruit set (as a proportion of flowers) was estimated at 20–25% in Java (Ferwerda, 1948), and 30% in Brazil (Silveira and Carvalho, 1996, as cited by DaMatta et al., 2007).

Pollen tube abundance was more than five times greater in open-pollinated compared to wind-pollinated flowers (Fig. 1). The importance of cross-pollination is also affirmed by the much lower pollination success, final fruit set and seed set in the self-pollination and no-pollination treatments (Fig. 1). The lower proportion of pea-berries in open-pollination treatments further emphasises the importance of insect pollinators in enhancing not only fruit production, but also coffee seed set.

The potential to further increase final fruit set by, for example, introducing managed bees, is likely to be limited as around 80% of flowers are already naturally pollinated (Fig. 1A). Increasing pollination beyond this depends on the increasingly diminishing returns on management interventions to secure pollination visits to an ever smaller fraction of unpollinated flowers. While 80% of flowers were pollinated, only 33% developed into fruit, suggesting some degree of resource limitation. Thus even if interventions to enhance pollinators do increase pollination success, the expected increase in fruit set might not necessarily be realised. Research on multiple scales, that is at both whole-plant and agro-forest scales, is required to ascertain the potential value of enhancing pollinators.

Initial fruit set (a measure of production used in some previous studies) was not a reliable measure of production: final fruit set (Fig. 1C) corresponds to pollination success (Fig. 1A) and pollen-tube abundance (see Appendix D: Fig. 1A), but initial fruit set reflected neither pollination nor final fruit set (see Appendix E: Table 1). Initial fruit set was high for wind-, open- and cross-pollination treatments, and also higher than one would have expected for no- and self-pollination treatments (especially since it is a self-incompatible species) (Fig. 1B), but this may simply reflect the commonly observed phenomenon of swelling of non-pollinated or self-pollinated ovules that are aborted at later stages of fruit development (Ghazoul and Satake, 2009; Seavey and Bawa, 1986). Coffee fruit set values derived a few weeks after pollination have, therefore, questionable value as a measure of final harvest.

In conclusion, we could confirm that bees are important in augmenting the final fruit set and seed set of coffee. Several earlier studies reached a similar conclusion, but many were based on early fruit development which, at least for *C. canephora* in Kodagu, is an unreliable measure of mature fruit set. The current dependency of coffee pollination on *A. dorsata* (58%) is a matter of some concern as this species nests mainly on large trees to which it has high nest-site fidelity; (Paar et al., 2000) in forest remnants that are increasingly threatened by degradation and conversion. The protection of such trees is likely to prove crucial for the continued profitable production of coffee, even though the large foraging range of *A. dorsata* might allow this species to persist in more intensively managed landscapes. The point at which landscape intensification and forest loss begins to affect pollination services has not yet been reached in Kodagu, making it challenging to advocate forest preservation to farmers in the region, especially when irrigation can be used to enhance pollination. Nevertheless, the positive relationship between forest size and pollination in irrigated agro-forests provides one pathway by which the dual objectives of conservation and crop production might be encouraged.

In view of concern over the potential decline of *A. dorsata*, coffee pollination services might be enhanced by increasing the number of domesticated *Apis cerana* colonies within coffee agro-forests and by protecting the existing nests of *Tetragonula iridipennis*. While the efficacy of this strategy might be marginal in the short term (as the pollination service is already high; Fig. 1A), it might provide farmers with protection from a sudden or gradual loss of wild pollinators (notably *A. dorsata*) through loss of nesting trees. Over-dependency on a single species, however, expose farmers to vulnerability of pollinator loss through diseases.

Acknowledgements

The College of Forestry, Ponnampet, provided research assistant support throughout the study. This work would have been impossible without the co-operation of coffee planters, and the help of field assistants, specifically Chengappa, S. K, Raghunath Gowda, and Shruthi, J. We are very grateful to A. M. Klein and the reviewers for their helpful comments. This study was funded by the North-South Centre, ETH Zürich and the Professorship of Ecosystem Management, ETH Zürich.

Appendix A–E:. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.baae.2012.03.007.

References

Baskett, C. A., Emery, S. M., & Rudgers, J. A. (2011). Pollinator visits to threatened species are restored following invasive plant removal. *International Journal of Plant Sciences*, *172*, 411–422.

Bidie, G. (1869). On the effects of forest destruction in Coorg. In *Proceedings of the Royal Geographical Society of London, 13* (pp. 74–83).

Coffee Board India. (2010). *Bangalore: Government of India*. Ministry of Commerce & Industry.

DaMatta, F. M., Ronchi, C. P., Maestri, M., & Barros, R. S. (2007). Ecophysiology of coffee growth and production. *Brazilian Journal of Plant Physiology*, *19*, 485–510.

Dyer, F. C., & Seeley, T. D. (1991). Dance dialects and foraging range in three Asian honey bee species. *Behavioral Ecology and Sociobiology*, *28*, 227–233.

FAOSTAT. (2010). *FAO Statistics Division*.

Ferwerda, F. P. (1948). Coffee breeding in Java. *Journal of Economic Botany*, *2*, 258–272.

Fletcher, T. B. (1915). Bees and the fertilisation of coffee. *Madras Bulletin*, *4*, 38.

Free, J. B. (1993). *Insect pollination of crops*. London: Academic-Press.

Garcia, C. A., Bhagwat, S. A., Ghazoul, J., Nath, C. D., Nanaya, K. M., Kushalappa, C. G., et al. (2010). Biodiversity conservation in agricultural landscapes: Challenges and opportunities of coffee agroforests in the Western Ghats, India. *Conservation Biology*, *24*, 479–488.

Garibaldi, L. A., Steffan-Dewenter, I., Kremen, C., Morales, J. M., Bommarco, R., Cunningham, S. A., et al. (2011). Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology Letters*, *14*, 1062–1072.

Ghazoul, J. (2004). Alien abduction: Disruption of native plant-pollinator interactions by invasive species. *Biotropica*, *36*, 156–164.

Ghazoul, J. (2005). Buzziness as usual? Questioning the global pollination crisis. *Trends in Ecology and Evolution*, *20*, 367–373.

Ghazoul, J., & Koh, L. P. (2010). Food security not (yet) threatened by declining pollination. *Frontiers in Ecology and the Environment*, *8*, 9–10.

Ghazoul, J., & Satake, A. (2009). Nonviable seed set enhances plant fitness: The sacrificial sibling hypothesis. *Ecology*, *90*, 369–377.

Greenleaf, S. S., & Kremen, C. (2006). Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. *Biological Conservation*, *133*, 81–87.

International Coffee Organization (ICO). (2010). *Mission: Importance of coffee*. London: International Coffee Organization (ICO).

International Coffee Organization (ICO). (2011). *Annual Review. 2009/10 [ICO Document]*. London: International Coffee Organization (ICO).

Jha, S., & Vandermeer, J. H. (2009). Contrasting bee foraging in response to resource scale and local habitat management. *Oikos*, *118*, 1174–1180.

Kearns, C. A., Inouye, D. W., & Waser, N. M. (1998). Endangered mutualisms: The conservation of plant–pollinator interactions. *Annual Review of Ecology and Systematics*, *29*, 83–112.

Kevan, P. G., & Wojcik, V. A. (2007). Pollinator services. In D. I. Jarvis, C. Padoch, & H. D. Cooper (Eds.), *Managing biodiversity in agricultural ecosystems* (pp. 200–223). New York: Columbia University Press.

Klein, A. M. (2009). Nearby rainforest promotes coffee pollination by increasing spatio-temporal stability in bee species richness. *Forest Ecology and Management*, *258*, 1838–1845.

Klein, A. M., Steffan-Dewenter, I., & Tscharntke, T. (2003). Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *Journal of Applied Ecology*, *40*, 837–845.

Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., et al. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 303–313.

Le Pelley, R. H. (1973). Coffee insects. *Annual Review of Entomology*, *18*, 121–142.

Millennium Ecosystem Assessment (MEA). (2005). *Ecosystems and human well-being: Synthesis*. DC: Island Press.

Paar, J., Oldroyd, B. P., & Kastberger, G. (2000). Entomology – Giant honeybees return to their nest sites. *Nature*, *406*, 475-475.

Philpott, S. M., Perfecto, I., & Vandermeer, J. (2006). Effects of management intensity and season on arboreal ant diversity and abundance in coffee agroecosystems. *Biodiversity and Conservation*, *15*, 139–155.

Quinn, G. P., & Keough, M. J. (2004). *Experimental design and data analysis for biologists* (3rd ed.). Cambridge: Cambridge University press.

R Development Core Team. (2010). *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ricketts, T. H. (2004). Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology*, *18*, 1262–1271.

Ricketts, T. H., Daily, G. C., Ehrlich, P. R., & Michener, C. D. (2004). Economic value of tropical forest to coffee production. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 12579–12582.

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., et al. (2008). Landscape effects on crop pollination services: Are there general patterns? *Ecology Letters*, *11*, 499–515.

Roubik, D. W. (1989). *Ecology and natural history of tropical bees*. New York: Cambridge University Press.

Roubik, D. W. (2002a). Feral African bees augment neotropical coffee yield. In P. G. Kevan, & V. L. Imperatriz-Fonseca (Eds.), *Pollinating bees – The conservation link between* *agriculture and nature* (pp. 255–266). Brasilia: Ministry of Environment.

Roubik, D. W. (2002b). Tropical agriculture – The value of bees to the coffee harvest. *Nature*, *417*, 708.

Seavey, S., & Bawa, K. (1986). Late-acting self-incompatibility in angiosperms. *The Botanical Review*, *52*, 195–219.

Veddeler, D., Klein, A.-M., & Tscharntke, T. (2006). Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos*, *112*, 594–601.

Vergara, C. H., & Badano, E. I. (2009). Pollinator diversity increases fruit production in Mexican coffee plantations: The importance of rustic management systems. *Agriculture Ecosystems & Environment*, *129*, 117–123.