Changes in partner traits drive variation in plant–nectar robber interactions across habitats

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Abstract

The frequency and outcome of biotic interactions commonly vary with environmental conditions, even absent changes to community composition. Yet the drivers of such environmentally-mediated change in biotic interactions are poorly understood, limiting our ability to predict how environmental change will impact communities. Studying nectar robbery by stingless bees of *Odontonema cuspidatum* (Acanthaceae) in a coffee agroecosystem, we documented a temporally consistent difference in nectar robbing intensity between anthropogenic and seminatural habitats. Plants growing in coffee fields (anthropogenic habitat) experienced significantly more nectar robbery than plants growing in forest fragments (seminatural habitat). Using a combination of field surveys and manipulative experiments, we found that nectar robbery significantly reduces fruit set in *O. cuspidatum*, and that the difference in nectar robbing intensity between habitats is due primarily to changes in robber behavior. Nectar robbers robbed plants in coffee more because of differences both in focal plant traits and floral community composition. Nectar robber density was higher in coffee fields, but changes in abundance had a weak effect on nectar robbery. These results suggest that trait changes stemming from environmental change may often drive changes in biotic interactions, despite being less well-studied than the effects of environmental change on organism density.

Keywords: nectar robbing, stingless bees, Odontonema cuspidatum, *anthropogenic environmental change, indirect interactions, plant-animal interactions*

Introduction

Understanding the effects of anthropogenic environmental change on biotic communities and ecosystem function is a key challenge for ecologists. Anthropogenic environmental change is resulting in striking biodiversity loss at a global scale (Matzke et al. 2011, Dirzo et al. 2014), with consequences for ecosystem function and ecosystem service provision at smaller scales (Hooper et al. 2012). But while the bulk of research on the effects of anthropogenic environmental change has focused on changes to community composition (Tylianakis et al. 2008, Valiente-Banuet et al. 2015), there is growing appreciation that the structure of interaction networks within communities can be altered by environmental change even when community composition is unaffected (Tylianakis et al. 2008, Poisot et al. 2015, 2017, Valiente-Banuet et al. 2015). Such interaction 'rewiring' may be mediated by changes to either the density (Figure 1, Pathways A-B) or traits (Figure 1, Pathways C-D) of one or more interacting species. These effects, moreover, may derive directly from altered environmental conditions (Figure 1, Pathways A, D), or be mediated by environmentally-driven changes to community context – i.e. changes to other species' density or traits (Figure 1, Pathways B-C).

Given the multiple pathways by which environmental change can impact interactions, it is perhaps not surprising that both theoretical (Valiente-Banuet et al. 2015) and empirical (Tylianakis et al. 2008, Valiente-Banuet et al. 2015) work suggest that changes in interaction structure are likely to occur more commonly, and more quickly, than species loss in response to environmental change. Yet, even where changes to the structure of interactions have been documented, we frequently lack understanding of the underlying drivers of these changes (Tylianakis et al. 2008). This limits our ability to predict how future environmental change will impact communities and ecosystems.

Nectar robbery (NR), in which a flower visitor extracts nectar from a flower via an opening other than the corolla mouth, is one interaction type that is likely to be strongly impacted by environmental context (Morris 1996, Irwin and Maloof 2002, Cuevas and Rosas-Guerrero 2016), since in many cases it is a facultative behavior (Morris 1996, Irwin et al. 2010, Richardson and Bronstein 2012). Nectar robbery is a widespread behavior in nectar-feeding animals (Irwin et al. 2010), but most robbers also act as legitimate pollinators of other species, or even of other individuals of the same species (Morris 1996, Irwin et al. 2010, Richardson and Bronstein 2012). The effects of NR on reproductive success of the robbed plant are variable, with negative, neutral, and positive effects reported (Maloof and Inouye 2000, Burkle et al. 2007).

Nectar-robbing intensity (NRI) – measured as the proportion of flowers that experience robbery – commonly varies both spatially (Morris 1996, Irwin and Maloof 2002, Cuevas and Rosas-Guerrero 2016) and temporally (Navarro 2000, Irwin and Maloof 2002, Cuevas and Rosas-Guerrero 2016). Multiple drivers of such variability have been postulated. These include both direct responses of robber or plant to environmental conditions and responses mediated by the broader community. Direct responses may include variation in the density of robbers (Navarro 2000, Irwin and Maloof 2002) or the density, flower number, or nectar quality or quantity of the focal plant (Krupnick et al. 1999) due to environmental conditions. Community-mediated responses may include altered foraging behavior depending on the availability of alternative floral resources (Irwin and Maloof 2002, Irwin et al. 2010) or density of other flower visitors (Roubik 1982). Yet to date, there has been little work documenting which of these mechanisms operate in specific instances to generate variation in NRI. Without a mechanistic

understanding of the ecological drivers of NR, it is difficult to predict the circumstances under which NR will occur and how it will be altered by environmental change.

In this study, we first assessed the intensity of NR by stingless bees of the shrub *Odontonema cuspidatum* (Nees) Kuntze (Acanthaceae) between a semi-natural habitat (forest fragments) and an anthropogenic one (coffee farm). We then evaluated the role of potential drivers of NR (Figure 1) in generating observed habitat-based spatial heterogeneity in the extent of NR experienced by *O. cuspidatum*. We also evaluated whether NR influenced either the likelihood of individual flowers setting fruit or plant-level reproductive output across both habitats. Our aim was to understand the extent to which variation in the intensity and outcome of NR across habitats is driven by 1) changes in population density of robber or plant (Figure 1 Pathways A-B), 2) direct effects of environmental conditions (i.e. light availability) on one or more traits of either partner (Figure 1 Pathway D), or 3) indirect effects on robber or plant traits via changes in community context (Figure 1 Pathway C). We interpret the term 'trait' to include both physical characteristics and behaviors, consistent with the definition used in the literature on trait-mediated indirect effects (e.g. Werner and Peacor 2003, Schmitz et al. 2004, Utsumi et al. 2010). Specifically, the traits we focus on are, for the plant, flower number and floral nectar characteristics and, for the nectar robbers, foraging behavior.

Methods

Study system

This research was conducted at Finca Irlanda, a shaded, organic coffee farm, approximately 300ha in size, located in the Soconusco region of southeastern Chiapas, Mexico. The farm ranges from 900-1150 masl; above ~1000 masl it is comprised primarily of *Coffea*

arabica plantations, with three small (<30 ha) forest fragments embedded within the farm. Forest fragments are characterized by a higher density and diversity of canopy trees in comparison to the coffee fields. As a result of the higher density of canopy trees in forest fragments, the amount of light reaching the ground is generally lower in the forest than the coffee farm (see Results). This difference in canopy cover likely represents the key environmental difference between these habitats, though soil fertility may also differ, given the high abundance of nitrogen-fixing *Inga* trees in the coffee farm and the additional nutrients from compost application to coffee plants.

Within this landscape, *O. cuspidatum*, a perennial shrub native to the region, grows both in areas under coffee cultivation and in forest fragments. In the study area, *O. cuspidatum* blooms primarily from June to August, in the early part of the rainy season. Slender red flowers, 2-2.5cm long, are borne on indeterminate branching racemes; individual plants produce from 1 to approx. 30 racemes, and each raceme holds from approx. 10 to hundreds of flowers (G. Fitch unpublished data). *Odontonema cuspidatum* is self-fertile but requires animal pollination for fertilization, due to spatial separation of anthers and stigma (G. Fitch unpublished data). Hummingbirds, particularly the blue-tailed hummingbird (*Amazilia cyanura*), are the most frequent legitimate floral visitors (G. Fitch unpublished data); this, together with the flower's morphology, suggests that hummingbirds are the primary pollinators of *O. cuspidatum*. The flowers also attract a wide range of nectar-feeding insects, most of which engage exclusively in nectar robbery, extracting nectar from animal-made holes in the base of the corolla tube. Primary nectar robbers – i.e. those that make the hole themselves, hereafter 'PNR' – are primarily two species of stingless bee in the genus *Trigona* (*T. fulviventris* and *T. nigerrrima*; Hymenoptera: Apidae: Meliponini). Other bees, ants, and wasps, as well as several species of Lepidoptera, secondarily rob by using previously-made holes, but were not observed to make their own holes.

Fertilized flowers produce explosively dehiscent capsules (Daniel 1995, Meyer and Lavergne 2004).

Data collection

Spatiotemporal patterns of nectar robbing intensity

Within a 25ha area that included both coffee fields and forest fragments, we haphazardly selected 109 individual *O. cuspidatum* for inclusion in the study. This represents ~50% of all individuals found in the survey area. Because of the spatial arrangement of the forest fragments, each plant was within 500m of a habitat edge. All plants were individually labeled and followed through both years of the study, except that thirty-three plants surveyed in 2017 either died or did not flower in 2018, and an additional 15 plants that flowered in 2018 but not 2017 were monitored in 2018 only. We recorded the GPS coordinates of each plant. Distance between plants included in the study ranged from 10-2200m.

In 2017-2018, plants were surveyed for NR weekly for the duration of the flowering period. At each survey, all inflorescences with open flowers were surveyed. Flowers >1.5cm long were checked for evidence of NR (characteristic hole at corolla base), and the number of robbed and unrobbed flowers on each inflorescence was recorded. The 1.5cm cutoff was chosen because flowers of this length were generally within 2 days of opening, and prior to this stage flowers experienced minimal NR (G. Fitch unpublished data). Results for open and unopened flowers were tabulated separately. In 2018 only, each monitored inflorescence was individually tagged.

Putative drivers of nectar robbing intensity

Odontonema cuspidatum *density*

To determine the density of *O. cuspidatum* in each habitat, in June 2018 we counted the number of *O. cuspidatum* inflorescences, at any stage of development, within a 20m radius of each of our target plants.

Odontonema cuspidatum *floral traits*

Each time we surveyed plants for NR, we recorded the total number of flowers present on the plant, for our measure of per-observation flower number. At the end of the flowering period, the season-long total number of flowers produced was determined by counting all mature fruits and persistent ovaries (i.e. flowers that had not set fruit) on each plant.

On a subset of monitored plants (49 in 2017, 19 in 2018 with 7 included in both years), we assessed nectar volume and sugar content. Because standing nectar crop was minimal in unbagged flowers, to measure nectar content we covered 2 inflorescences/plant with a bag made of 0.5cm tulle mesh to exclude floral visitors. During the flowering period, we checked bagged inflorescences for open flowers 2x/week. To assess nectar volume, we removed all nectar from a flower using a 75μL microcapillary tube (Drummond Scientific, Broomall PA), then measured the height of the nectar in the tube using digital calipers (Thomas Scientific, Swedesboro NJ) and converted this measure to nectar volume. We then used a pocket refractometer (Eclipse 45-81, Bellingham & Stanley, Tunbridge Wells UK) to assess the nectar sugar content of each sample. From each plant, we assessed nectar characteristics of at least 4 flowers (range: 4-32 flowers, mean ± SE: 12.9 ± 0.3 flowers).

Primary nectar robber (PNR) density and foraging behavior

To determine PNR population density in each habitat, in 2018 we conducted surveys for PNR nests. Surveys were conducted along 30m x 10m transects oriented in one of the cardinal directions and centered on a target *O. cuspidatum* plant. We conducted surveys along 32 transects, 16 in coffee and 16 in forest. Collectively, these transects encompassed 44 monitored *O. cuspidatum* plants. The PNR species nest either in trees or in the ground at the base of trees (Fierro et al. 2012), so our nest search focused on trees ≥15cm dbh (Hubbell and Johnson 1977). On all such trees within each transect, we scanned the trunk and major limbs from 0-20m above the ground for evidence of nesting. Surveys were conducted between 0700-1100, when nest activity was highest. Both species of PNR of *O. cuspidatum* in the study area have prominent nests with high activity levels, so we are reasonably confident that we located all nests of these species within our transects.

To determine PNR forager density, in addition to nest density, in 2017 and 2018 we surveyed local PNR abundance for each focal *O. cuspidatum* plant. Surveys occurred during peak bee activity (0700-1100h) on sunny days. Each survey consisted of two 10min periods. The first focused on flowers from 2-5 inflorescences of the focal *O. cuspidatum*, and the second focused on flowers within 10m of the focal plant, with a 10 min break in between surveys. Prior to beginning the survey, we counted the number of open flowers on focal inflorescences, and located all bee-attractive flowering plants in the 10m neighborhood. During surveys, all insect flower visitors were caught using resealable plastic bags and held, one insect per bag, for the duration of the survey. At the end of each 10min survey, captured insects were identified to species or morphospecies and then released. Insects were classified as primary nectar robbers, secondary nectar robbers, legitimate visitors, or unknown, based on prior observation of insect visitation to *O. cuspidatum*. These observations provided us with several measures of PNR

forager abundance: total site-level abundance, abundance on focal *O. cuspidatum*, per-flower abundance on focal *O. cuspidatum*, abundance on non-focal flowers, and the proportional abundance on target plants (number of individuals caught on target plants divided by total number of individuals caught during that survey). We interpret the latter metrics as a measure of PNR foraging behavior, while we consider the others to be measures of PNR foraging behavior.

In 2018, we assessed within-plant PNR foraging behavior on 23 monitored plants, 13 in coffee and 10 in forest. On each plant, we monitored 5 foraging bouts by individual PNRs at focal *O. cuspidatum* plants, recording the species of PNR, the length of time spent on the inflorescence, and the number of flowers robbed. We then calculated the proportion of potentially robbable flowers that had been robbed.

Environmental conditions – Canopy cover

We measured canopy cover directly above the crown of each focal plant (N=109) in June 2017 (June 2018 for plants added in 2018) using CanopyApp 1.0.3 (University of New Hampshire, Durham, NH USA).

Community context – Floral resource availability

In 2017, floral surveys were conducted to determine floral resource availability in the neighborhood of 95 focal plants. For each plant, four 10m x 2m transects were established, each one beginning at the focal plant and extending 10m in one of the cardinal directions. Along each transect, all blooms were counted and identified to species or genus. Surveys occurred once for each focal plant, near the peak bloom for each focal plant.

Effect of nectar robbery on reproduction

For monitored plants that had >20 flowers and had both robbed and unrobbed flowers (N=44), we evaluated the difference in probability of producing fruit and in seed set between robbed and unrobbed flowers. Flowers were marked with either red (robbed) or blue (unrobbed) nail polish on the pedicel. Robbed flowers were marked either on the day before they were to open or soon after opening, while unrobbed flowers were marked only as they were beginning to senesce, to ensure that robbing occurred before pollination and that flowers marked as unrobbed were not subsequently robbed. The number of marked flowers of each type (robbed and unrobbed) varied across plants depending on the availability of robbed and unrobbed flowers (robbed flowers: range = 1-26 flowers, mean ± SE = 4.7 ± 0.7 flowers; unrobbed flowers: range = 1-16 flowers, mean ± SE = 2.9 ± 0.4 flowers).

We assessed fruit set by counting the number of fruit and number of persistent ovaries on mature inflorescences. The fate of each marked robbed or unrobbed flowers was recorded. Inflorescences that had been damaged by insect herbivores [primarily *Chlysone* sp. (Nymphalidae: Lepidoptera) larvae] were excluded from further analyses. To measure seed set, up to 5 fruits (in 2017) or all undamaged fruits (in 2018) were collected from each inflorescence. These fruits, segregated by plant, were placed in small bags made of 0.5 cm mesh fabric and left in a drying oven at 50 deg C until all had dehisced (approximately 24h), at which time seeds were counted.

Data analysis

All analyses were conducted in R v.3.5.1 (R Core Team 2018). All models were checked for conformity to assumptions.

Influence of habitat on nectar robbing intensity

We evaluated the influence of habitat on NRI using GLMMs with habitat and year as fixed effects and plant and date as random effects. We separately considered three components of NRI: number of open flowers robbed, number of unopened flowers robbed, and total number of flowers robbed. In all cases, we included log(total number of flowers of that class) as an offset, and used a Poisson error distribution with log-link function. To check for spatial autocorrelation (SA) in NRI, we fit a parallel set of GLMs, and calculated Moran's I for the residuals of each of these models using package `ape` (Paradis and Schliep 2018). We found no evidence for SA in these data ($p > 0.1$ in all cases).

Influence of habitat on putative drivers of nectar robbing intensity

We tested for a significant effect of habitat on the following putative drivers of NRI focal plant flower number, focal plant nectar volume and nectar sugar content, canopy cover, neighborhood floral density, PNR density at focal plants and in the 10m neighborhood, and PNR nest density. Focal plant flower number (assessed as the total number of flowers produced over the flowering period) and PNR nest density were fitted with a GLM with Poisson distribution and log-link function. Focal plant nectar volume and nectar sugar content, as well as canopy cover, were fitted using linear models. For other putative drivers, we had multiple measures per plant, and therefore used GLMMs with plant as a random effect and a Poisson distribution with log-link function. We calculated Moran's I for the residuals of all models to check for SA. We detected significant SA for three putative drivers: focal plant flower number, canopy cover, and neighborhood PNR density. For these drivers, we then used the `spdep` package to calculate

Moran eigenvectors to account for SA (Bivand and Wong 2018). These eigenvectors were included as additional predictors in an updated model. Where significant SA was found, the estimates we provide for the effect of habitat on the relevant driver come from the model including Moran eigenvectors.

We also tested whether canopy cover (and therefore light availability) could account for differences in floral traits (flower number and flower nectar volume) between habitats. For flower number, we used a GLM with negative-binomial distribution, and for nectar volume we used a linear mixed-effects model with plant as a random effect.

Influence of putative drivers on nectar robbing intensity

The number of plant-year combinations for which we had observations of a particular putative driver varied substantially across drivers. Therefore, we initially assessed the relationship between NRI and each putative driver (that is, all the variables listed in Table 1) separately, then included those drivers that had an effect on NRI at $p \leq 0.2$ in a combined model (see below). All models used the number of robbed flowers as the response variable, and included log(total number of flowers surveyed) as an offset; error distributions were either Poisson or negative binomial, depending on whether the data were overdispersed, and used a log-link function.

We initially evaluated the effects of several floral neighborhood metrics: floral richness, total flower density, and *O. cuspidatum* flower density. These metrics were strongly correlated and total flower density was the best predictor of NRI, so for all subsequent analyses we used total floral density to assess effects of floral neighborhood. The relationship between flower visitation and both flower number and floral neighborhood is often nonlinear and unimodal

(Rathcke 1983, Ghazoul 2006). Beyond unimodality, we did not have *a priori* expectations for the relationship between these drivers and NR. Therefore, to assess their effect on NRI, we used general additive models (GAMs). To test for nonlinearity, we compared model versions with linear and smoothed relationships using AICc. In both cases, the nonlinear model indicated a unimodal relationship and improved fit over the linear model (for flower number, ΔAICc = 19; for floral neighborhood ΔAICc = 25). For all other drivers we assumed a linear response of NR; we used a GLM to evaluate the effect of canopy cover and floral neighborhood and GLMMs to evaluate the effect of all other drivers (since for these drivers we had multiple observations per plant).

These single-predictor models indicated that at the $p \leq 0.2$ level, the putative drivers that affected NRI were focal plant flower number, neighborhood floral density, and per-flower robber density at focal plants. To test whether these putative drivers had independent effects, we used a GAM with all three predictors; smooth terms were applied to flower number and floral neighborhood, while robber density was constrained to a linear function.

Based on GAM results for the relationship between NRI and neighborhood floral density, we classified neighborhood floral densities as low (less than the lower bound of the 95% confidence interval for the predicted maximum of the function relating NRI to floral density), moderate (within the 95% confidence interval), or high (higher than the upper bound of the 95% confidence interval).

Effects of nectar robbery and habitat on Odontonema cuspidatum *reproduction*

To determine the effect of NR on *O. cuspidatum* reproduction, we compared differences in fruit set between robbed and unrobbed flowers using a binomial GLMM with plant as a

random effect. We additionally tested for an effect of habitat on fruit set, and of differences in the effect of NR on fruit set between habitats, by including habitat and a robbed status × habitat interaction term as fixed effects. To control for differences across plants in their ability to produce fruit, independent of the effects of NR, we only included plants for which we had data on the fate of both robbed and unrobbed flowers in a single year (N = 44).

To test the effect of NRI and habitat on measures of reproductive output, we examined fruit set, seed set, and seeds produced per plant. To model fruit set, we used a Poisson generalized linear mixed-effects model (GLMM) with number of fruit as the response variable, offset by log(total number of flowers produced), as counted at time of fruit harvest. Fixed effects were habitat, NRI, and a habitat × NRI interaction term, and plant was included as a random effect. Seed set and seeds per plant were assessed only In 2018, so for those metrics we used a negative binomial generalized linear model (GLM) with number of seeds as the response; the model for seed set additionally included log(total number of flowers) as an offset. Because there was no effect of the habitat × NRI interaction term in the model for fruit set, this predictor was omitted from our models of seed set and seeds per plant.

Results

Spatiotemporal patterns in nectar robbing intensity (NRI)

Plants growing in forest fragments were robbed significantly less than those growing in coffee, consistent across years (proportion of flowers robbed in forest: 0.35 ± 0.01; in coffee: 0.35 ± 0.01; $z = -2.26$, $p = 0.02$). This was due primarily to flowers being robbed earlier in their development when growing in coffee: incidence of nectar robbery (NR) of unopened flowers

was significantly higher in coffee (forest: 0.15 ± 0.01; coffee: 0.27 ± 0.02; $z = -2.8$, $p = 0.005$), while NR of open flowers trended higher in coffee but did not differ significantly between habitats (forest: 0.58 ± 0.02; coffee: 0.68 ± 0.02; $z = -1.13$, $p = 0.26$). Across both habitats, NRI was significantly higher in 2018 than 2017 (2017: 0.32 ± 0.02; 2018: 0.46 ± 0.01; $z = 8.70$, $p < 0.001$) and, in both years, increased over the survey period (ß = 0.19±0.02, $z = 10.49$, $p < 0.001$).

Relationships between putative drivers and habitat

Partner densities

Density of *O. cuspidatum* did not differ between habitats (Table 1). Density of primary nectar robbers (PNRs), on the other hand, showed a complex response to habitat. There was no difference in nest density of the two PNR species between habitats (Table 1). Nevertheless, PNR density at monitored *O. cuspidatum* plants was higher in coffee than in forest (Table 1). However, PNR density at all other flowers within 10m of focal plants (most of which received legitimate visits from PNRs) was equivalent across habitats (Table 1). Because of the high density of PNRs at target *O. cuspidatum*, total PNR density was still significantly higher in coffee than in forest (Table 1).

Partner traits

Both *O. cuspidatum* flower number and flower nectar volume were significantly lower in forest (Table 1). Nectar sweetness was slightly lower for plants growing in coffee, but this difference was not significant (Table 1). These changes in floral traits stem largely from reduced light availability in forest fragments: canopy cover was significantly higher in the forest (Table

1), and, across habitats, had a negative effect on both total flower number (ß = –0.31±0.08, z = –3.72, $p < 0.001$) and nectar volume (ß = –0.19±0.08, t = –2.82, p = 0.03).

Primary nectar robber foraging behavior at *O. cuspidatum* plants differed between habitats. On a per-visit basis, PNRs robbed a significantly higher proportion of flowers on plants growing in coffee (Table 1), despite those plants have more flowers. Moreover, since PNR density on target *O. cuspidatum* was higher in coffee, while PNR density in the floral neighborhood was equivalent between habitats, the relative density of PNRs on target *O. cuspidatum* was higher in coffee, though this difference was only marginally significant (Table 1).

Relationships between putative drivers and nectar robbery

NR responded nonlinearly to both *O. cuspidatum* flower number and neighborhood floral density (Figure 2, Table 2). While the precise shape of this nonlinear response differed between flower number and neighborhood floral density, in both cases NRI initially increased with floral availability, then decreased as floral availability increased further (Figure 2). For *O. cuspidatum* flower number, the position of the predicted maximum was 645 blooms [95% confidence interval (CI): 467-850 blooms; Figure 2A]. For neighborhood floral density, the predicted maximum was 1.8 blooms/m^2 (95% CI: 1.1–1.9 blooms/m^2; Figure 2B). Significantly, the neighborhoods of most forest-growing *O. cuspidatum* had low floral densities (i.e. below the lower bound of the 95% CI for the predicted maximum: 38 plants below, 11 within, 3 above, with a further 7 of the 11 plants within the 95% CI falling outside the 90% CI). The floral neighborhoods of coffee-growing plants varied more in density (10 plants below the CI; 16

within; 23 above; Figure 2B), with a substantially higher percentage of coffee-growing plants found in floral neighborhoods within the 95% CI for peak NRI (33% versus 22%).

Beyond *O. cuspidatum* flower number and neighborhood floral density, the only other putative driver to significantly affect NRI was per-flower PNR density on target plants, which was higher for coffee-growing plants (Table 1). When these three significant drivers were combined into a single model, each retained their significance level and showed a qualitatively similar effect on NRI as when they were considered independently (Table 2). Moreover, when habitat was added as a linear predictor to this model, it did not have a significant effect on NRI and did not improve model fit (Table 2).

Effects of nectar robbery and habitat on Odontonema cuspidatum *reproduction*

Nectar robbery significantly reduced the probability of a flower setting fruit, from 0.32±0.04 (mean±SE) for unrobbed flowers to 0.18±0.03 for robbed flowers, representing a 43% decrease in fruit set ($z = 3.41$, $p < 0.001$). The effect of NR on probability of setting fruit was equivalent between habitats (robbed status × habitat interaction: ß = 0.16±0.60, $z = 0.27$, $p = 0.8$), as was the overall probability of setting fruit (coffee: 0.23±0.03; forest: 0.23±0.04; $z = -0.10$, $p = 0.9$).

Consistent with the negative effect of NR on the probability of individual flowers setting fruit, we found a significant negative relationship between NRI and fruit set at the plant level (Table 3). This effect was consistent across habitats, and fruit set did not differ between habitats (Table 3). By contrast, neither seed set nor seeds per plant were influenced by NRI. Seed set was higher in forest-growing plants, though due to the smaller number of flowers produced by forest-growing plants, seeds per plant did not differ between habitats (Table 3).

Discussion

Changes in the frequency or outcome of biotic interactions across ecosystems can be explained by 1) differences in the density of the interacting species and/or 2) differences in the traits of the interacting species, either stemming directly from abiotic conditions or mediated by changes in the broader community. In this study, we document a temporally consistent pattern of habitat-based heterogeneity in nectar robbing intensity (NRI) of the plant *O. cuspidatum*. Our results show that higher levels of NR in coffee-growing *O. cuspidatum* are primarily the result of partner trait differences, with a lesser contribution of density differences. From the plant's perspective, these trait differences are driven directly by differing environmental conditions between habitats, while on the robber's side, trait differences are primarily the result of altered biotic community context. Specifically, we found that, together, 1) a nonlinear response of PNRs to the availability of alternative floral resources, 2) greater flower production for *O. cuspidatum* growing in coffee, and 3) higher density of foraging PNRs and/or greater preference for *O. cuspidatum* by PNRs in the coffee farm than in adjacent forest fragments can fully explain differences in NRI between habitats.

Of these three factors, field survey data indicate that floral neighborhood composition has the greatest influence on NRI. Response to floral neighborhood was unimodal, with NRI initially increasing with increasing floral density – presumably because areas of very low floral density are avoided by bees – then peaking at ~0.9 blooms/m^2. Further increases in floral density likely draw potential PNRs away from focal plants, thereby reducing NRI. Most (65%) of the plants whose neighborhood floral density fell within the 95% CI for predicted maximal NRI grew in

coffee. Thus, disparities in floral density between habitats contribute to the on-average higher NRI experienced by coffee-growing plants, via effects on PNR foraging behavior.

Similarly, differences between habitats in the number of flowers produced by *O. cuspidatum*, likely due to differences in light availability, contributed to higher NRI for plants growing in coffee. The relationship between NRI and *O. cuspidatum* flower number was unimodal with an intermediate peak. In both habitats, flower number for nearly all plants was equal to or smaller than that at which the model predicted peak NRI. Thus, since flower number was on average larger for coffee-growing plants, the preference by PNRs for plants with more flowers contributed to higher NRI in coffee-growing plants.

Per-flower density of PNRs caught on focal *O. cuspidatum* was, not surprisingly, the final significant predictor of NRI. As expected, given higher levels of NRI for plants in coffee, per-flower PNR density was higher on plants growing in coffee than those in forest fragments. This higher density on focal plants appears to be due to both increased PNR density in coffee versus forest and differences in PNR foraging behavior between habitats.

The total number of PNRs caught during surveys of both target plants and their floral neighborhood was higher in coffee, providing evidence that higher per-flower PNR density on target plants in coffee is the result of higher overall PNR densities there. Since PNR nest density was equivalent between the two habitats, higher density of PNR foragers in coffee is likely the result of preferential foraging in coffee fields over forest fragments (the small size of forest fragments in the study area mean that bees leaving a nest in the forest can readily move into coffee fields to forage). Alternatively, it may be that nests found in coffee tend to be larger, with more foragers, than those found in forest fragments; we did not attempt to assess the size of the nests we found. Whether the higher density of PNRs in coffee is due to preferential foraging in

this habitat or discrepancy in nest size between the two habitats, this finding highlights the importance of shade coffee as a habitat for stingless bees, a result consistent with other studies (Jha and Dick 2010, Fisher et al. 2017).

In addition to higher PNR density in coffee fields, PNR foraging behavior differed between habitats in two ways, both of which contributed to increased NRI for plants growing in coffee. First, PNRs showed increased preference for *O. cuspidatum* over alternative floral resources in coffee compared to forest fragments – i.e. the proportion of all PNRs caught on *O. cuspidatum* was higher in coffee than in forest. Second, per foraging bout, individual PNRs robbed a greater proportion of all available flowers from plants in coffee, perhaps because these flowers were more rewarding. Thus, while it is difficult to evaluate the relative importance of differences in PNR density and foraging behavior in driving differences in NRI between habitats, it is clear that both factors play a role.

Both flower-level and plant-level data indicate that NR significantly reduces fruit set. The negative correlation between fruit set and NRI is striking, given that PNR attraction to *O. cuspidatum* is associated with floral traits – i.e. floral display size and nectar quantity and quality – that are frequently positively correlated with both attractiveness to pollinators (Adler and Bronstein 2004, Theis et al. 2014) and the availability of resources to allocate to reproduction (Bazzaz et al. 1987, 2000). In this population, negative effects of NR are sufficient to outweigh any benefits of increased pollination and/or resource availability, and result in a net reduction in fruit set. Interestingly, this effect does not translate to seeds per plant, likely because NRI is positively related to the number of flowers produced; even if a smaller proportion of these flowers produces seeds, the net outcome is a consistent number of seeds across a range of NRIs. The two years of data presented here do not represent lifetime reproductive output for most of

the surveyed plants, so we cannot say conclusively whether there is a cost to either future reproduction or survival of producing large numbers of flowers that, due to the effects of NR, go unpollinated.

These results highlight that environmental change – in this case conversion of forests for coffee cultivation – can influence biotic interactions via multiple interacting pathways. Crucially, only some of the change in interaction frequency between *O. cuspidatum* and its PNRs across habitats can be attributed to changes in the density of the interacting species. Of greater importance to determining the frequency of these interactions are details of the biotic community and abiotic environment within which the interaction is embedded. This is in line with a large body of research on the relative importance of trait-mediated vs. density-mediated indirect interactions in determining the effect of one species on another species with which it does not directly interact (Werner and Peacor 2003, Schmitz et al. 2004). Yet it suggests that the importance of trait-mediated effects of anthropogenic environmental change – on species interactions and, ultimately, species persistence and ecosystem function – have been underappreciated. As this work highlights, detecting and understanding such trait-mediated effects may require fine-grained analysis of interacting organisms across environmental contexts. Thus, on the one hand we heartily endorse recent calls to use network approaches to better understand how environmental change can impact communities beyond just species loss (Tylianakis et al. 2008, Poisot et al. 2015, 2017, Valiente-Banuet et al. 2015). On the other, we caution that the empirical data upon which networks are built often lack the level of detail needed to describe the effects of environmental change on biotic communities.

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Works cited

Adler, L. S., and J. L. Bronstein. 2004. Attracting antagonists: does floral nectar increase leaf herbivory? Ecology 85:1519–1526.

Bazzaz, F. A., D. D. Ackerly, and E. G. Reekie. 2000. Reproductive allocation in plants. Pages 1–29 *in* M. Fenner, editor. Seeds: the ecology of regeneration in plant communities. CABI.

Bazzaz, F. A., N. R. Chiariello, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. BioScience 37:58–67.

Bivand, R. S., and D. W. S. Wong. 2018. Comparing implementations of global and local indicators of spatial association. TEST 27:716–748.

Burkle, L. A., R. E. Irwin, and D. A. Newman. 2007. Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. American Journal of Botany 94:1935–1943.

Cuevas, E., and V. Rosas-Guerrero. 2016. Spatio-temporal variation of nectar robbing in Salvia gesneriflora and its effects on nectar production and legitimate visitors. Plant Biology 18:9–14.

Daniel, T. F. 1995. Flora of Chiapas Part 4: Acanthaceae. (D. E. Breedlove, Ed.). California Academy of Sciences, San Francisco, CA.

Dirzo, R., H. S. Young, M. Galetti, G. Ceballos, N. J. B. Isaac, and B. Collen. 2014. Defaunation in the Anthropocene. Science 345:401–406.

Fierro, M., L. Cruz-López, D. Sánchez, R. Villanueva-Gutiérrez, and R. Vandame. 2012. Effect of biotic factors on the spatial distribution of stingless bees (Hymenoptera: Apidae, Meliponini) in fragmented neotropical habitats. Neotropical Entomology 41:95–104.

Fisher, K., D. J. Gonthier, K. K. Ennis, and I. Perfecto. 2017. Floral resource availability from groundcover promotes bee abundance in coffee agroecosystems. Ecological Applications 27:1815–1826.

Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology 94:295–304.

Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. a Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt, and M. I. O'Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105–108.

Hubbell, S. P., and L. K. Johnson. 1977. Competition and nest spacing in a tropical stingless bee community. Ecology 58:949–963.

Irwin, R. E., J. L. Bronstein, J. S. Manson, and L. Richardson. 2010. Nectar robbing: ecological and evolutionary perspectives. Annual Review of Ecology, Evolution, and Systematics 41:271–292.

Irwin, R. E., and J. E. Maloof. 2002. Variation in nectar robbing over time, space, and species. Oecologia 133:525–533.

Jha, S., and C. W. Dick. 2010. Native bees mediate long-distance pollen dispersal in a shade coffee landscape mosaic. Proceedings of the National Academy of Sciences 107:13760–13764.

Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to Isomeris arborea. Ecology 80:125–134.

Maloof, J. E., and D. W. Inouye. 2000. Are Nectar Robbers Cheaters or Mutualists? Ecology 81:2651–2661.

Matzke, N., E. A. Ferrer, A. D. Barnosky, G. O. U. Wogan, B. Mersey, C. Marshall, K. C. Maguire, S. Tomiya, E. L. Lindsey, J. L. McGuire, T. B. Quental, and B. Swartz. 2011. Has the Earth's sixth mass extinction already arrived? Nature 471:51–57.

Meyer, J.-Y., and C. Lavergne. 2004. Beautés fatales: Acanthaceae species as invasive alien plants on tropical Indo-Pacific Islands. Diversity and Distributions 10:333–347.

Morris, W. F. 1996. Mutualism denied? Nectar-robbing bumble bees do not reduce female or male success of bluebells. Ecology 77:1451–1462.

Navarro, L. 2000. Pollination ecology of Anthyllis vulneraria subsp. vulgaris (Fabaceae): nectar robbers as pollinators. American Journal of Botany 87:980–985.

Paradis, E., and K. Schliep. 2018. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics.

Poisot, T., C. Guéveneux-Julien, M.-J. Fortin, D. Gravel, and P. Legendre. 2017. Hosts, parasites and their interactions respond to different climatic variables. Global Ecology and Biogeography 26:942–951.

Poisot, T., D. B. Stouffer, and D. Gravel. 2015. Beyond species: why ecological interaction networks vary through space and time. Oikos 124:243–251.

R Core Team. 2018. R: a language and environment for statistical computing.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305–329 *in* L. Real, editor. Pollination Biology. Academic Press, Inc.

Richardson, L. L., and J. L. Bronstein. 2012. Reproductive biology of pointleaf manzanita (Actostaphylos pungens) and the pollinator - nectar robber spectrum. Journal of Pollination Ecology 9:115–123.

Roubik, D. W. 1982. The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. Ecology 63:354–360.

Schmitz, O. J., V. Krivan, and O. Ovadia. 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecology Letters 7:153–163.

Theis, N., N. A. Barber, S. D. Gillespie, R. V. Hazzard, and L. S. Adler. 2014. Attracting mutualists and antagonists: Plant trait variation explains the distribution of specialist floral herbivores and pollinators on crops and wild gourds. American Journal of Botany 101:1314–1322.

Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363.

Utsumi, S., Y. Ando, and T. Miki. 2010. Linkages among trait-mediated indirect effects: a new framework for the indirect interaction web. Population Ecology 52:485–497.

Valiente-Banuet, A., M. A. Aizen, J. M. Alcántara, J. Arroyo, A. Cocucci, M. Galetti, M. B. García, D. García, J. M. Gómez, P. Jordano, R. Medel, L. Navarro, J. R. Obeso, R. Oviedo, N. Ramírez, P. J. Rey, A. Traveset, M. Verdú, and R. Zamora. 2015. Beyond species loss: The extinction of ecological interactions in a changing world. Functional Ecology 29:299–307.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84:1083–1100.

Figure 1. Conceptual diagram of the pathways by which environmental change can result in changes to interactions without changing species composition. Pathways are not mutually exclusive. Each line type represents one pathway. Bullet points indicate the aspects of each category assessed in this study. Pathway A-B: environmental conditions (e.g. insolation) directly affect the abundance (Pathway A) or one or more traits (Pathway B) of one or both of the interacting species. Pathway C-D: environmental conditions affect the traits or density of other species in the community, which in turn influences the abundance (Pathway C) or trait(s) (Pathway D) of one or more interacting species.

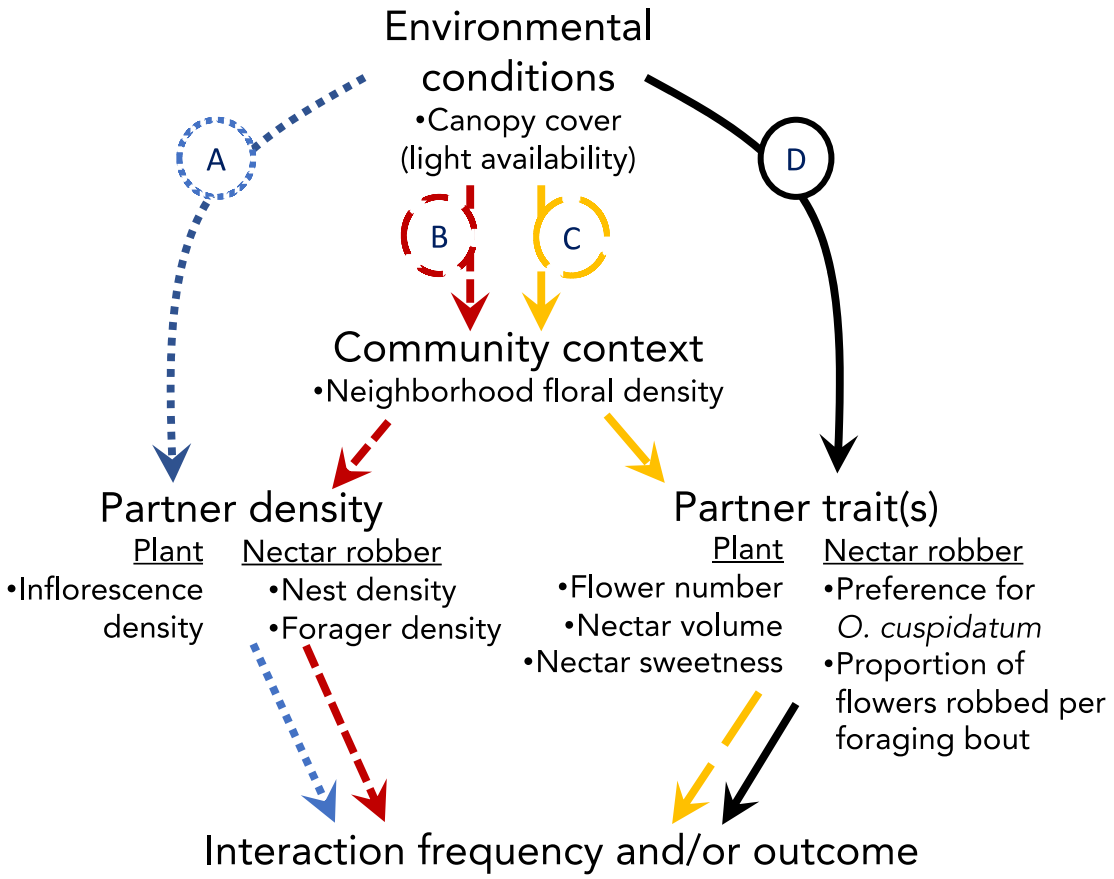

Figure 2. Relationship between nectar robbing intensity (NRI) of *O. cuspidatum* and A) focal plant flower number and B) floral neighborhood. In both panels, points represent individual plants, colored according to habitat. Black line represents best-fit from a general additive model; gray-shaded area represents standard error. Blue shaded area represents 95% confidence interval about a) *O. cuspidatum* flower number or b) the neighborhood floral density where maximum NRI is predicted. Dashed vertical lines and shaded area represent the mean and standard error for a) focal plant flower number and b) neighborhood floral density in each habitat. 'Neighborhood floral density' refers to the mean number of blooms of all species per transect. In a), the x-axis is square-root transformed to improve legibility.

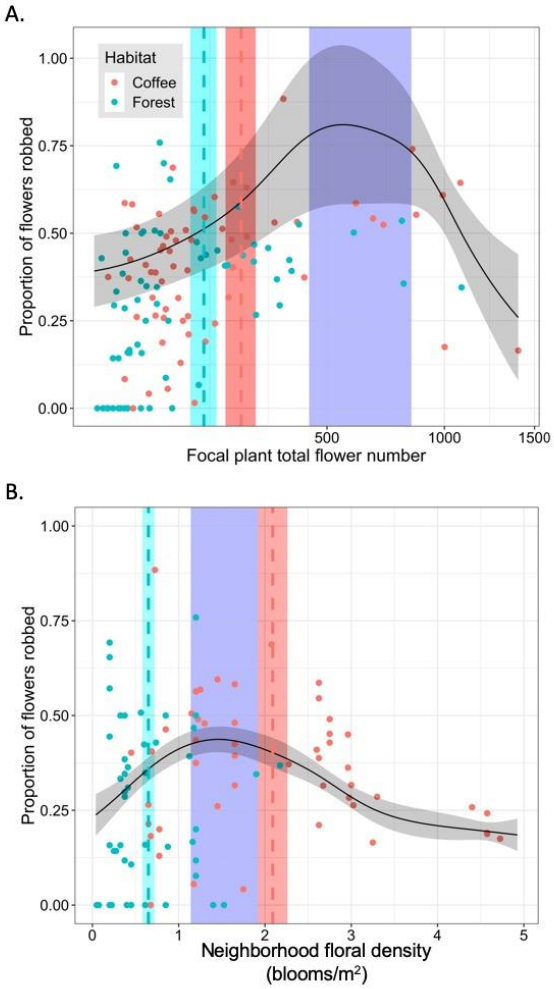

Table 1. Relationship between habitat and putative drivers of nectar robbery. Model results are from generalized linear models (canopy cover, neighborhood floral density, *O. cuspidatum* density, and PNR nest density) or generalized linear mixed-effects models with plant as a random effect (all other variables). PNR: primary nectar robber; DF: residual degrees of freedom; %DE: percent of the null deviance in the relevant response variable explained by habitat; †: includes spatial eigenvectors as additional predictors to account for spatial autocorrelation. Boldface indicates a significant difference between habitats in levels the putative driver, at $p < 0.05$.

Putative driver	**Mean ± SE**		**Test statistic (t or z)**	**DF**	**p**	**%DE**
	Coffee	**Forest**				
Environmental conditions						
Canopy cover (%)†	**70.8 ± 2.6**	**90.5 ± 1.0**	**7.01**	**83**	**< 0.001**	**51.0**
Community context						
Neighborhood floral density (blooms per transect)†	**74 ± 8**	**28 ± 5**	**–5.46**	**51**	**< 0.001**	**25.4**
Partner densities						
O. cuspidatum density (inflorescences per survey)	18 ± 3	11 ± 2	–1.05	54	0.3	0.0
PNR nest density (nests per transect)	0.2 ± 0.1	0.1 ± 0.1	1	15	0.7	0.0
Total PNR density (individuals per observation)†	**1.9 ± 0.2**	**0.8 ± 0.2**	**–4.62**	**40**	**< 0.001**	**31.4**
PNR density – target *O. cuspidatum* (individuals per observation)	**2.2 ± 0.3**	**0.8 ± 0.2**	**–4.10**	**46**	**< 0.001**	**27.0**
Per-flower PNR density – target *O. cuspidatum* (individuals per observation)	**0.2 ± 0.03**	**0.1 ± 0.03**	**–2.40**	**46**	**0.02**	**12.5**

PNR density – 10m neighborhood (individuals per observation)†	1.6 ± 0.3	1.0 ± 0.3	0.89	39	0.4	0.0
Partner traits						
***O. cuspidatum* flower number†**	**243 ± 37**	**160 ± 26**	**–9.22**	**156**	**< 0.001**	**40.6**
***O. cuspidatum* nectar volume (µL)**	**6.6 ± 0.3**	**4.4 ± 0.4**	**–2.70**	**65**	**0.009**	**10.1**
O. cuspidatum nectar sweetness (Brix)	25.4 ± 0.1	24.7 ± 0.3	–1.14	63	0.3	2.0
% PNRs caught on *O. cuspidatum* (% of total PNRs caught per observation)†	57.5 ± 6.4	45.0 ± 10.5	–1.37	43	0.06	7.4
% of available *O. cuspidatum* flowers robbed per visit	**80.2 ± 9.5**	**40.0 ± 10.8**	**2.74**	**57**	**0.007**	**2.3**

Table 2. Relative importance of the significant drivers of nectar robbing intensity (NRI). Results from a general additive model combining all of the putative drivers that significantly influenced NRI when considered independently. Boldface indicates a significant effect on NRI at $p < 0.05$. % DE: percent of the null deviance in NRI explained by each driver; PNR: primary nectar robber; ***$p < 0.001$.

Driver	% DE	ΔAICc for omitting this variable	p
***O. cuspidatum* flower number**	**15.0**	**37.9**	**< 0.001*** **
Neighborhood floral density	**22.1**	**52.0**	**< 0.001*** **
Per-flower PNR density – target plant	**15.2**	**31.3**	**< 0.001*** **
Habitat	0.0	–2.3	0.25

Table 3. Relationship between reproductive output and 1) nectar robbing and 2) habitat. Boldface indicates a significant relationship at $p \leq 0.05$. DF: residual degrees of freedom; %DE: percent of the null deviance in the measure of reproductive output that is explained by each predictor; NRI: nectar robbery intensity. Boldface indicates statistical significance at $p < 0.05$; . p = 0.05, **p<0.01.

Predictor	**Coefficient (ß ± SE)**	**Test statistic (t or z)**	**DF**	**%DE**
Fruit set (GLMM)				
NRI	**–0.63 ± 0.21*** **	**–2.94**	**123**	**8.0**
Habitat (Forest)	–0.34 ± 0.23	–1.45	123	0.8
NRI × Habitat	0.31 ± 0.31	1.00	123	0.0
Seeds per plant (2018 only; GLM)				
NRI	0.88 ± 0.52	1.71	56	2.7
Habitat (Forest)	–0.05 ± 0.30	–0.17	56	1.5
Seed set (2018 only; GLM)				
NRI	–0.33 ± 0.36	–0.91	56	1.2
Habitat (Forest)	0.38 ± 0.20 **.**	1.92	56	5.5