



Nocturnal Bees Feed on Diurnal Leftovers and Pay the Price of Day – Night Lifestyle Transition

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Bees exemplify flights under bright sunlight. A few species across bee families have evolved nocturnality, displaying remarkable adaptations to overcome limitations of their daylight-suited apposition eyes. Phase inversion to nocturnality in a minority of bees that co-exist with diurnal bees provides a unique opportunity to study ecological benefits that mediate total temporal niche shifts. While floral traits and sensory modalities associated with the evolution of classical nocturnal pollination syndromes, e.g. by bats and moths, are well-studied, nocturnality in bees represents a poorly understood, recently invaded, extreme niche. To test the competitive release hypothesis, we examine how nocturnality shapes foraging by comparing pollen loads, nest pollen, and flower visitation of sympatric nocturnal and diurnal carpenter bees. We predicted that nocturnal bees primarily use night-blooming flowers, show little/no resource overlap with diurnal species and competitive release favors night-time pollen collection for provisioning. Contrarily, we found substantial resource overlap between nocturnal and diurnal bees. Flower opening times, floral longevity and plant abundance did not define nocturnal flower use. Smaller pollen loads on nocturnal foragers suggest subsistence on resource leftovers largely from diurnal flowers. Greater pollen types/diversity on nocturnal foragers indicate lower floral constancy compared to diurnal congeners. Reduced activity during new moon compared to full moon suggests constraints to nocturnal foraging. Invasion and sustenance within the nocturnal niche is characterized by: (i) *opportunistic foraging* on residual resources as indicated by smaller pollen loads, extensive utilization of day-blooming flowers and substantial overlap with diurnal bees, (ii) *generalization at two levels*—between and within foraging trips as indicated by lower floral constancy, (iii) *reduced foraging on darker nights*, indicating visual constraints despite sensitive optics. This together with smaller populations and univoltine breeding in nocturnal compared to multivoltine diurnal counterparts suggest that nocturnality imposes substantial fitness costs. In conclusion, the evolution of nocturnality in bees is accompanied by resource generalization instead of specialization. Reduced floral constancy suggests differences in foraging strategies of nocturnal and diurnal bees which merits further investigation. The relative roles of competition, floral rewards and predators should be examined to fully understand the evolution and maintenance of nocturnality in bees.

Keywords: carpenter bees, floral rewards, floral constancy, nocturnality, nocturnal niche, nocturnal pollination networks, pollination syndromes, specialization

INTRODUCTION

Partitioning along the time niche axis is an uncommon mechanism for reducing competition or predation avoidance (MacArthur and Levins, 1967; Schoener, 1974; Wiens et al., 1986; Kronfeld-Schor and Dayan, 2003). Temporal niche partitioning usually manifests as within-phase shifts, while complete phase inversions, for example from day to night, are extremely rare with its rarity attributed to evolutionary constraints imposed on extreme modifications in species physiology, anatomy and behavior (Kronfeld-Schor and Dayan, 2003).

Bees are typically day-active and their flights are affected by reduced light levels (Kelber et al., 2006) or the obscuring of solar cues, e.g. during a solar eclipse (Galen et al., 2018). Nocturnality in bees is uncommon, but has evolved repeatedly in several families (Wcislo and Tierney, 2009) and is proposed as a strategy to gain resources from night-blooming flowers (Hopkins et al., 2000; Wcislo et al., 2004; Wcislo and Tierney, 2009), minimize competition or predation (Bohart and Youssef, 1976; Smith et al., 2003; Wcislo et al., 2004) or avoid high temperatures (Gerling et al., 1989; Gottlieb et al., 2005). These hypotheses have not been tested explicitly in detail (but see Wcislo et al., 2004). Bees possess daylight-adapted apposition compound eyes that limit vision under dim-light conditions. That some bees have made the transition to nocturnality is noteworthy, as are investigations into how they function within the nocturnal niche. Some species are facultatively nocturnal (Dyer, 1985; Somanathan et al., 2009b) to exploit a transiently available resource. Best studied amongst dim-light bees are the crepuscular halictid *Megalopta genalis* in Panama (Greiner et al., 2004a,b; Warrant et al., 2004; Kelber et al., 2006; Warrant, 2008) and the truly nocturnal carpenter bee *Xylocopa tranquebarica* in India (Somanathan et al., 2008a,b, 2009a, 2019). Recent research on dim-light vision has revealed remarkable anatomical and neurophysiological adaptations in a range of insects, including nocturnal bees (Warrant et al., 2004; Kelber et al., 2006; Somanathan et al., 2008a,b, Warrant and Dacke, 2011; Foster et al., 2017; Narendra and Ramirez-Esquivel, 2017; Stöckl et al., 2017). Dim-light adaptations that contribute to greater sensitivity of apposition eyes include enlarged compound eyes and ocelli, large facet lenses and rhabdoms, slower integration times, wider acceptance angles and lateral branching of the first order neurons (Greiner et al., 2005; Berry et al., 2011; Warrant, 2017). How these dim-light adaptations shape behaviors such as foraging in nocturnal bees is unknown. For example, although color vision even under starlight levels has been reported in the carpenter bee *Xylocopa tranquebarica* (Somanathan et al., 2008b), the ecological consequences of nocturnality in bees is yet unknown.

The sensory and physiological basis of dim-light flower search in two classical nocturnal pollinators: moths (Heinrich, 1971; Raguso and Pichersky, 1995; Kelber et al., 2002; Raguso and Willis, 2003; Goyret et al., 2008; Okamoto et al., 2008; Kuenzinger et al., 2019; Stöckl and Kelber, 2019) and bats (Heithaus et al., 1975; von Helversen and von Helversen, 1999; Winter et al., 2003; Fleming et al., 2009; Simon et al., 2011) are well understood. Distinct floral traits associated with bat (Baker, 1961; von Helversen and von Helversen, 2003; Fleming et al., 2009) and

moth pollination are well studied (Raguso et al., 1996; Svensson et al., 2011), although these pollinators may also opportunistically exploit flowers that do not strictly bear these characteristics (Ollerton et al., 2009; Borges et al., 2016; Borges, 2018). Nocturnal bees co-occur with bats and moths in tropical habitats, but how they find flowers and the floral traits that define their foraging choices are unclear. Are they specialists, generalists or opportunistic foragers? Is foraging limited by moon phase?

We integrate patterns of nocturnal flight activity with floral resource use to comprehensively examine for the first time, what shapes and constrains foraging in nocturnal bees by studying a group of sympatric carpenter bees in India. *Xylocopa tranquebarica* is truly nocturnal while *X. tenuiscapa* and *X. leucothorax* are sympatric diurnal species (Somanathan et al., 2008a, 2019). We evaluate the costs and benefits of nocturnality by examining flight activity across moon phases and resource use and we used population sizes and number of breeding episodes per year (voltinism) as a proxy for fitness across the bee species. We asked the following questions: (A) Does variation in light levels from full moon to new moon impact flight and foraging activity, given that *X. tranquebarica* is known to be capable of navigation under dim starlight? (B) Does *X. tranquebarica* largely or exclusively restrict foraging to night-opening flowers and is there significant floral resource partitioning between the nocturnal *X. tranquebarica* and its diurnal congeneric species *X. tenuiscapa* and *X. leucothorax*? (C) Since phase inversion to a nocturnal lifestyle in bees is rare, are there obvious fitness costs associated with nocturnality in *X. tranquebarica*?

MATERIALS AND METHODS

This study was carried out during the flowering periods from February–May and November–December 2008 and February–March 2017. This study was conducted in the Bhimashankar Wildlife Sanctuary (19°21′–19°11′N, 73°31′–73°37′E, elevation 900 m), Maharashtra State, in the Western Ghats of India. See **Supplementary Section S1a,b** for details on the study site and biology of carpenter bees.

Flight Activity

All three species construct nest tunnels inside dead wood of trees (**Figure 1**). During flowering seasons (2007 and 2008), 12 nests of *X. tranquebarica* were watched by 2–3 observers using night vision binoculars (US Night Vision, United States) to record departures, returns and flight durations from 1800 to 0600 h during full and new moon phases (\pm 0–4 nights). Observers positioned themselves at a distance of 1–2 m from nest trees. Tree trunks bearing nests that were within heights of 1 m from the ground were selected for ease of observation in all three bee species making it possible to clearly observe departing and arriving bees. Moreover, the loud buzzing of the bees informed observers about the departure and arrival of bees at the nest site at night. On corresponding dates, such data were also obtained during the daytime at 12 *X. tenuiscapa* and 10 *X. leucothorax* nests. For further details see **Supplementary Section S1c**.



FIGURE 1 | (A) Tree with nests of the nocturnal carpenter bee *X. tranquebarica*. Pollen traps consisting of opaque plastic tubes lined on the inner surface with sticky tape were placed at nest entrances to strip pollen from returning foragers. (B) The carpenter bee *X. tenuiscapa* carrying orchid pollinia on its head and (C) tree pollen on its legs.

Pollen Utilization

Bee Pollen Loads

Carpenter bees were captured at nest entrances when they were returning from foraging trips on 12 nights in the nocturnal bee and on 12 corresponding days in the two diurnal bee species. After the bee left the nest to forage, a cotton ball was inserted about an inch inside the nest entrance and a long thin thread which was attached to the cotton ball was allowed to hang freely outside (Figure 1). On arrival, the bee landed on the nest entrance and a plunger was placed over the nest entrance, trapping the bee between the cotton ball and the plunger. By pulling on the thread, the bee was quickly moved into the plunger and covered. At the netted end of the plunger, pollen was extracted from the dorsal and ventral sides and the legs of the bee by thoroughly swabbing with cotton buds dipped into glycerin jelly after which the bees were released (31 individuals per species). Pollen thus harvested were stored in 70% ethanol. By this method we could not completely remove all the pollen but we could harvest as much as possible without injuring the bee. Hence, total pollen counts were not obtained. Instead we estimated pollen numbers and identified pollen types/species on all captured bees. Refer to **Supplementary Section S1d** for further details of pollen estimation and identification.

Nest Pollen Traps

We used a non-invasive method to assess pollen use by the three bee species by placing traps at nest entrances of the three bee species. Each nest trap consisted of a black plastic tube (3 cm long, 1 cm diameter) attached to the nest entrance of all three species

($n = 12\text{--}18$ nests per bee species) (Figure 1). The inner surface of the trap was lined with sticky tape which stripped small amounts of pollen from returning foragers' head, thorax abdomen and legs, as they passed through the tube to enter the nest. The traps were replaced at 5-day intervals from February–May and November–December 2007 and stored for later estimation of pollen counts and diversity. The samples thus collected were acetolysed and then stored for identification and counting (for further details see **Supplementary Section S1e**).

For every plant species i we obtained quantitative measures of pollen use from the nest traps of the three bee species j , which we term as the fractional pollen utilization index (PI), calculated as

$$PI = \frac{\text{Total pollen of plant species } i \text{ in all nest traps of bee species } j}{\text{Sum of pollen of plant species } i \text{ in nest traps of all 3 bee species}}$$

Using this method, we compared relative quantitative pollen usage for a given plant species across the three bee species.

Nocturnal Foraging on Night-Blooming *H. quadriloculare* Flowers

Heterophragma quadriloculare trees (Bignoniaceae) produce white, night-opening flowers that last only one night, and are exclusively pollinated by carpenter bees at night (Somanathan and Borges, 2001). The flowers have touch-sensitive stigmas (TSS) that close rapidly after bee visitation (Somanathan and Borges, 2001; unpublished data). This provided a convenient read-out to score visitation by noting the stigma state (open/closed) in the morning following full-, half-, and new moon nights in 2017. Trees were observed between sunset and sunrise (1800–2100 h, 0000–0300 h, and 0500–0700 h) for 10–30 min per session, resulting in 30–80 observation minutes per tree per night ($n = 4\text{--}8$ trees) in February and March 2017. Nocturnal visits by *X. tranquebarica* were observed across new moon ($n = 5$ trees/1 night), half ($n = 6\text{--}8$ trees/4 nights) and full moon phases ($n = 8$ trees/1 night). Number of flowers visited by *X. tranquebarica* during each foraging bout (the time an individual spent on the tree from entry to exit) was recorded. The number of flowers visited during a bout was determined by listening to the distinct loud buzzing while in flight which stopped each time a bee entered a flower and resumed when the flower visit was over. These auditory measures have been shown to correspond to visual counts of number of flowers visited at night in *H. quadriloculare* (Somanathan and Borges, 2001). In the mornings following full and new moon (± 5 nights), the TSS states of the stigmas were scored ($n = 10$ flowers, 4–8 trees).

Community Flower Visitation

Direct observations of flower visits were carried out during flowering months in 2007 and 2008 for 71 plant species (2–3 individuals/species) by 2 or 3 observers from 0600 to 2100 h for one day during peak flowering for each species. Only the identities of flower visitors and their visit timings were recorded. Frequencies could not be obtained as visits were low for most plant species.

Floral Resource Use Networks

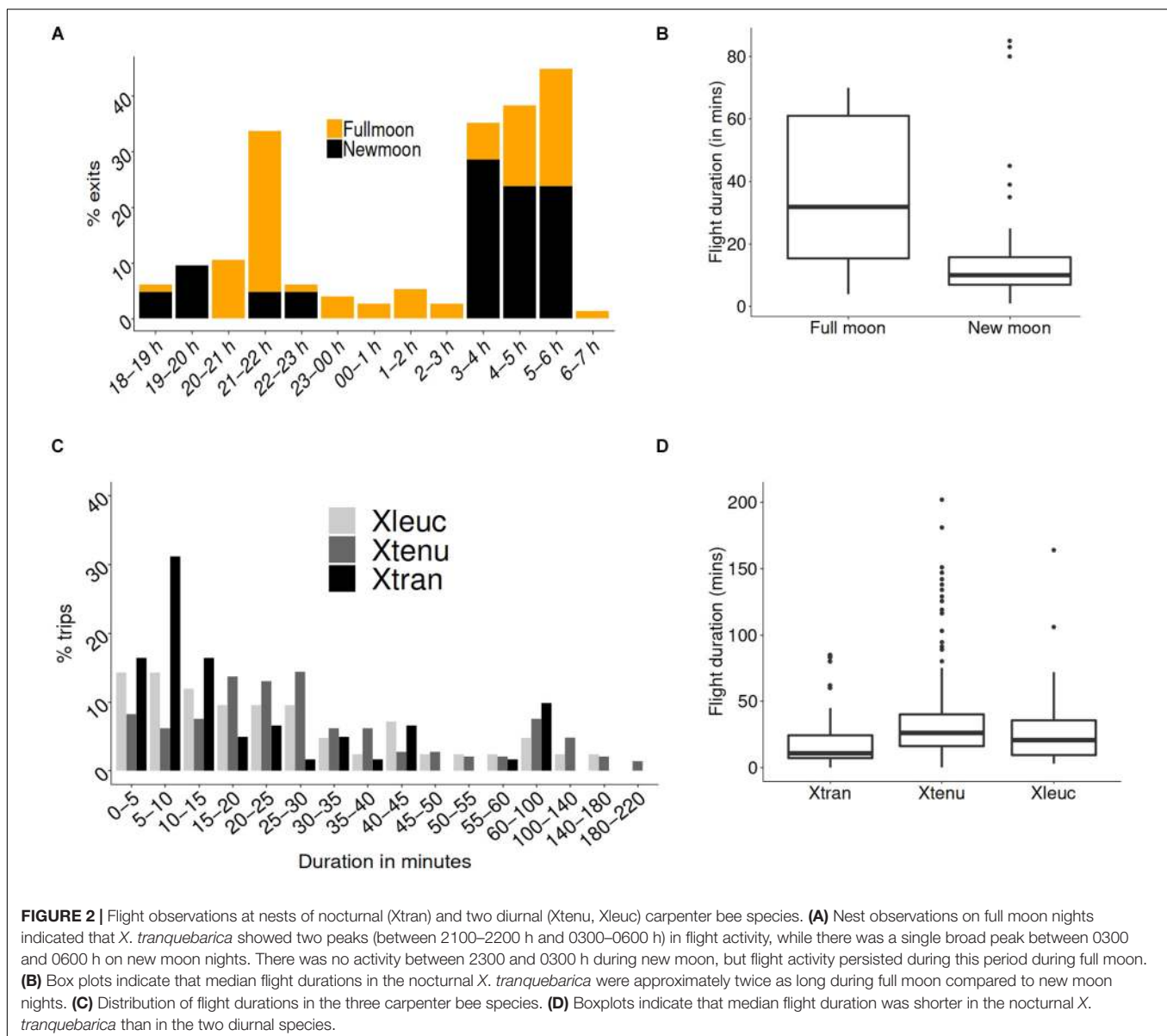
Bipartite networks for overall floral use, pollen and nectar use were built (Bipartite package in R (2.9.1), Dormann et al., 2008) from a combination of body pollen loads, nest pollen traps and flower observations of the plant community ($n = 71$ species) as described above. We constructed three kinds of networks and compared metrics for all three networks.

- (i) *Overall floral use network*: These are qualitative networks of total floral resource use that combine visitor information from direct flower observations, pollen collected and identified from bee bodies and nest traps.
- (ii) *Pollen network*: Pollen resources were identified as those plants whose pollen was collected from bees' bodies or nest traps. The fractional pollen utilization index (PI; described above) for each bee species was employed to

denote interaction strengths for each observed link in a quantitative bipartite network of pollen use.

- (iii) *Nectar network*: Plant species that were visited by a bee species during flower observations (described above), but whose pollen was not recovered from nest traps or from bee bodies were classified as exclusive nectar species. While this method allows us to deduce which plants were visited exclusively for nectar, it was not possible to distinguish plants that were visited for both pollen and nectar.

Indices obtained from the overall visitation network (i above) were compared with 1,000 null models generated with the Patefield algorithm (Patefield, 1981). In these null models, the size of the network along with the total number of interactions (marginal total) was held constant. For the pollen and nectar networks (ii and iii above), the shuffle web function (bipartite



package, Dormann et al., 2008), which holds the total number of interactions constant and shuffles the matrix randomly was used. Z-scores were calculated for each index to compare the extent of deviation from randomly generated models. Positive z-values suggest that the mean obtained from null models is lower than the observed values and negative z-values indicate that the mean from null models is higher than the observed index value.

Czekanowski's index of similarity was used to estimate overlap between bee species in the use of flowers [Vegan package in R (3.1.1), Oksanen et al., 2017].

Influence of Time of Flower Opening and Longevity on Floral Resource Use by Bees

Opening times and longevity of flowers were measured (20–30 flowers from 2 to 3 individuals for 83 plant species). Buds were enclosed in muslin bags and inspected shortly after sunrise between 0600 and 0700 h, in the afternoon (between 1200 and 1300 h) and after sunset (between 1800–2000 h). The morning and evening inspections marked the beginning of the day and the beginning of the night respectively. Accordingly, all plant species were assigned to one of four bins: *Diurnal* (D) when all flowers opened only during the day time, *Largely diurnal* (Ld) when 75% of flowers opened during the day and the remaining opened between sunset and sunrise, *Nocturnal* (N) when all flowers opened at night, and *Largely nocturnal* (Ln) when 75% of flowers opened at night and the remaining after sunrise. To determine longevity of individual flowers, the bags were opened between 0600–0700 h and 1700–1800 h daily until petals wilted or discolored.

A generalized linear model (GLM) was performed in R (3.1.1) to examine flower utilization (binary response variable obtained by combining body pollen loads, pollen from nest traps and

flower visitation observations) in relation to explanatory variables such as bee species, flower opening time, flower longevity, length of flowering season for plant species (which is the number of months a species was observed to flower) and tree species abundance (estimated in transects and classified as low, medium and high; see below for details).

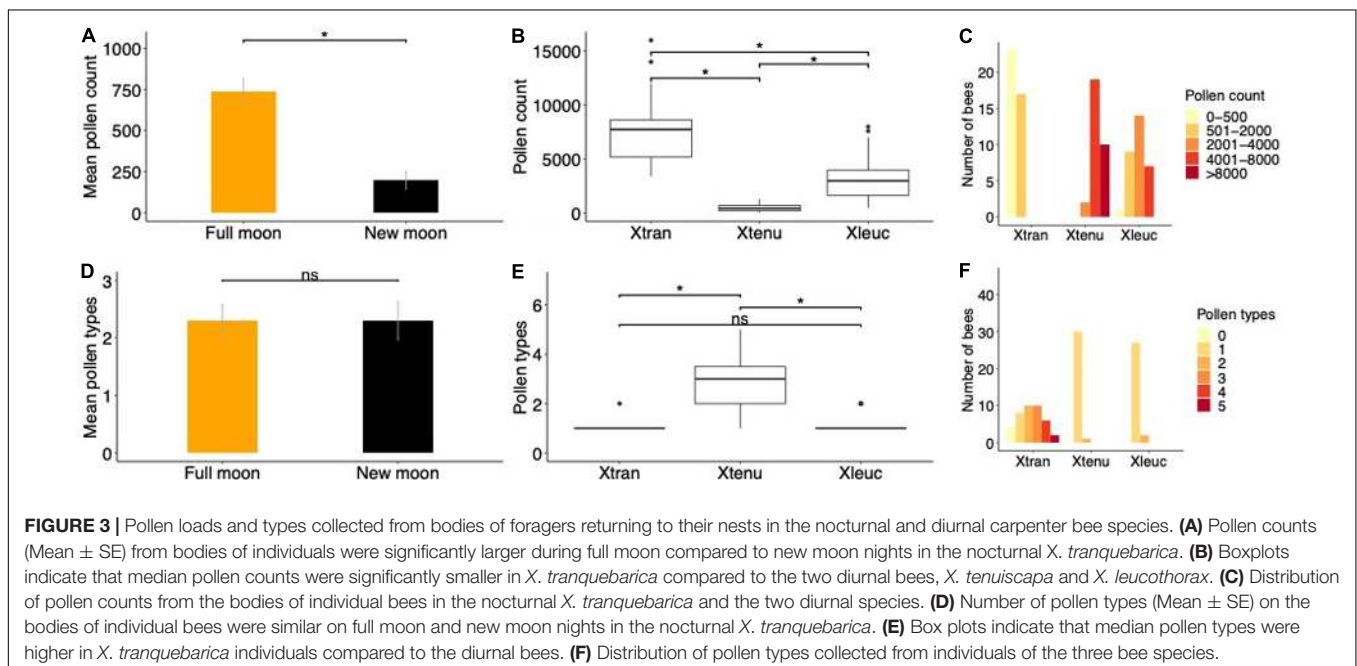
Nest Densities in Diurnal and Nocturnal Carpenter Bees

As a measure of fitness, population sizes were estimated from nest densities in 66 transects (20 × 20 m²) laid randomly in an east–west direction in a 5 km² area. For each transect we recorded the number of nests of each bee species as well as the abundance of tree species.

RESULTS

Moon Phase, Flight Activity, and Durations

Nocturnal flight activity, measured as the number of exits from nests, was higher on full moon than on new moon nights in *X. tranquebarica* (Figure 2A; Wilcoxon rank sum test with continuity correction, $W = 41.5$, $p = 0.02$; $n = 76$ for full moon and $n = 21$ for new moon nights). Flight durations were significantly longer during full moon [Figure 2B; Welch *t*-test for unequal variance, $t = 1.91$, $p = 0.04$, $n = 7$ full moon exits (from 2 nights) and 46 new moon exits (from 10 nights)]. Flight durations varied significantly between the bee species (Figure 2C; Kruskal-Wallis $\chi^2 = 19.015$, d.f. = 2, $p < 0.001$) and was significantly shorter in the nocturnal *X. tranquebarica* than the diurnal *X. tenuiscapa* (Figure 2D; *post-hoc* pairwise Wilcoxon test with Bonferroni correction, *X. tranquebarica*–*X.*



tenuiscapa: $p < 0.0001$, *X. tenuiscapa*–*X. leucothorax*: $p = 0.37$, *X. tranquebarica*–*X. leucothorax*: $p = 0.19$). Fifty percentage of flight trips were between 5 and 10 min in the nocturnal *X. tranquebarica*, 15 and 20 min in *X. leucothorax*, and 20 and 25 min in *X. tenuiscapa*.

Pollen Load Size and Diversity in Relation to Moon Phase

Pollen loads of individual bees (mean pollen counts) were larger on full moon compared to new moon nights in *X. tranquebarica* (Figure 3A). The sizes of pollen loads collected on closely corresponding dates (± 1 –2 days) differed between the three bee species, with the nocturnal bee carrying significantly smaller loads (Figures 3B,C; Kruskal-Wallis $\chi^2 = 72.803$, d.f. = 2, $p < 0.001$). The number of pollen types (plant species) on bodies of individual *X. tranquebarica* foragers returning to the nest was similar on full moon and new moon nights (Figure 3D; Wilcoxon

rank sum test, $W = 193.0$, $p = 0.85$). The number of pollen types on individuals differed across bee species (Figure 3E; Kruskal-Wallis $\chi^2 = 53.823$, d.f. = 2, $p < 0.001$); most pollen loads were composed of a single pollen type in the two diurnal bee species, while in the nocturnal bee, pollen loads were predominantly composed of 2–5 species (Figure 3F; Kruskal-Wallis $\chi^2 = 36.90$, d.f. = 2, $p < 0.001$).

Flower Visitation by Nocturnal Bees to *H. quadriloculare* Flowers in Relation to Moon Phase

The number of flowers visited per foraging bout by *X. tranquebarica* was higher on full moon compared to the half and new moon phases (Kruskal-Wallis $\chi^2 = 7.45$, d.f. = 2, $p = 0.02$, Supplementary Figure S1A). *Post-hoc* pairwise comparisons were not possible due to low sample sizes on new moon nights. TSS states were significantly correlated with moon phase, with a higher proportion of stigmas closed following full moon compared to new moon (Kruskal-Wallis $\chi^2 = 9.96$, d.f. = 1, $p = 0.002$; Supplementary Figure S1B).

Community Flower Opening and Longevity

Approximately 70% of the plant community ($n = 83$ species) had *Diurnal* (D) or *Largely diurnal* (Ld) opening, while the remaining had *Nocturnal* (N) or *Largely nocturnal* (Ln) opening when binned into these categories (Figure 4A and Supplementary Table S1). Overall flower utilization by combining pollen and flower observation data showed that the nocturnal *X. tranquebarica* visited both day- (D, Ld) and night-blooming (N, Ln) flowers (Figure 5A), as also indicated by the pollen utilization index (*PI*; Figure 5C). Proportional flower use between the four categories was similar across bee species (Supplementary Table S2). Over 60% of the plant community produced flowers that lasted ≥ 2 days (Figure 4B). Flower utilization by bees (overall and *PI*) and longevity of flowers were unrelated (Figures 5B,D and Supplementary Table S2).

A GLM with overall flower utilization as a binomial response variable and with stepwise backwards removal of explanatory variables resulted in the best fit, with plant species abundance as the explanatory variable that significantly influenced visitation (Table 1 and Supplementary Figures S2, S3). Fifteen plant species that were highly abundant in the community were visited by the diurnal bee *X. leucothorax*, while the nocturnal bee visited ten of the highly abundant plant species (Supplementary Figure S2A). In the full model, bee species, flower opening, flower longevity, length of flowering and interaction terms between these variables did not significantly explain floral use (Supplementary Table S3 and Supplementary Figures S3, S4).

Floral Resource Use Networks

A total of 62 of 71 plant species were visited by at least one bee species (Supplementary Table S1). Bipartite networks, network measures and indices of resource overlap suggest substantial overlap in flower use between the bee species (Figure 6 and Supplementary Tables S4, S5).

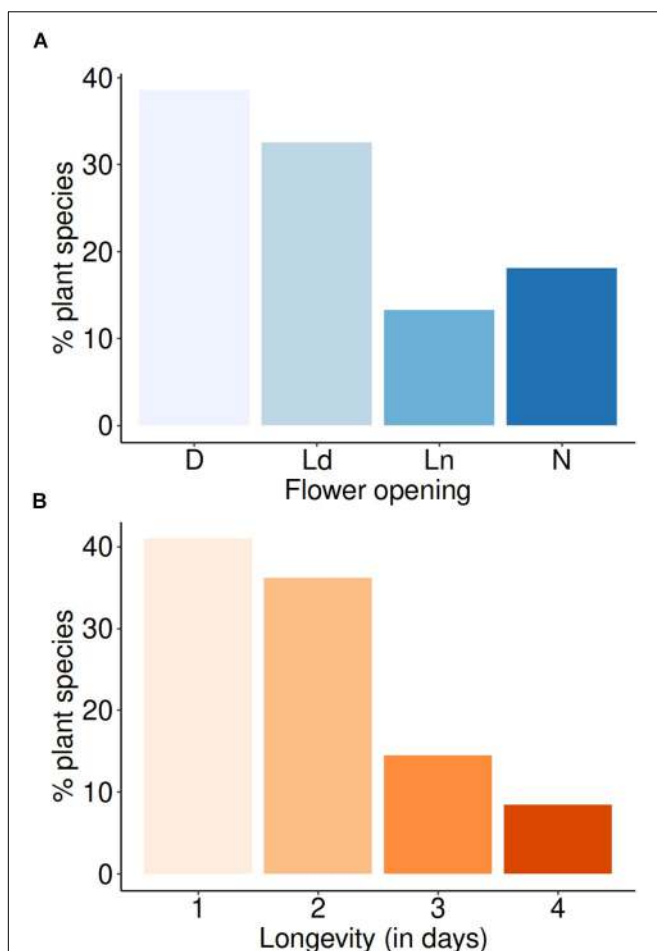


FIGURE 4 | Flower opening times and longevity in the plant community. **(A)** Distribution of opening times indicated that flowers opened largely during the day in this community. D, diurnal opening; Ld, Largely diurnal opening; Ln, Largely nocturnal opening; N, nocturnal opening. **(B)** Distribution of flower longevity in this community indicated that more than 60% of plant species produced flowers that lasted 2 or more days.

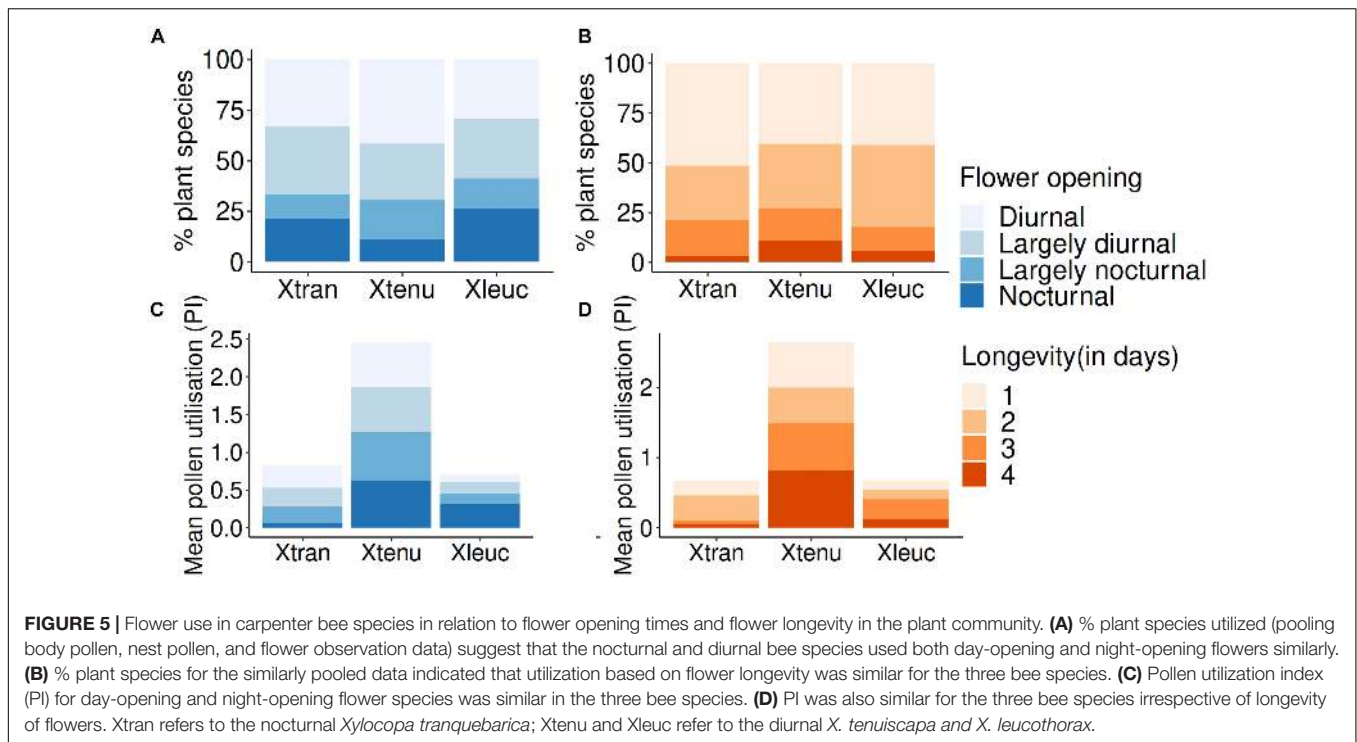


TABLE 1 | Generalized linear model for flower utilization by the three species of carpenter bees using data combined from nest traps, body pollen, and flower observations.

Predictors	Odds ratios	CI	P
Intercept	2.20	0.39–12.63	0.369
Flower opening time (anthesis)	1.31	0.62–2.81	0.482
Bee-Xtran	0.85	0.38–1.89	0.682
Bee-Xleuc	1.43	0.62–3.31	0.400
Length of flowering season (months)	1.32	0.43–4.55	0.639
Plant abundance_low	0.36	0.14–0.90	0.033
Plant abundance_medium	0.30	0.11–0.81	0.019
Flower longevity (days)	1.07	0.72–1.59	0.752

The explanatory variables included in the model were flower opening time (diurnal, largely diurnal, nocturnal, and largely nocturnal), flower longevity (in days), length of flowering season (number of months) for plant species and plant species abundance (binned as low, medium or high). Diurnal anthesis, Bee = *X. tenuiscapa*, Plant abundance = high were considered as base values by the model. A stepwise backwards removal model yielded the best fit and plant species abundance was the only variable that significantly explained flower utilization by the bee species. Xtran, Xleuc refer to the nocturnal *X. tranquebarica* and *X. leucothorax*, respectively (the full model is presented in the **Supplementary Table S3**). $P < 0.05$ indicated in bold.

Z scores for degree and normalized degree suggest that the null models were similar to the observed values (**Table 2**). Z scores for paired differences index in the overall network (**PDI**; **Table 2**), which indicates the level of generalization (0–1: generalist to specialist) suggest that the null models were significantly more specialized than the observed **PDI**. **PDI** was lowest in *X. tranquebarica* indicating greater generalization than the other bee species in the overall network. Czekanowski's index

indicated that overlap between pairs of bee species was similar (**Supplementary Table S5**).

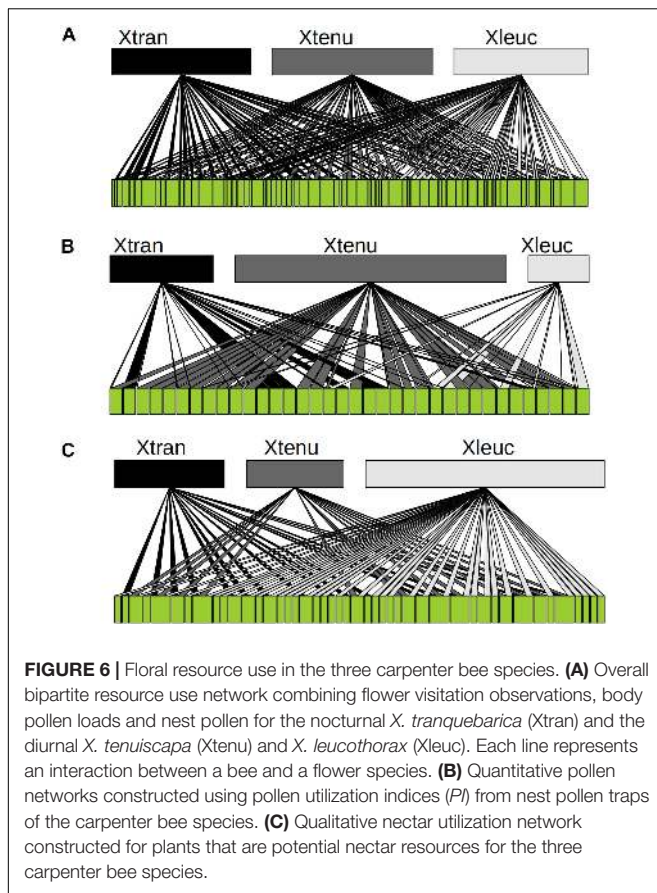
Nest Abundances

Mean number of nests (\pm SD) per transect differed between the three species (Kruskal-Wallis $\chi^2 = 35.9$, $p < 0.001$). *X. tenuiscapa* had significantly higher nest densities per plot (14.34 ± 17.33) than the nocturnal *X. tranquebarica* (3.28 ± 1.59) or *X. leucothorax* (2.05 ± 2.47 ; *post-hoc* pairwise Wilcoxon test with Bonferroni correction for all three species pairs, $p < 0.001$).

DISCUSSION

We found that resource use by the nocturnal *X. tranquebarica* is characterized by opportunistic and generalized feeding on day- as well as night-blooming flowers and by substantial overlap with the diurnal congeneric bees. However, foraging activity in the nocturnal bees is affected at very low light levels during moonless nights. Interestingly, the nocturnal bee showed lower floral constancy (for pollen) relative to diurnal bees, suggesting that the nocturnal lifestyle in this bee is accompanied by a change in foraging behaviors involved in the search and detection of flowers.

Recent studies have shown that transitions to nocturnality in bees, as in the paleotropical carpenter bee *X. tranquebarica* we studied and the neotropical sweat bee *Megalopta genalis*, are accompanied by remarkable visual adaptations in their apposition eyes (Greiner et al., 2004a,b, Warrant et al., 2004; Theobald et al., 2006; Somanathan et al., 2008b, 2009a, 2019; Warrant, 2008), including color discrimination under dim



light (Somanathan et al., 2008b). Though *X. tranquebarica* performed flights even on new moon nights, we found that flight activity and durations reduced significantly on new moon

nights compared to full moon nights. Moreover, flights of the nocturnal bee were shorter than those of the two diurnal species. A recent study on neotropical nocturnal bees reported that light intensity influenced flower visits more than other factors such as temperature, humidity, wind speed and flower availability (Liporoni et al., 2020). Hence we conclude that despite its remarkable extent of nocturnality, flights in *X. tranquebarica* are constrained on darker nights.

A comparative analysis of resource use by combining flower visitation, forager pollen loads and nest trap data revealed the following: Firstly, pollen loads of the nocturnal *X. tranquebarica* are smaller than pollen loads of *X. tenuiscapa* by a factor of 17 and *X. leucothorax* by a factor of 7, though their body sizes are similar and *X. tranquebarica* is covered with dense pubescence (greater pollen carrying potential) compared to the diurnal species. Secondly, the number of pollen species/types in pollen loads on individuals was higher in the nocturnal bee compared to the diurnal species, indicating lower flower constancy in the nocturnal bee compared to the diurnal species. This is particularly interesting because diurnal bees are generally known to show flower constancy during foraging bouts (Grant, 1950; Waser, 1986). Moreover, diurnal bees including carpenter bees are known to trap line during foraging bouts by moving between flowering individuals of a species in a fixed sequence (Saleh and Chittka, 2007; Somanathan et al., 2019). However, during a single foraging trip from the nest, the nocturnal *X. tranquebarica* collected pollen from more flower species than the diurnal species. Constancy and trap lining behaviors are hypothesized to be adaptive behaviors in pollinators leading to greater foraging efficiency or may be attributed to memory constraints (Chittka et al., 1999). From the plant's perspective, constancy is proposed to result in pollination efficiency by reducing the probability of heterospecific pollen transfer and negative effect on fitness through stigma clogging (Chittka et al., 1999;

TABLE 2 | Network metrics of floral resource use by nocturnal and diurnal carpenter bees.

	<i>X. tranquebarica</i> (Nocturnal)		<i>X. tenuiscapa</i>		<i>X. leucothorax</i>	
	Observed	z	Observed	z	Observed	z
Overall floral network						
Degree	38	-1.041	42	0.158	48	1.658
Normalized degree	0.535	-1.054	0.591	0.161	0.676	1.680
Paired differences index	0.471	-4.46**	0.414	-6.05**	0.328	-4.144**
Pollen network						
Degree	24	0.143	32	1.146	18	-1.289
Normalized degree	0.558	0.143	0.74	1.146	0.41	-1.289
Paired differences index	0.452	-0.14	0.26	-1.14	0.59	1.28
Nectar network						
Degree	16	-0.603	13	-0.805	27	1.409
Normalized degree	0.470	-0.603	0.382	-0.805	0.794	1.409
Paired differences index	0.545	0.603	0.636	0.805	0.212	-1.40

Three separate networks were constructed for overall floral resource use, pollen use, and nectar use. Overall and nectar networks are qualitative networks while pollen use was a quantitative measure (*PI*; see methods for details). Normalized degree in the overall network, which is a measure of the number of plant species utilized by a bee species out of the total number of plant species, was lowest in nocturnal *X. tranquebarica* and highest in *X. leucothorax*. The normalized degree for the pollen network was highest for *X. tenuiscapa*, followed by the nocturnal *X. tranquebarica* and was lowest for *X. leucothorax*. In the nectar network, the normalized degree was highest for *X. leucothorax*, followed by the nocturnal *X. tranquebarica* and lowest for *X. tenuiscapa*. Paired differences index (*PDI*) values in the pollen network were lowest for *X. tenuiscapa* indicating greater generalization than the other two bee species. *PDI* in the nectar network indicates greater generalization in *X. leucothorax* compared to the other two species. Standard scores (*z*) and *p*-values indicated by asterisks (***p* < 0.001) are derived from comparisons with 1,000 null models. These comparisons are significant only for *PDI* values in the overall network and suggest greater generalization in the observed means compared to the means from the null models.

Morales and Traveset, 2008) which may contribute to loss of paternal fitness due to erroneous pollen placement (Moreira-Hernández and Muchhala, 2019). Our finding opens up avenues for further enquiry on learning, memory and foraging efficiencies of this and other dim-light bee species. Thirdly, the nocturnal bee utilizes day- or night-opening, and short- or long-lived flowers in a community that comprises largely of day-blooming flowers. Finally, we found substantial dietary overlap between the three species. Thus, we conclude that the truly nocturnal *X. tranquebarica* is a resource generalist, an opportunistic forager on diverse flowers and subsists on partially depleted resources from day-opening flowers. Although the nocturnal *X. tranquebarica* collected fewer pollen, we do not know whether they also collected lower nectar quantities compared to the diurnal species. Nevertheless, since developing brood crucially depend on stored pollen, the paucity of pollen may have fitness consequences for *X. tranquebarica*. Indeed, nest densities of *X. tranquebarica* are lower than in the diurnal *X. tenuiscapa* and comparable to *X. leucothorax*. Also *X. tranquebarica* is univoltine while the two diurnal species are multivoltine (HS pers. obs.) suggesting fitness costs. To determine voltinism, females returning to nests with pollen and presence of brood in a few nests that were opened up through the year were noted.

It is not known when nocturnality evolved in *X. tranquebarica*, although the Oriental/Palaearctic subgenus *Nyctomellita* to which it belongs dates back to 33 mya (Leys et al., 2002). The temporal transition to nocturnality in bees is commonly assumed to be related to an escape from competitors and/or enemies and is presumed to have been facilitated by the availability of flowers bearing bat-pollination syndromes (Roulston, 1997; Wcislo and Tierney, 2009). However, the “rich nocturnal resource niche” hypothesis, proposed to explain evolution of nocturnality in bats (Rydell and Speakman, 1995) does not appear to define resource use in *X. tranquebarica*.

Given the work on visual adaptations in nocturnal bees (Greiner et al., 2004a,b, Warrant et al., 2004; Kelber et al., 2006), we propose several avenues of research that arise from our findings. (1) At the mechanistic level, how does the nocturnal *X. tranquebarica* detect flowers? Color is an important sensory modality in diurnal bees, though odor, shape, texture, patterns, temperature, etc. are also attractants (Kevan and Lane, 1985; Chittka and Raine, 2006; Dyer et al., 2006; Schiestl and Johnson, 2013; Balamurali et al., 2015). *X. tranquebarica* can discriminate color landmarks during nocturnal homing (Somanathan et al., 2008b) and they visit flowers with varied display attributes (color, size, and shape) in our site and in Thailand (Burgett et al., 2005), which suggests involvement of varied sensory cues during foraging. Two recent studies have demonstrated that nocturnal halictid bee species belonging to the genus *Megalopta* are attracted to odor baits (Carvalho et al., 2012; Knoll and Santos, 2012). Furthermore, the lower flower constancy in this species, at least with respect to pollen collection, merits further investigation into how nocturnal bees detect and memorize features of flowers and their spatial locations. Whether nocturnal carpenter bees are capable of trap lining between specific flower

locations as has been suggested for diurnal carpenter bees (Somanathan et al., 2019) is unknown. (2) The contribution of nocturnal bees to pollination services at night is unknown. This study indicates that *X. tranquebarica* is a flower generalist, as are nocturnal sweat bees *Megalopta genalis* and *M. ecuadoria* in the neotropics (Wcislo et al., 2004). The role of nocturnal bees in pollination requires wider investigation. This has been studied so far only for one night-blooming species (Somanathan and Borges, 2001). (3) Does operating in the night environment constrain fitness in nocturnal bees? Smaller population size, smaller pollen loads, the flight constraints on darker nights and the univoltine lifecycles in *X. tranquebarica* suggest fitness costs compared to diurnal conspecifics. (4) If *X. tranquebarica* largely subsists on residual diurnal floral resources with possible fitness consequences, then why is nocturnality maintained? The answer could lie within the realms of greater competition and higher risk of predation during the daytime for which we have accumulated evidence (unpublished results). The relative roles of competition, rewards and predators in mediating nocturnality in bees remain to be fully understood. Resource use in nocturnal bees must be examined in other geographical locations to confirm if the flight and foraging constraints we found in this site applies to other habitats.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.5061/dryad.34tmpg4g0>.

AUTHOR CONTRIBUTIONS

HS, AK, and RB formulated the study. HS, SK, and EJ performed the field work. HS and VG analyzed pollen samples. HS and SK analyzed data. HS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.566964/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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