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ORIGINAL ARTICLE

# Chemical components change along the ontogeny of a bat fruit (*Neolamarckia cadamba*) with ripening asynchrony in favour of its fruit selection and seed dispersal

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## Abstract

An asynchronous fruit-ripening strategy can enhance the chance of seed dispersal by providing ripe fruits for an extended period to foragers. However, mechanisms associated with this strategy that can facilitate seed dispersal are understudied. This study aimed to investigate whether the chemical components (minerals and secondary metabolites) of a bat fruit with ripening asynchrony change along its ontogeny (Rubiaceae: *Neolamarckia cadamba*). We predicted that the seed-to-pulp ratio would increase along with fruit ripeness. The chemical components of the fruit were also predicted to change along their ontogenesis in favour of fruit selection and seed dispersal by fruit bats. Our study shows that the asynchronous fruiting strategy limited the number of ripe fruits daily so that fruits were available at a steady rate. As predicted, the seed-to-pulp ratio of each fruit increased along with fruit development. A fruit's mineral concentration also increased as fruit developed, with a sharp jump at full ripeness, when fruit colour also changed. In contrast, the concentration of secondary metabolite compositions decreased gradually during the process of ontogeny. Fruit bats (Pteropodidae: *Pteropus giganteus* and *Cynopterus sphinx*) were the only nocturnal frugivore visitors of these trees and their fruit selection was driven by fruit size and colour. Both bats preferably consumed ripe fruits, which had a higher concentration of attractants (essential minerals) and a lower concentration of deterrents (secondary metabolites), supplemented with a higher seed-to-pulp ratio. The bats exhibited different foraging patterns and home ranges resulting in dispersal (as measured by feeding roost location) occurring across different spatial scales. Our study shows that the chemical components involved in an asynchronous fruit-ripening process could select for extended fruit availability by intensifying the demand for each ripe fruit among legitimate seed dispersers, which increases the likelihood of fruits being dispersed away from parent crowns.

**Keywords** Bat fruits · Fruit bats · Fruit chemistry · Fruit colour · Seed-to-pulp ratio · Seed dispersal

## Introduction

Plants have evolved different mechanisms for attracting their seed dispersers. Synchrony and asynchrony are the two major strategies of fruit ripening exhibited by zoochorous fruits, and both strategies are common in the tropics (Poulin et al. 1999). Synchronously fruiting trees exhibit a "big bang" or mass fruiting pattern for a shorter duration (Gentry 1974; Corlett 2006) and often attract frugivores in plenty through vivid displays enabling visual cues (Bawa 1983; Fleming 1988; Lambert and Marshall 1991; Elangovan et al. 1999). In contrast, asynchronously ripening trees exhibit a "steady-state" pattern of producing few fruits for a longer duration (Gentry 1974; Corlett 2006), which facilitates the

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reliable and continuous supply of fruits to legitimate seed dispersers (Ohashi and Thomson 2009).

Asynchrony may improve the chances of seed dispersal by ensuring the regular visits of foragers (Gorchov 1988). For example, the long-lasting fruit availability within the tree could suit the foraging behaviour of some bat seed dispersers, i.e. trapline foraging, which is repeated sequential visits to a series of feeding locations for a prolonged duration (Smythe 1970; Sazima et al. 1999). Furthermore, asynchrony limits ripe fruit availability, which increases the demand for the fruits and thereby reduces the chances of decay and seed predation (Thompson and Willson 1979; Herrera 1982). In short, asynchrony lengths the availability of ripe fruits and maximises the chance of each fruit attracting a frugivore and increasing the likelihood of seed dispersal (Janzen 1979; Korine et al. 2000).

Among the animal-mediated dispersal systems within angiosperms, at least 549 species in 191 genera of 62 families have traits that match the bat-fruit syndrome (Geiselman et al. 2002; Lobova et al. 2009); these fruits often exhibit strong odour, dull colour and exposed positioning (van der Pijl 1957). Bat fruits commonly exhibit the asynchronous ripening strategy (Lambert and Marshall 1991; Justiniano and Fredericksen 2000; Thies and Kalko 2004), which could be an evolutionary adaptation to the behaviour of dispersers, such as trapline foraging (Janson 1983; Marshall 1983; Sazima et al. 1999; Hodgkison et al. 2013). Pteropodid bats are one of the main seed disperser groups in the Old World tropics (Corlett 2011), and they prefer pulpy nutrient-rich fruits with high proportions of carbohydrates, proteins, lipids and essential minerals (Ruby et al. 2000; Barclay 2002). Thus, fruit bats and chiropterochorous plants have mutualistic relationships, and these are essential to the health of the ecosystems in which they occur (Jordano 2000; Bollen et al. 2004; Aziz et al. 2021).

The sense of taste allows animals to evaluate and predict the quality of many food resources (Muñoz et al. 2020; Jiao et al. 2021; Demi et al. 2021). Defence against seed predation, particularly before the fruit becomes fully ripe, is a requirement of plants for successful reproduction. Hence, many plants have evolved secondary compounds such as alkaloids and tannins, to make the fruits less attractive and prevent the removal of unripe fruits (Heil et al. 2002; Hornoy et al. 2012). In many species, chemical-mediated defences, especially alkaloid based, play a significant role as deterrents and help to discourage frugivores from consuming unripe fruits (Cazetta et al. 2008; Matsuura and Fett-Neto 2015). Thus, it is a common phenomenon that many unripe fleshy fruits hold a higher concentration of secondary metabolites that are often noxious (McKey 1974; McKey et al. 2010; Whitehead and Bowers 2014). In case the preventive taste-driven barriers are overridden, the noxious compounds (i.e. alkaloids and tannins) of immature fruits can cause physiological constraints on frugivore metabolism that are detrimental to their health (Detzel and Wink 1993) are often neutralised by frugivores through adaptive behaviours (Mahandran et al. 2016).

Fruit chemistry is known to change during the ripening of fruits from higher to lower concentrations of secondary metabolites (e.g. alkaloids and tannins) (Cipollini and Levey 1997a, b; Schaefer et al. 2003; Cazetta et al. 2008; Whitehead and Poveda 2011) while it is vice versa in the case of essential minerals (e.g. Na, Ca, Fe, K and Mg) (Diamond 1999; Voigt et al. 2008; Mahandran et al. 2016). The chemical components are generally known to have the function of attraction and defence; however, the interrelationship between these compounds during their ontogeny has not been fully explored.

Since we have a limited understanding of the chemo-ecological aspects of bat-plant interactions, this study was aimed at answering the following questions with reference to a typical bat fruit, *Neolamarckia cadamba*: (i) Does ripening asynchrony limit the availability of ripe fruits and result in spatiotemporal partitioning of resources among sympatric fruit bats in the foraging tree? (ii) How do the seed-to-pulp ratio and the concentration of chemical components change along their ontogenesis in trees that have asynchronous fruit-ripening? (iii) How does the difference in the foraging behaviour of sympatric bats contribute to the transportation of seeds at different spatial scales?

First, due to the presence of two sympatric bats and the limited availability of ripe fruits per night, we expected these two bat species to exhibit both spatial and temporal partitioning to minimise interspecific interference in the foraging tree. At the same time, we expected intraspecific competition to occur during foraging bouts by the two bat species. Second, we predicted that the seed-to-pulp ratio and the concentration of chemicals in fruits would change during the process of ripening. Specifically, we predicted that the seed-to-pulp ratio and concentration of essential minerals in fruits would increase as fruit size increased, whereas the concentration of secondary metabolites would decrease. Finally, the difference in behavioural repertoires (viz. feeding time, time spent in the tree and aggressive interactions) of sympatric bats, perhaps driven by asynchronous ripening, might enhance seed dispersal to different distances, viz. short and long distances.

## Materials and methods

### Plant species

*Neolamarckia cadamba* (Rubiaceae) is a large-sized deciduous tree that attains a height of around 20–22 m and is widely distributed across tropical and subtropical countries in southern and south-eastern Asia (Orwa et al. 2009). This is an important tree in Ayurveda, an Indian indigenous system of medicine, in which its various parts (viz. barks, roots, leaves and fruits) are known to have a multitude of remedial functions against various ailments such as fever, uterine complaints, skin diseases, inflammation, anaemia, dysentery, leprosy and cancer (Mondal et al. 2009). Fruits are fleshy globose pseudocarps, orangish when ripe with muriculate seeds (2229 ± 159 seeds; $n = 30$ ripe fruits; mean ± SD). The flowering of these trees occurred in the late spring (from October to November) and fruiting in the early summer (from February to March) (Fig. S1). These trees exhibited an asynchronous fruit-ripening pattern and the fruit availability lasted for about 4–6 weeks.

## Bat species

Two sympatric Old World pteropodid bats, *P. giganteus* (*ca.* 1500 g) and *C. sphinx* (*ca.* 60 g), were the only nocturnal frugivore visitors of *N. cadamba* fruits. Both the bat species have a wide geographical distribution. The former species is distributed across the tropical regions of South Central Asia, including India, Pakistan, China and as far south as the Maldive Islands (Nowak 1999). The latter species is widely distributed across the Indo-Malayan biogeographic region making up South Asia, Southeast Asia and Southern China including Taiwan (Storz and Kunz 1999).

## Study site and study period

We conducted the field observations on bat foraging and fruit sample collections from the *N. cadamba* trees ($n = 10$) in the Vaigai riverbanks (geographical coordinates: 9°93′N and 78°12′E; 101 m.asl), Tamil Nadu, India. During the study period (2019–2020), the study site experienced warm and humid subtropical weather (29–42 °C, min–max). This site was predominantly comprised of agricultural fields with crops such as cotton (*Gossypium hirsutum*) and groundnut (*Arachis hypogea*). Besides, there were other trees such as *Alstonia scholaris* (Apocynaceae), *Azadirachta indica* (Meliaceae), *Ceiba pentandra* (Malvaceae), *Delonix regia* (Fabaceae), *Pongamia pinnata* (Fabaceae), *Ficus benghalensis* (Moraceae), *Ficus religiosa* (Moraceae), *Madhuca latifolia* (Sapotaceae) and *Polyalthia longifolia* (Annonaceae) along with *N. cadamba*.

## Fruit sampling

Fruits were categorised into two groups based on their size and ripeness: unripe fruits were dark green and small (weight: 76.99 ± 14.25 g, diameter: 51.43 ± 8.64 mm; $n = 60$; subjected to morphometry), while ripe fruits were dark yellow and large (weight: 119.63 ± 6.09 g, diameter: 68.30 ± 1.87 mm; $n = 30$; subjected to morphometry only). There were no large unripe fruits and/or small ripe fruits; hence, it was not possible to separate attraction based on fruit size from attraction based on ripeness. For further studies, we collected three fruits from a single tree with differences in size and ripeness. The ripeness was judged by the palpable softening of fruits. We did the same for all the 10 trees, and thus, we obtained 30 fruits from 10 trees, for which we estimated the seed-to-pulp ratio and quantified the chemical components (i.e., secondary metabolites and essential minerals). For each fruit, measurements (weight and diameter) were taken immediately after collection. Thereafter, the fruits were diagonally cut into eight equal parts, and from them, two diagonal parts were randomly selected to count the number of seeds. The number obtained for two parts was multiplied by four to estimate the total number of seeds present in the fruit. This number was divided by total fruit weight to estimate the seed-to-pulp ratio per gram. Fruits that weighed < 105 g were hard and green in colour and were grouped as unripe and those > 105 g were soft and yellow in colour and were grouped as ripe. All the 10 trees selected for fruit sampling were located at least 500-m away from each other.

For the estimation of mineral and secondary metabolite contents present in the fruits, the collected fruits were further cut into smaller pieces and the seeds were manually removed with forceps and needles. The remaining pulp was oven-dried at 60 °C for about 96 h, powdered and then stored at – 20 °C in zip lock bags until used for the chemical analysis (samples were stored for about 2 months). The prepared fruit samples were subjected to atomic absorption spectrometry (iCE 3000 AA1143001 v1.30; Thermo Scientific, USA) to estimate the concentration of five essential minerals, viz. sodium (Na), calcium (Ca), potassium (K), iron (Fe) and magnesium (Mg), based on Bravo et al. (2012). The same fruit samples were further subjected to UV–Vis Spectrophotometry (UV-2301; Thermo Scientific, USA) to estimate the concentration of four secondary metabolites, viz. total alkaloids, total tannins, total coumarins and total saponins based on respective standard methods (Roberts and Link 1937; Peri and Pompei 1971; Wink 1993; Harborne 1998). The values of both the mineral and secondary metabolite concentrations were represented in parts per million (ppm).

## Ripe-unripe ratio

The availability of ripe and unripe fruits on the focal tree was visually estimated by counting fruits on six branches selected at random and multiplying them with the total number of tree branches (Chapman et al. 1992). This was performed before each observation schedule, which began at around 17:30 h (at least 1 h before the sunset to ensure precise estimation of ripe and unripe fruits before the focal observations on bats).

## Field observations on frugivory

Pilot field observations were conducted on a tree both during the day (from 05:30 to 18:30 h; $n=3$ days) and night (from 18:30 h to 05:30 h; $n=3$ nights) to identify the main frugivore visitors to these fruits. Since these fruits were mostly visited by bats at night time, we subsequently limited our field observations to nighttime from dusk to dawn (from 18:30 to 05:30 h), with the support of red-filtered headlamps (Tikka Plus 2; Petzl, USA). In the results, we summarise the daytime data collected during this pilot study to provide a brief overview of all frugivores, whereas our detailed data for bats were collected anew from other fruiting trees ($n=4$). Our observations were limited to four trees as the other six trees were in a place that was not suitable for conducting focal observations and feeding roost observations for estimating the minimum seed dispersal distances as there were other adult conspecifics in its vicinity. Two observers simultaneously conducted focal observations on the selected four fruit-bearing trees (weekly once per tree, for 4 weeks) and obtained 256 h of focal animal observations (8 h per night × 16 nights × 2 observers). While the first observer focused on the smaller bats (*C. sphinx*), the second observer focused on the larger bats (*P. giganteus*). Each observer conducted focal observations on one individual bat at a time, focusing on bats that fed on branches observed to have clear visibility from different angles. We omitted data from those individuals that were lost from our sight before it left the tree. An additional observer (8 h per night × 16 nights = 128 h) focused on counting the entry and exit of individuals of each bat species on an hourly basis (temporal variation) and their location of landing and feeding territories of two bat species in the foraging tree (spatial partitioning).

The focal animal sampling method (Altmann 1974) was used to measure and quantify the foraging activities of bats. Specifically, we recorded feeding time, total time spent on the tree and aggressive interactions. Species of bats were distinguished based on their distinctive morphological characters (Bates and Harrison 1997) when they alighted on the tree to feed. The number of bouts made by the smaller bats (*C. sphinx*) to the foraging tree was manually counted using a handheld tally counter. A "bout" was defined as the entry of a bat to the tree in search of fruits and its exit with or without consuming a fruit. The presence of larger bats (*P. giganteus*) in the tree was estimated by scanning the tree every 10 min and the number of bats counted during each scan, as these bats usually spent a longer duration in the tree, unlike the smaller bats. Meanwhile, the fruit type (ripe or unripe) consumed by each bat species was also recorded. Feeding time denotes the time taken to consume a single fruit. Total time spent by individual bats on the foraging tree was recorded as the time from arrival to departure and including fruit consumption. Only those bats that consumed a major proportion of each fruit (roughly 90%) were considered as consuming an entire fruit and these observations were used to estimate feeding time.

## Feeding roosts and seed dispersal distances

Considering the difficulty in tracking the visitor bats and bat-dispersed seeds in the natural settings, we searched for feeding roosts around the focal trees during the daytime to calculate minimum seed dispersal distances. In the subsequent morning hours (between 06:00 and 07:15 h), we walked around the parent tree in concentric circles to a maximum radius of 500 m in search of fresh boluses (Tang et al. 2012). Feeding roosts of bats were distinguished by the presence of fresh remnants of chewed boluses (with few seeds) that were dropped by bats during the previous nights. Follow-up observations at night were made at these feeding roosts to confirm the identity of the bat species using the roost. Seed dispersal distances were recorded by measuring the distances between feeding roosts and the nearest fruit-bearing trees. By this method, we could only estimate the minimum seed dispersal distances and not the average or maximum seed dispersal distances.

## Statistical analyses

We tested all datasets for normality using the Shapiro–Wilk test, and wherever the data deviated from normality we performed non-parametric tests (Zar 2007). To test the temporal variation in the foraging hours within and between two bat species, we grouped the number of bat visits at hourly intervals across the nighttime; the pattern was tested using a Kruskal–Wallis test and the peak foraging time was determined using Dunn's post hoc test. The prediction that ripe fruits were consumed more often by frugivores (because they were of higher quality), than unripe fruits, the number of consumption of each fruit type (unripe vs. ripe) was compared using the Mann–Whitney $U$-test assuming a $z$ distribution, for each bat species. To test the prediction that the seed-to-pulp ratio increased with an increase in fruit size, we subjected the data (seed-to-pulp ratio and fruit weight) to least-squares regression analysis (Pearson's $r$). We predicted that large ripe fruits were of higher quality because they had a higher concentration of minerals and a lower concentration of secondary metabolites. To test this prediction, associations between the fruit weight and corresponding concentration of minerals and secondary metabolites were assessed using two regression analyses, polynomial fit (Adj. $R^2$) and a linear fit (Pearson's $r$), respectively. The difference in feeding time, time spent on the feeding tree and the seed dispersal distance of the two sympatric bat species was compared using a Mann–Whitney $U$-test assuming a $z$ distribution. All the statistical tests were performed using OriginPro ver.2020b (OriginLab Corporation, Northampton, MA 01,060, USA).

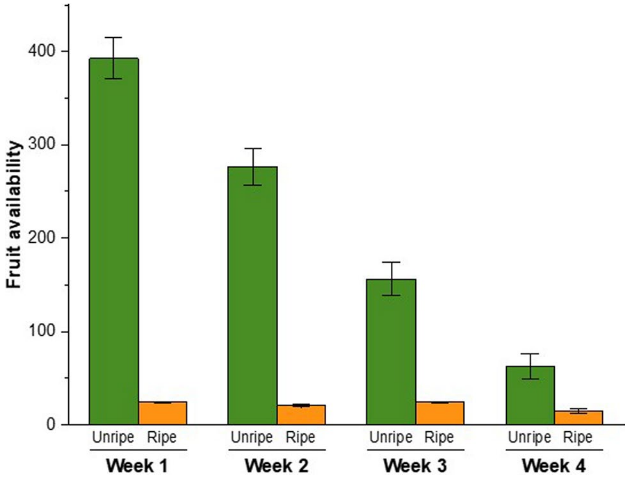


**Fig. 1** Asynchronous fruit-ripening pattern and fruit availability of *N. cadamba* ($n = 4$) observed in southern India. Values are represented as mean ± SD (refer Fig. S2 for additional visualisation of asynchronous ripening of fruits at the day level on individual trees)

## Results

### Fruit ripeness, frugivore visitors and fruit selection

At the start of the fruiting ripening phase, a single medium-sized *N. cadamba* tree held about 500 fruits. For each individual tree, the supply of ripe fruits each day was observed to be at a steady rate (21.1 ± 4.7 ripe fruits/tree/day; $n = 16$ days, i.e. 4 days each for four trees) throughout the whole fruit season, regardless of the total number of fruits on a tree. This confirms that these trees exhibit a typical asynchronous fruit-ripening pattern (Fig. 1). Furthermore, this pattern was consistent among the individual trees (Fig. S1 and S2).

The fruit-bearing trees of *N. cadamba* received more frugivore visits during the nighttime compared to the daytime. Daytime visitors comprised squirrels ($n = 19$), koels ($n = 9$) and mynahs ($n = 5$) that visited these trees infrequently over the 3 days of the pilot study and consumed fruits in situ, especially during the early morning hours. Whereas throughout the night, two species of pteropodid bats, namely *P. giganteus* and *C. sphinx*, were frequent visitors (Fig. 2). Foraging activities of both bat species began *ca.* 45 min after the sunset (18:45 h). On average, each fruit-bearing tree received about 140.0 ± 11.0 bouts/night from the smaller *C. sphinx* and 24.0 ± 3.0 visits/night from the larger *P. giganteus* ($n = 16$ nights). However, all these bat visits did not necessarily result in successful fruit consumption.

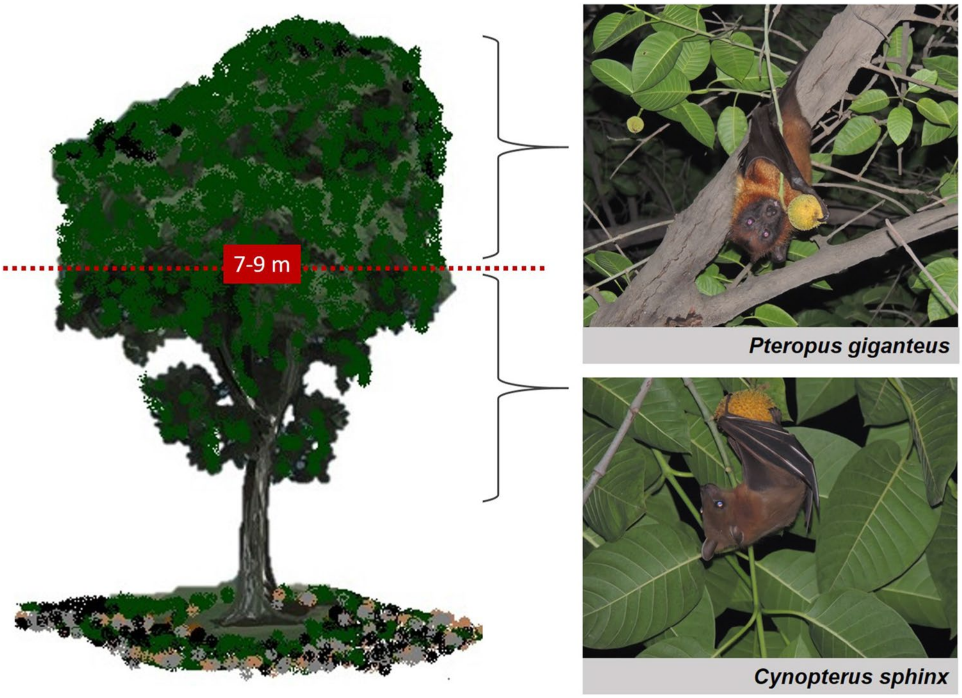


**Fig. 2** Diagrammatic illustration showing the spatial segregation of two species of sympatric fruit bats, *P. giganteus* and *C. sphinx* while foraging on fruits of *N. cadamba*. The dotted red line indicates the spatial boundary at about 7–9 m from the ground. While the lower portion is predominantly used by the smaller bats, the upper portion canopy is used by the larger bats

Both the bat species visited the *N. cadamba* trees continuously throughout the nighttime and both exhibited significant within-species temporal variation in foraging activity across the night (measured at hourly intervals) (i.e. *C. sphinx*: Kruskal–Wallis $H = 169.9$, $df = 11$, $P = 0.001$ and *P. giganteus*: Kruskal–Wallis $H = 160.50$, $df = 11$, $P = 0.001$). There was also temporal variation between the two bat species, as they displayed different periods of peak foraging time; the peak activity of the larger bats (*P. giganteus*) occurred at 20:00 h, whereas the peak activity of the smaller bats (*C. sphinx*) occurred at 21:00 h (Fig. S3).

Spatial partitioning was observed within the foraging tree between the two bat species. The large-bodied *P. giganteus* foraged from the upper to the middle (i.e. $\geq$ 7–8 m) canopy layers, whereas the small-bodied *C. sphinx* used lower to middle (i.e. $\leq$ 7–8 m) canopy layers (see Fig. 2 for diagrammatic illustration). The foraging behaviour of these two bats also differed; *P. giganteus* searched for fruits on the different branches using biped and quadruped movements, while *C. sphinx* searched for suitable fruits by hovering around the fruits for a few seconds (3–5 s) before alighting on it for consumption. However, both the bats selected ripe fruits over unripe fruits (*C. sphinx*: $z = -5.69$, $P = 0.001$ and *P. giganteus*: $z = -5.69$, $P = 0.001$) (Fig. 3). The consumption of unripe fruits by bats usually occurred after the peak foraging hours.

## Relationship between the fruit size and seed-to-pulp ratio

As predicted, the seed-to-pulp ratio increased with an increase in the fruit size (i.e., fruit weight) and there was a significant positive correlation (Pearson's $r = 0.90$, $P < 0.05$; Fig. 4), indicating that large ripe fruits have the highest seed-to-pulp ratio.

## Chemical basis of ontogeny in fruit ripening

The concentration of selected mineral compounds was strongly associated with increases in fruit weight during the process of ontogeny from unripe to ripe (i.e. Na, Adj $R^2 = 0.87$; C, Adj $R^2 = 0.85$; K, Adj $R^2 = 0.82$; Fe, Adj $R^2 = 0.88$; Mg, Adj $R^2 = 0.89$ ($n = 30$ fruits); the fitting function was statistically significant at $P < 0.05$ level). A sharp increase in the concentration of all the selected minerals occurred when the fruit colour changed from dark green (unripe) to dark yellow (fully ripe) (Fig. 5). Conversely, the concentration of secondary metabolites was strongly, negatively correlated with fruit size (i.e., alkaloids, Pearson's $r = -0.96$; tannins, $r = -0.84$; coumarins, $r = -0.77$; saponins, $r = -0.76$ ($n = 30$ fruits); the fitting function was statistically significant at $P < 0.05$ level) with reference to an increase in fruit weight (Fig. 6).

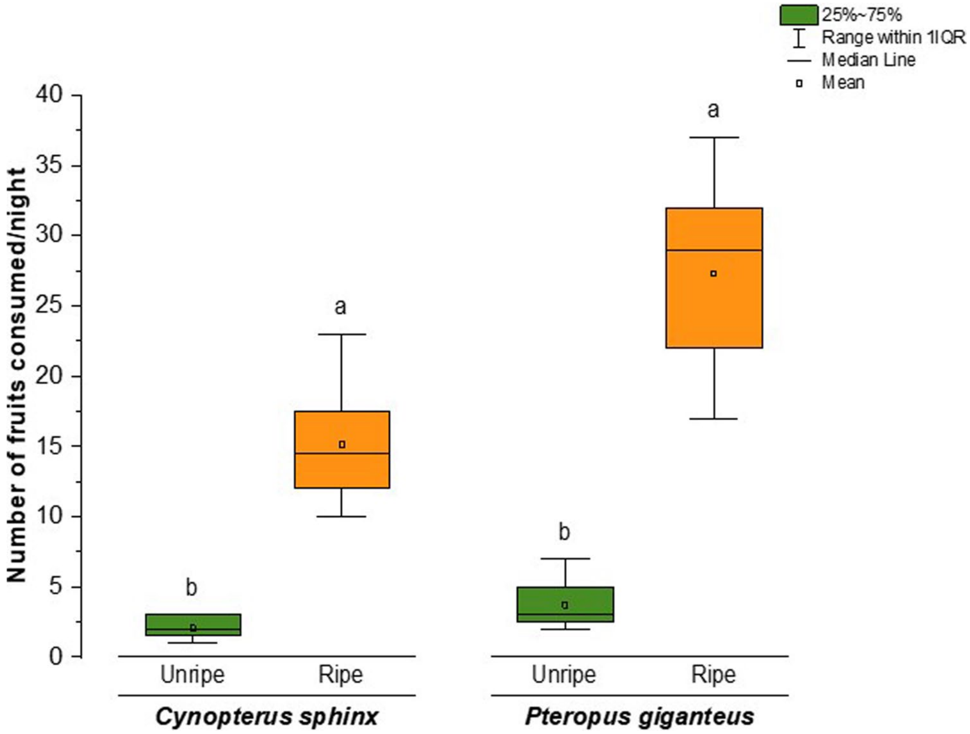


**Fig. 3** Fruit selection by two sympatric fruit bats (*C. sphinx* and *P. giganteus*) while foraging on the asynchronous fruiting trees (*N. cadamba*). Lowercase alphabets denote significant within-species differences at 0.001 levels

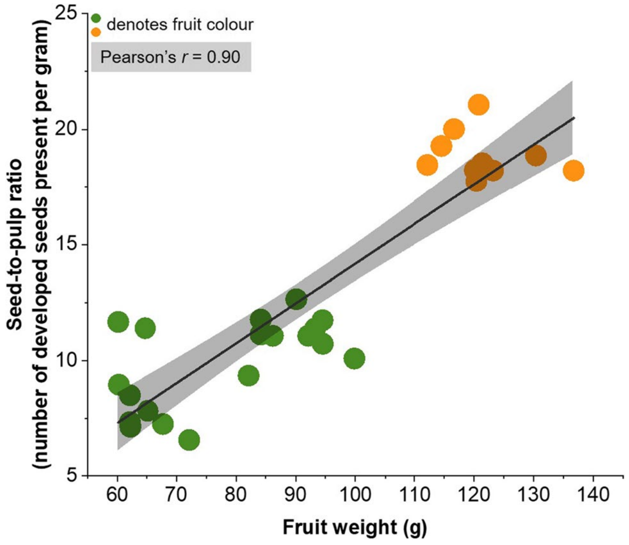
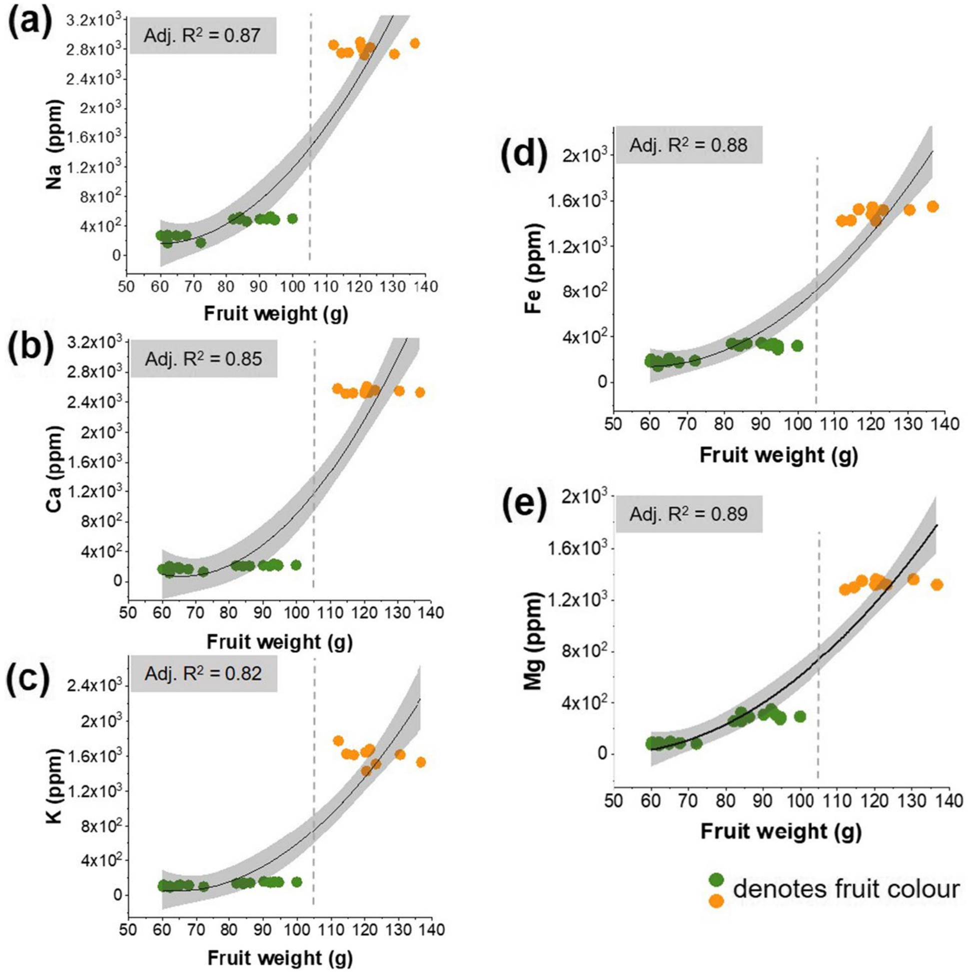


**Fig. 4** A least-squares regression plot (linear fit line with 95% CI) showing a positive correlation between the fruit weight and seed-to-pulp ratio

## Feeding behaviour of pteropodid bats

After alighting on a branch, the large bats (*P. giganteus*) searched for the ripe fruits by biped and quadruped movements and retrieved them by pulling the fruit-bearing long stalks close to them using their forewings and clawed thumbs. They held the fruits using their forearms, consumed them in situ by biting, chewing and sucking the juice along with the soft tissues comprising its seeds, and then, they spat out the boluses along with few seeds. The smaller bats (*C. sphinx*) appeared to evaluate the fruit quality by hovering near a fruit. After that, they alighted on the fruit of their choice and consumed the fruits *P. giganteus*. The small bats took more time to consume the fruits (20.52 ± 3.07 min, $n = 125$; consumption of ripe and unripe fruits combined), compared to the large bats (05.19 ± 1.08 min, $n = 151$; consumption of ripe and unripe fruits combined, $z = 15.21$, $P = 0.001$). This resulted in longer periods of time spent on the tree by smaller bats (28.76 ± 3.42 min, $n = 125$) when compared to the larger bats (12.36 ± 1.91 min, $n = 151$, $z = 13.24$, $P = 0.001$; Fig. 7).

**Fig. 5** Least-squares regression plots (polynomial fit line with 95% CI) showing a sharp increase (a significant positive correlation) in the concentration of the selected minerals (viz. Na, Ca, K, Fe and Mg) along the ontogeny of fruits (see the dotted line at a 105-g level in the x-axis that divides fruits based on ripeness)



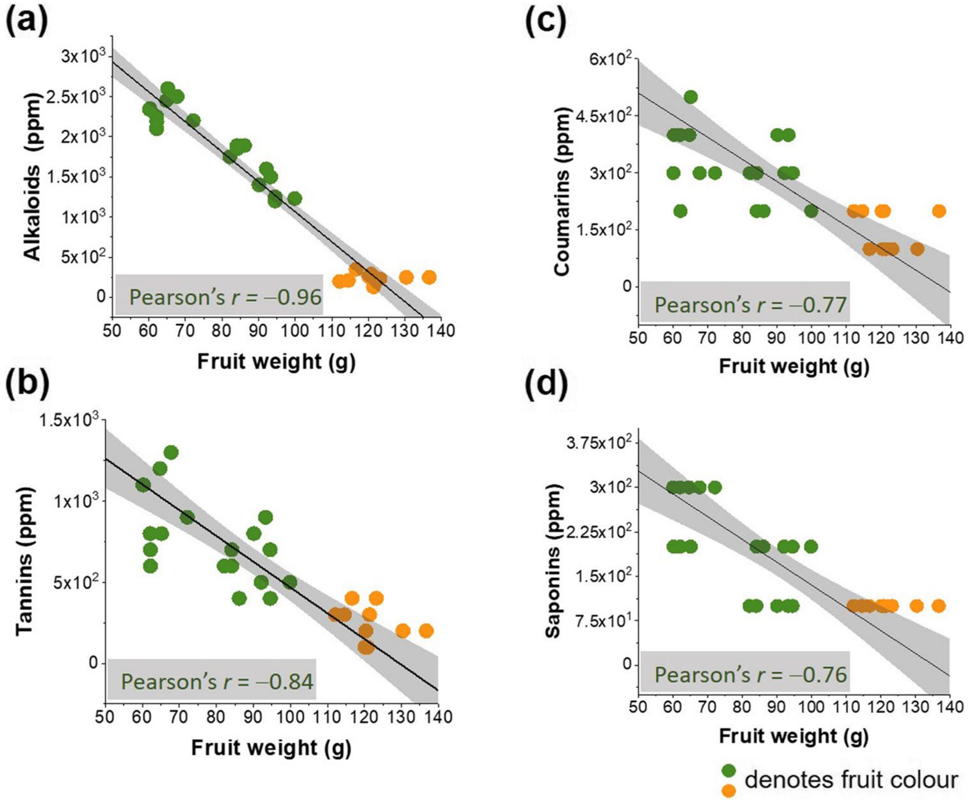


**Fig. 6** Least-squares regression plots (linear fit line with 95% CI) showing a gradual decrease (a significant negative correlation) in the concentration of the selected secondary metabolites (viz. alkaloids, tannins, coumarins and saponins) along the stages of ontogeny in the process of getting ready for dispersal

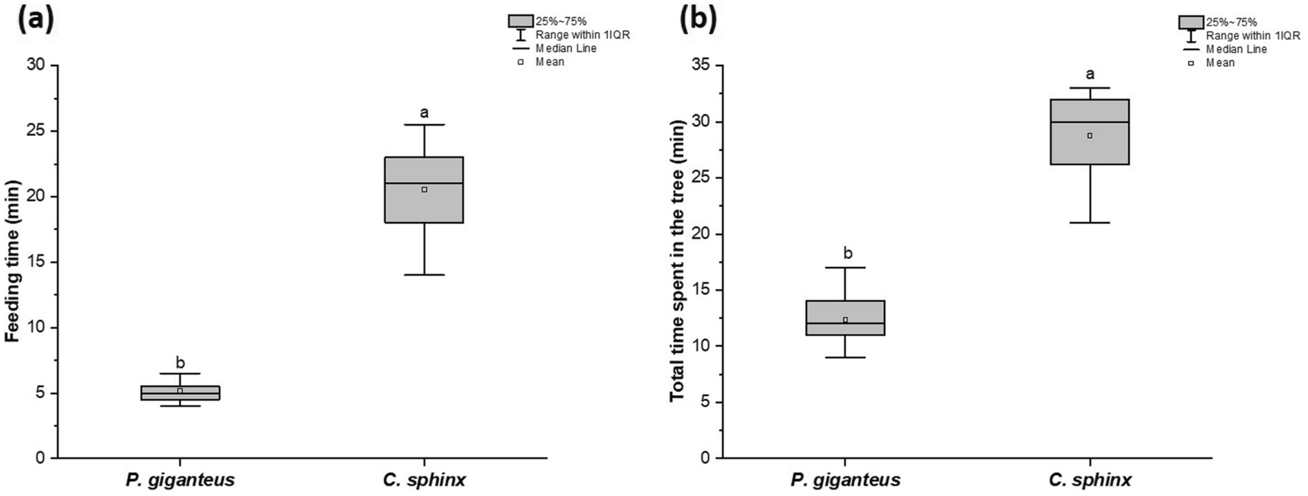


**Fig. 7** Differences in **a** feeding time and **b** total time spent on the fruit-bearing trees of *N. cadamba* by two sympatric bat species. Different lowercase alphabets show significant differences at 0.001 levels

Competition for resources occasionally resulted in aggressive encounters, especially among the conspecifics of the large bats. When this happened, an individual would leave the fruiting tree with or without consuming fruits, unless they were dominant enough to defend fruits on their feeding branches (bats often emitted loud vocalisations to guard fruits from conspecifics). For the large bats, these aggressive interactions forced the less dominant individuals to carry entire fruits away for ex situ consumption at feeding roosts ($n = 63$; i.e. 29.43% instances). The minimum distance between the parent tree and the adjacent feeding roosts and/or day roosts where the boluses of these fruits were found was between 180 and 450 m ($n = 23$ feeding roosts found around four parent trees); the maximum distance could not be estimated since it was hard to manually track and trace feeding roosts over long distances. Similarly, when the smaller bats encountered interferences from their conspecifics they departed from the tree with a mouthful of fruit to their feeding roosts ($n = 27$; i.e. 17.76% instances). The minimum distance between the parent tree and the feeding roosts of these bats ranged between 45 and 72 m ($n = 45$ feeding roosts around four parent trees). There was a significant difference in the minimum seed dispersal distances achieved by these two bats ($z = -6.72$, $P = 0.001$), with the larger bats dispersing seeds further (Fig. 8).

## Discussion

Numerous studies have demonstrated the production of distinctive fruit scents by bat fruits to signal ripeness (e.g. Hodgkison et al. 2003, 2013; Ripperger et al. 2019), but only a few studies have focused on the function of other chemical components of the fruits (e.g. Whitehead et al. 2016; Baldwin et al. 2020). Our study investigated the chemical basis of ontogenesis in a typical bat fruit (*N. cadamba*). The asynchronous fruit-ripening strategy exhibited by these plants ensured a limited, but steady supply of ripe fruits. For the bats, this meant, fruits were available over a long period, but it also intensified competition for ripe fruits each night. The chemical components of the fruits played dual roles as repellents and attractants, and these components varied in their concentration during the ontogeny of the fruits. Only a few fruits (i.e. 21.1 ± 4.7) were available at a time for frugivores; the higher availability of minerals in these fruits was signified by the ripe fruit colour. In contrast, the remaining immature fruits were chemically defended from seed predators (from premature fruit consumption) by higher investment in secondary metabolites. This suggests a functional shift of chemicals for predator defence to frugivore attraction when seeds are ready for dispersal. Hence, the asynchronous fruit ripening resulted in a continuous and steady supply of ripe fruits for about 4–6 weeks, which suited the trapline foraging strategy of bats (Sazima et al. 1999) and ensured the bats could forage on almost all the fruits in the crop.

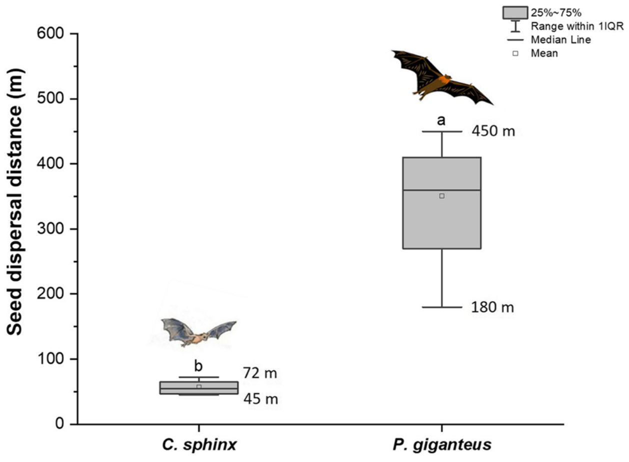


**Fig. 8** Differences in the minimum seed dispersal distance of *N. cadamba* by two sympatric bat species. Values near the whiskers indicate the seed dispersal distance range of each species (as measured by feeding roost location). Different lowercase alphabets show significant differences at 0.001 levels

Both the bat species preferably consumed ripe fruits. Peaks in foraging activity occurred early in the night for both species, possibly to increase the likelihood of individuals accessing the limited supply of ripe fruits. The change in fruit colour from dark green to dark yellow indicated fruit ripeness. Fruit colour is known to have a functional role in driving fruit selection in other nocturnal frugivores such as mouse lemurs (Valenta et al. 2013) and could similarly be a visual cue for bats to determine fruit ripeness (Mahandran et al. 2021). Ripe fruits were most beneficial to the bats because of the sharp jump in the concentration of minerals on ripening. The consumption of unripe fruits usually happened after the peak foraging hours, perhaps due to the limited availability of ripe fruits later in the night. However, this also shows that pteropodid bats have the propensity to override the preventive taste-driven barriers once there is a demand for resources (another example, bees: Tiedeken et al. 2014). As predicted, secondary metabolites in the unripe fruits were higher than in the ripe fruits, which conforms to the optimal defence theory (McKey 1974). The secondary components function as a defence mechanism, to prevent fruit consumption before the seeds are fully developed (viable) and can germinate. This may be the reason for the tree to invest more in chemical-mediated defensive compounds during the early phase of fruit development (Detzel and Wink 1993; Rojas et al. 2021).

Alkaloids and tannins are generally known to be highly concentrated in those plant parts upon which any predatory attack would have the greatest impact on the plant's fitness (McKey 1974; Rojas et al. 2021). Hence, as we observed in this system as well, it is likely that these plants also invest in chemical defences to deter predation of immature seeds by frugivores. Higher levels of secondary metabolites can limit the bioavailability of essential nutrients in fruits (Kamchan et al. 2004). It is therefore essential for frugivores to avoid consuming fruits that have these compounds at high levels, by assessing the quality of fruits before consumption. Animals consuming plant materials (e.g. immature fruits) with secondary metabolites often ingest soil-based minerals later to detoxify the metabolites and avoid physiological distress (Voigt et al. 2008; Bravo et al. 2010, 2012; Ghanem et al. 2013; Mahandran et al. 2016). However, lower levels of secondary compounds (e.g. tannins) can be helpful by binding with proteins in the guts of mammals and bringing benefits by, for example improving amino acid availability or reducing nitrogen loss as urea (Goel et al. 2005; Barbehenn and Constabel 2011).

On the other hand, minerals are essential supplements that are physiologically important for the survival and reproduction of animals in general and mammals in particular (Ghanem et al. 2013). Minerals could act as an attractant to ensure fruits are selected and dispersed by bats. All mineral supplements are known to have vital physiological functions. For example, Na predominantly helps osmoregulation, nerve impulses and muscular function in animals (Michell 1995), whereas Ca is essential for animals during reproduction and lactation (Bravo et al. 2010). The most common explanation for the availability of basic cations such as Ca, Mg, K and Na is that they could detoxify the deleterious effects of secondary plant metabolites (Diamond 1999; Voigt et al. 2008; Mahandran et al. 2016).

In this study, we found that the seed-to-pulp ratio increased with the increase in fruit size, which is expected for fruits having muriculate seeds (Shanahan 2000; Rojas et al. 2021). Fruits with a higher number of fertilised seeds are known to develop faster (Gorchov 1985) and it is, therefore, most likely that the seeds play a vital role in regulating intra-plant asynchrony in this species. Fruit size can act as an indicator of fruit quality as larger fruits offer more nutritional support to would-be dispersers, while the higher seed numbers associated with the large size enhance the chance of a seed being successfully dispersed. This is an example of the reciprocal benefits that are essential to plant-animal mutualism, whereby each can enhance the others' reproductive output (Fleming 1988; Valenta et al. 2017; Mahandran et al. 2018).

Foraging by two sympatric bats resulted in spatial partitioning of foraging spaces, where the upper to middle canopy layers were occupied by larger bats, and the lower to middle canopy layers were occupied by smaller bats. This kind of spatial segregation helps to avoid interspecific interferences. The fruits in the upper canopy are mostly consumed by larger bats and are more likely to get dispersed farther, as these flying foxes (genus: *Pteropus*) often have a wider home range (Thornton et al. 1996; Shilton et al. 1999). In contrast, the fruits in the lower canopy are mostly consumed by smaller bats (e.g. *C. sphinx*) and are often dispersed in close vicinity to the fruiting trees (Mahandran et al. 2021; Sangavi et al. 2021). However, the full spatial distribution of seed dispersal events (seed rain) remains overlooked, as it is incredibly challenging to track individual seeds (Nathan and Muller-Landau, 2000). In this study, we estimated the minimum seed dispersal distance by tracking boluses (with few seeds) from the feeding roosts of bats.

Even though bats avoided interspecific inferences by spatial partitioning, intraspecific interferences still occurred, and these often resulted in aggressive encounters among conspecifics for the ripe fruit resources. These encounters resulted in bats being forced to vacate the parent tree, often with a fruit or a part of a fruit in their mouth, which they consumed at a safer place (feeding roosts). The nearby feeding roosts of *P. giganteus*, with boluses of these fruits, were found between 180 and 450 m from the parent trees. These values represent the minimum seed dispersal distances since feeding roosts were likely to be located much further than our search area. Flying foxes have a large home range as they commute distances up to 50 km at a speed of 40 km/h (Richards 1990; Olesky et al. 2015). These bats have been recorded to carry fruits for distances exceeding a kilometre (Shilton et al. 1999; McConkey and Drake 2007; Drake and McConkey 2021). In the genus *Pteropus*, aggressive interactions can increase the chances of seed transportation to longer distances (Richards 1990; McConkey and Drake 2015; Mahandran et al. 2018). Similarly, the smaller bats were also forced to vacate the parent tree with a mouthful of fruit following interferences with conspecifics, and their feeding roosts were located at a minimum distance of between 45 and 72 m. A study using radio-telemetry has shown that the home range of *C. sphinx* to be about 0.75 ± 0.27 km$^2$ (Gopukumar et al. 1999). Hence, it is likely that both *C. sphinx* and *P. giganteus* provide complementary seed dispersal services by dispersing seeds at different spatial scales, i.e. at short and long distances, respectively.

The actual seed dispersal potential of pteropodid bats could be much more than what we have estimated by locating feeding roosts. Few seeds were embedded in the spat out boluses under these trees and, considering the small size of these muriculate seeds, it is probable that some seeds were also ingested while feeding. The gut retention time of Old World bats' reportedly ranges anywhere from an hour to a day and seeds can theoretically be dispersed over hundreds of kilometres (Shilton et al. 1999). However, recent studies have shown that secondary metabolites directly control the frugivores' physiology and behaviour by regulating the gut retention time (Morales et al. 2013; Baldwin and Whitehead 2015; Baldwin et al. 2020). The consumption of fruits containing a higher concentration of secondary metabolites could negatively impact gut retention time and dispersal distances. On the contrary, fruits containing a lower concentration of secondary metabolites have a positive effect on gut retention time and can facilitate long-distance seed dispersal (Wahaj et al. 1998; Tewksbury et al. 2008).

In conclusion, this study demonstrates the chemical basis of ontogeny and fruit-ripening asynchrony of a bat fruit, *N. cadamba*. The fruit traits (bat-fruit syndrome) ensured these fruits were appealing to the fruit bats. Remarkably, the asynchronous fruit-ripening strategy intensified the demand for ripe fruits by restricting their availability at a steady rate. This limited availability caused interferences among conspecifics of two sympatric bats which effectuated seed dispersal at different spatial scales.

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**Author contribution** V.M. conceived the project ideas, G.W. and J.C. conceptualised the methods and further improvised the project ideas, C.M.M. and V.M. performed the field works and lab works, P.S.A. provided additional support to the field works, P.T.N. supervised the study, V.M. performed data analyses and led the writing of the manuscript.

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**Data availability** All the data supporting the results are made available.

## Declarations

**Ethics approval** No animals were captured as part of this study; hence, no approval was needed.

**Consent to participate** Not applicable.

**Consent for publication** All the authors contributed substantially to the draft and gave consent for the publication.

**Competing interests** The authors declare no competing interests.

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