Temperature is a regulator of leaf production in the family Dipterocarpaceae of equatorial Southeast Asia

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PREMISE: Leaf phenology is an essential developmental process in trees and an important component in understanding climate change. However, little is known about the regulation of leaf phenology in tropical trees.

METHODS: To understand the regulation by temperature of leaf phenology in tropical trees, we performed daily observations of leaf production under rainfall-independent conditions using saplings of *Shorea leprosula* and *Neobalanocarpus heimii*, both species of Dipterocarpaceae, a dominant tree family of Southeast Asia. We analyzed the time-series data obtained using empirical dynamic modeling (EDM) and conducted growth chamber experiments.

RESULTS: Leaf production by dipterocarps fluctuated in the absence of fluctuation in rainfall, and the peaks of leaf production were more frequent than those of day length, suggesting that leaf production cannot be fully explained by these environmental factors, although they have been proposed as regulators of leaf phenology in dipterocarps. Instead, EDM suggested a causal relationship between temperature and leaf production in dipterocarps. Leaf production by *N. heimii* saplings in chambers significantly increased when temperature was increased after long-term low-temperature treatment. This increase in leaf production was observed even when only nighttime temperature was elevated, suggesting that the effect of temperature on development is not mediated by photosynthesis.

CONCLUSIONS: Because seasonal variation in temperature in the tropics is small, effects on leaf phenology have been overlooked. However, our results suggest that temperature is a regulator of leaf phenology in dipterocarps. This information will contribute to better understanding of the effects of climate change in the tropics.

KEY WORDS ambient temperature; aseasonal tropics; climate change; convergent cross mapping (CCM); dipterocarp; empirical dynamic modeling (EDM); leaf phenology; rainfall; thermomorphogenesis; tropical rainforest.

Leaf phenology (i.e., the timing of leaf production) has increasingly attracted attention in plant biology in relation to climate change (Richardson et al., 2013; Abernethy et al., 2018). Many studies have reported that leaf phenology in temperate trees is sensitive to climate change and has increasingly changed in recent decades (Richardson et al., 2006; Vitasse et al., 2009; Jeong et al., 2011). At the same time, leaf phenology has been suggested to affect global climate in the longer term by influencing heat, water, and carbon budget through photosynthesis and evapotranspiration (Pielke et al., 1998; Pitman, 2003; Bonan, 2008). Therefore, to evaluate this feedback between vegetation and climate, it is important to understand how leaf phenology is regulated. In temperate regions, where the timing of leaf production in trees has been well studied, it has been clearly demonstrated that temperature and photoperiod are key environmental factors that regulate leaf phenology (Morin et al., 2009; Vitasse et al., 2009; Hänninen and Tanino, 2011). However, compared with temperate forests, our understanding of leaf phenology in tropical rainforests near the equator, where seasonal variations in environmental factors are much smaller than those in temperate regions, is much more limited (Reich, 1995; Abernethy et al., 2018).

Adult trees in tropical rainforests have been observed at regular intervals (e.g., 1 mo) to evaluate leaf phenology (Medway, 1972; Ng, 1981; Aide, 1988; Reich, 1995; Okuda et al., 2003; Ichie et al., 2004). These studies showed that leaf production fluctuates

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at the community level, and some have suggested that leaf production is associated with environmental factors such as precipitation/drought, light intensity, and day length (Medway, 1972; Ng, 1981; Wright and Schaik, 1994; Okuda et al., 2003; Ichie et al., 2004; Elliott et al., 2006). However, the number of these studies is limited because it is difficult to observe continuously for enough years to detect general trends in leaf production. Furthermore, because correlation does not indicate causality, it remains to be clarified whether these environmental factors really regulate leaf phenology.

Recently, a mathematical framework has been developed using empirical dynamic modeling (EDM) to predict behavior of nonlinear dynamic systems and to test causal interactions based on time-series data: a process called convergent cross mapping (CCM) (Sugihara et al., 2012). Because CCM can detect causal relationships between two time series without explicit equations, it has been applied to multiple complex ecosystems for identifying causality (Sugihara et al., 2012; Ushio et al., 2018). Recently, CCM has also been applied to understand the regulation of plant phenology (Zhang et al., 2018; Satake et al., 2019; Tani et al., 2020; Ushio et al., 2020). Thus far, to our knowledge, there has been no application of this method to leaf phenology in the tropics. However, we consider that there is the potential to use CCM to identify the environmental factors that regulate leaf phenology in tropical rainforests if sufficient time-series data are available on leaf production and environmental factors.

In this study, we (1) conducted daily observations of leaf production to obtain long periods of time-series data, (2) applied CCM to identify the environmental factors regulating leaf production, and (3) performed growth chamber experiments to confirm the results from the CCM analysis. We used species of Dipterocarpaceae, which is the dominant canopy tree family in lowland tropical rainforests in Southeast Asia and includes >500 species (Ashton, 1988; Ghazoul, 2016). Therefore, understanding leaf phenology in dipterocarps is crucial for precise evaluation of feedback between vegetation and climate in Southeast Asia. Studies using monthly observations in Southeast Asian tropical rainforests near the equator have shown that trees, including dipterocarps, tend to have higher community-level leaf production around the vernal and autumnal equinoxes, although the timing of the peaks varied from year to year (Medway, 1972; Ng, 1981; Okuda et al., 2003). Furthermore, based on observations of similar patterns of leaf production and rainfall, these studies proposed that either rainfall following a dry spell or the dry spell itself is a key environmental factor that regulates the timing of leaf production in the tree species in this region. However, Elliott et al. (2006) conducted leaf phenology observations in a seasonal tropical forest in northern Thailand and suggested that leaf flushing of dipterocarps and other tree species around the vernal equinox is induced by increasing day length rather than by rainfall. Because of these inconsistencies, it remains unclear how fluctuations in leaf production in dipterocarp species are regulated. With this in mind, we focused on two dipterocarp species from different genera, *Shorea leprosula* and *Neobalanocarpus heimii*. We conducted daily observations of population-level leaf production by multiple saplings under fully watered conditions. Then we asked the following four questions:

1. Does leaf production by dipterocarp saplings vary even if plants are grown under fully watered conditions?

2. If dipterocarp saplings still show fluctuation in leaf production under rainfall-independent conditions, can the observed patterns of leaf production be fully explained by the cycle of day length?

3. What environmental factors could be candidates for regulating the timing of leaf production in dipterocarps if rainfall and day length cannot fully explain the observed pattern of leaf production, and can we identify such candidate environmental factors by the application of CCM? In particular, could temperature be such a candidate factor?

4. Can we experimentally modify leaf production by dipterocarps by changing temperature alone if temperature is a candidate factor for regulating the timing of leaf production?

By addressing these points, we demonstrated how leaf production is regulated in dipterocarp species in Southeast Asian tropical rainforests near the equator.

MATERIALS AND METHODS

Plant materials and growth conditions for daily observations

The saplings of *S. leprosula* and *N. heimii* used for daily observations were derived from the same mother trees and were planted in a nursery at the Forest Research Institute Malaysia (FRIM) on October 23, 2013, and April 1, 2014, respectively (Appendix S1). They were grown in plastic pots (diameter 23 cm, height 28 cm) under shade cloth. They were watered twice a day, at 0700 hours and 1700 hours, using an automatic irrigation system.

Definition of the timing of leaf production

It is not obvious when a leaf is produced because leaves develop gradually. Therefore, to avoid ambiguity in this study, we defined the timing of leaf production as the day upon which a folded young leaf unfolded (Fig. 1A–C). We adopted this definition because this morphological change in a developing leaf is common among dipterocarp species and is a qualitative characteristic.

Observation of leaf production using time-lapse digital photography

We daily observed leaf production by 54 *S. leprosula* and 32 *N. heimii* saplings using time-lapse digital photography (Fig. 1D). For this study, the saplings of each species were separated into two groups, identified as Sl batches 1 and 2 for the two groups of *S. leprosula* saplings and Nh batches 1 and 2 for the *N. heimii* saplings. To minimize the positional effects related to sunlight exposure, these batches were arranged south to north in the following order: Sl batch 1, Nh batch 1, Nh batch 2, and Sl batch 2. To eliminate the effect of daily fluctuations of rainfall on leaf production, the plants were placed inside a tunnel covered with transparent plastic sheets. To obtain digital images of the four batches of plants at hourly intervals, four waterproof digital cameras with a time-lapse function (WG-40; Ricoh Imaging Company, Tokyo, Japan) were installed on the ceiling of the observation system. For collection of temperature and photosynthetically active radiation (PAR) data, sensors S-THB-M002 and S-LIA-M003 (Onset,
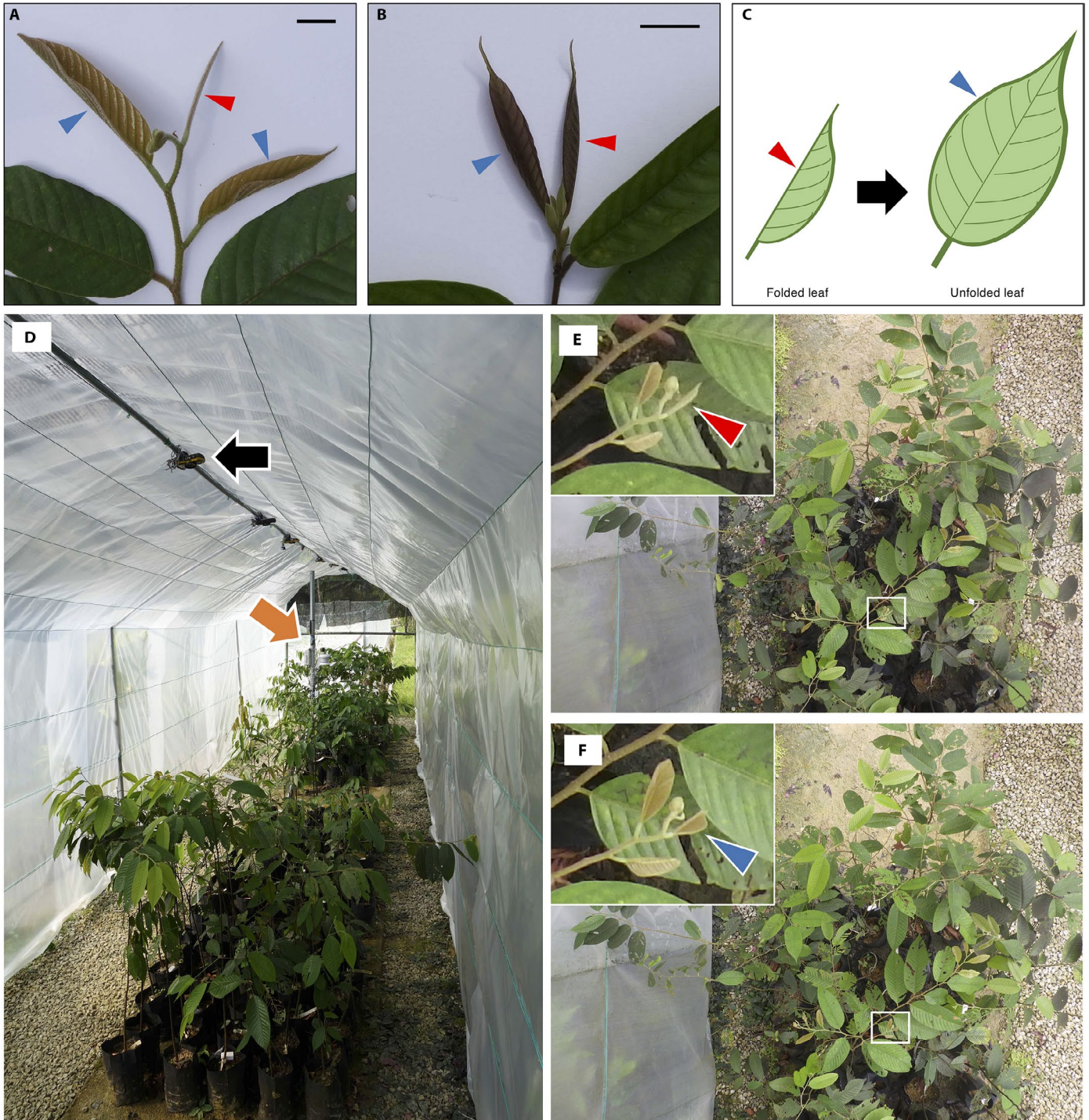
FIGURE 1. Leaf production observation system using time-lapse digital cameras. The pictures show folded and unfolded leaves in (A) *Shorea leprosula* and (B) *Neobalanocarpus heimii* (scale bars = 1 cm). (C) Schematic image of the folded and unfolded leaves of dipterocarps. (D) Overview of the observation system with time-lapse digital cameras. The observation system was covered by transparent plastic sheets to exclude rainfall. Black and orange arrows show a digital camera and sensors placed at the center of the observation system, respectively. The four batches of saplings were arranged in the order *S. leprosula* batch 1, *N. heimii* batches 1 and 2, and *S. leprosula* batch 2 from the near to the far side. Also shown are representative images of two consecutive days obtained from the digital camera for *S. leprosula* batch 1 on (E) December 7 and (F) December 8, 2017; insets show the timing of production of a leaf. The folded leaf indicated by a red arrow in E was unfolded by the next day, as indicated by a blue arrow in F. The latter photo was from December 8, so we considered that the leaf was produced on that day. Red and blue arrowheads in all the figures indicate folded and unfolded leaves, respectively.

Bourne, Massachusetts, USA), respectively, were placed at the center of the observation system between Nh batches 1 and 2 with data collection at 5 min intervals. The collected data were stored in a data logger, H21-USB (Onset). The temperature, PAR, and image data were recorded for ~7 mo, from June 24, 2017, to January 31, 2018.

To detect the timing of production of a leaf, we compared images obtained at ~1200 hours on consecutive days and checked the morphologies of all the young leaves by eye on the computer display. If a folded leaf became unfolded, we recorded that the leaf was produced on that day. An example can be seen in Fig. 1E–F. Leaf production data could not be obtained for Nh batch 2 from December 2, 2017, to December 20, 2017, because the camera malfunctioned during this period. See more details in Appendix S2.

Causality tests by EDM using the time-series data sets

To identify the environmental factors that regulated the timing of leaf production, we applied EDM (Sugihara and May, 1990; Sugihara et al., 1994; Sugihara et al., 2012) to the time-series data. The analyses were conducted by using package "rEDM" version 0.7.1 in R 3.5.0 (R Core Team). In the CCM analysis, if X influences Y, the states of X can be estimated from the time series of Y. Therefore, to test whether the candidate environmental factors regulate leaf production in dipterocarps, we checked estimation skills (cross-map skills) from leaf production to the candidate factors. To understand time lags in the regulation of leaf production by the candidate environmental factors, we conducted the CCM analysis at lags of 0 to −45 d for the daily observation data and lags of 0 to −12 mo for the monthly observation data at the Ulu Gombak Forest Reserve. We considered the results of the CCM analysis significant if the estimation skills from the original time series were greater than the 95% quantile of the null distributions of the surrogates generated from randomized leaf production data with four different methods (Appendix S3).

For the analysis of the daily observation data by digital photography, we concatenated the time-series leaf production data in the order Nh Batch1, Nh batch 2, Sl batch 1, and Sl batch 2, based on the methods of Hsieh et al. (2008) and Clark et al. (2015), and conducted the CCM analysis using the obtained composite time series.

In addition to the analysis of saplings, we also applied EDM to the previously reported leaf-flushing data obtained using adult trees. For the analysis of adult trees, we focused on Medway (1972), because that study contained long-term leaf-flushing data without missing values. The monthly leaf-flushing data of a hill dipterocarp forest reported by Medway (1972) were obtained not only from dipterocarps but also from some non-dipterocarp trees in the Ulu Gombak Forest Reserve from July 1963 to July 1969, as described in Medway (1972: table 6). The species studied by Medway (1972) consisted of 61 canopy trees, representing 45 species in 17 families, and their foliar activity was monitored at intervals of ~2 wk using a platform 43 m above ground. Among the 61 trees used for the observation, 18 trees belonged to the family Dipterocarpaceae (13 dipterocarp species), which was the most abundant family in the data (Medway, 1972).

The times of sunrise and sunset at the FRIM (latitude: 3.235339; longitude: 101.634269) and the Ulu Gombak Forest Reserve (latitude: 3.35000000; longitude: 101.78333333) during the study periods were calculated using a spreadsheet, (NOAA_Solar_Calculations_year.xls) provided by the National Oceanic and Atmospheric Administration (NOAA) (https://www.esrl.noaa.gov/gmd/grad/solcalc/calcdetails.html), by specifying the time zone as +8. Day length was calculated as the difference between the times of sunset and sunrise. For the CCM analyses, we used the values at the end of the month.

The monthly average temperature data and the monthly rainfall data for the Ulu Gombak Forest Reserve during the study period were downloaded from the Climate Change Knowledge Portal (https://climateknowledgeportal.worldbank.org) by specifying the same latitude and longitude as the sunrise and sunset data.

The details of the CCM analyses are summarized in Appendix S3.

Plant materials and growth conditions for the growth chamber experiments

We used 50 saplings of *N. heimii* for growth chamber experiments. These were derived from the same mother trees and were planted at the FRIM on May 12, 2017. We grew them in a single growth chamber (Percival model PGC-15; Percival Scientific, Perry, Iowa, USA) under 75% humidity, 200 µmol m^{-2} s^{-1} light intensity, 12 h light cycle (12 h light from 0700 hours/12 h dark from 1900 hours), and 50 mL water daily. Baseline light intensity was set to 200 µmol m^{-2} s^{-1} but was increased to 600 µmol m^{-2} s^{-1} from February 2, 2018, to March 16, 2018. To test the effects of temperature on the regulation of leaf production, we conducted two temperature experiments consisting of multiple sequential temperature treatments. Each temperature treatment was performed for ~3 wk, and we performed 20 and 5 consecutive temperature treatments in the first and the second experiments, respectively. The experiments were conducted from October 11, 2017, to December 19, 2018, and from August 10, 2019, to December 5, 2019, respectively. In these experiments, we separately changed the growth temperatures during light and dark periods from 24.0°C to 32.0°C to understand the effects of temperature under light and dark conditions.

To evaluate the effects of temperature treatments, leaf production of each sapling was measured by manually counting total leaves and defoliated leaves at the end of each 3 wk treatment and subtracting the total number of leaves present at the beginning of the treatment. When we counted leaves, we used the same criteria as for the time-lapse digital image analysis and did not include folded leaves. The mean initial plant height for the first and second experiments were 23.5 cm (~5 mo old) and 44.7 cm (~27 mo old), respectively.

To test for differences between the warming treatments and control, we performed one-sided Wilcoxon signed-rank tests in R (false discovery rate: 0.05).

RESULTS

Fluctuations of leaf production in dipterocarp species at the population level in the absence of rainfall variation

During our daily observation using time-lapse digital photography under the rainfall-excluded condition, Sl batches 1 and 2 and Nh batches 1 and 2 produced 348, 408, 375, and 390 leaves, respectively (Appendix S4). The daily observation data showed that the numbers of leaves produced fluctuated, but these changes were not completely random. Although the plants were grown under conditions without variation in rainfall, their 7 d moving average of leaf
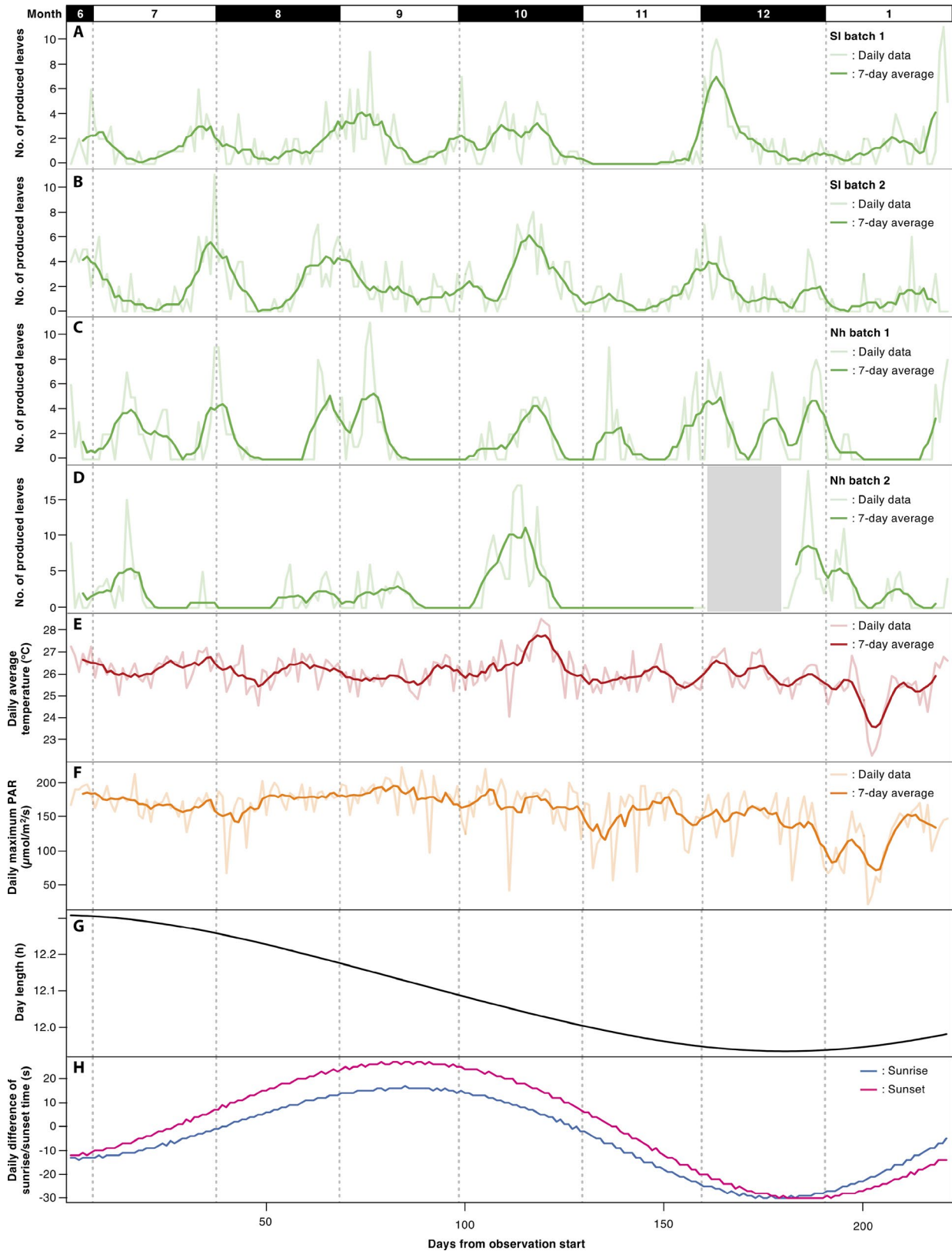
FIGURE 2. Daily observations of leaf production by digital cameras and the temperature, light intensity, day length, sunrise, and sunset data during the observation period. (A) Daily leaf production by *Shorea leprosula* (Sl) batch 1. (B) Daily leaf production by Sl batch 2. (C) Daily leaf production by *Neobalanocarpus heimii* (Nh) batch 1. (D) Daily leaf production by Nh batch 2. The shaded area represents the period during which the data could not be obtained for Nh batch 2. (E) Daily average temperature. (F) Daily maximum photosynthetically active radiation (PAR). (G) Day length. (H) Daily changes in sunrise and sunset times.

production showed clear peaks (Fig. 2A–D). The time series of leaf production in each batch showed a positive autocorrelation at lags up to ~1 wk, a negative autocorrelation at lags of 15 to 40 d, and a second peak of positive autocorrelation at lags of ~1.5 mo, although the autocorrelation of the second peak was weak (Appendix S5). This result indicates that periods of higher and lower leaf production tended to alternate at ~1.5 mo intervals in all the batches of plants, although the duration and interval of the periods varied. The periods of higher and lower leaf production tended to be synchronized between species and between batches within species (Fig. 2A–D).

Day length and changes in sunrise and sunset time have been proposed as regulators of phenology in the tropics (Borchert et al., 2005; Elliott et al., 2006), however, patterns of leaf production did not coincide with any of these variables (Fig. 2G–H). On the other hand, peaks of the 7 d moving average of temperature tended to be observed at timings similar to those of leaf production (Fig. 2A–E). The analysis of the autocorrelation of temperature showed results similar to those of the leaf-production data (Appendix S5), suggesting that peaks of temperature also occurred at ~1.5 mo intervals during the study period. As a comparison, we also analyzed the daily maximum PAR (Fig. 2F) and found that its autocorrelation was not similar to that of the leaf-production data (Appendix S5).

Causality tests for leaf production and candidate environmental factors using the daily observation data

To test the possibility that temperature regulates leaf production in dipterocarps, we conducted the CCM analysis. The results showed higher estimation skills from leaf to temperature at lags of up to about −1 wk and between around −3 and −5 wk and peaks of significant estimation skill compared with the null distributions at lags of −6, −24, and −27 d (Fig. 3A), providing evidence that leaf production is influenced by temperature changes that occurred approximately 6 d previously and 24–27 d previously.

The direction of these temperature effects was examined by comparing the pattern of observed leaf production with that of the daily average temperatures, offset by 6, 24, or 27 d (Fig. 3). This revealed that leaf production tended to increase (decrease) 6 d after an increase (decrease) in temperature (Fig. 3F). Conversely, the leaf production tended to show an inverse relationship with that of the temperatures 24 and 27 d before the observation (Fig. 3G–H). In short, increases in leaf production tend to occur 24–27 d after a decline in temperature and/or 6 d after an increase in temperature.

To avoid the possibility that these results were obtained because of observation errors by our digital photography approach, we also applied the CCM analysis to leaf-production data obtained by daily manual observation of 20 *S. leprosula* saplings for 45 d (Appendix S6). The estimation skills from leaf to temperature showed a similar pattern to those obtained by digital photography (Appendix S7), suggesting that the observed patterns of estimation skills were not simply artifacts.

By contrast, we did not observe significant estimation skills for the daily maximum PAR (Fig. 3B) or for day length, sunrise, and sunset (Fig. 3C–E). Therefore, in this analysis, we could not obtain clear results supporting that these variables influence the timing of leaf production.

Effect of temperature on leaf production of adult trees in Southeast Asian tropics near the equator

As described above, we studied the effect of temperature on leaf production using saplings of dipterocarps, but it was not clear whether our results could be applied to adult trees grown in natural environments in Southeast Asia. To test this, we applied EDM to the data for leaf flushing of a hill dipterocarp forest in the Ulu Gombak Forest Reserve previously reported by Medway (1972) (Fig. 4). The CCM analysis showed a peak of significant estimation skill from leaf to temperature at a lag of −6 mo (Fig. 5A). Comparing the pattern of leaf flushing with the monthly average temperatures 6 mo previously, leaf flushing tended to show a pattern inverse to that of these temperatures (Fig. 5F), suggesting that more individuals showed leaf flushing if temperatures declined ~6 mo previously. It is noteworthy that despite the difference in time scales, both adult trees and saplings showed similar results, both of which suggested that a decline in temperature must precede leaf production for leaf production to become high.

Although the main objective of this analysis was to test the involvement of temperature in the regulation of leaf production by adult trees, it was helpful to study the effects of other environmental factors to provide a complete picture of the mechanism of leaf flushing. Therefore, we conducted similar CCM analyses using the time-series data for rainfall, day length, and the times of sunrise and sunset (Fig. 5B–E). Among these variables, monthly rainfall also showed significantly high estimation skills at lags of −5 and −6 mo, although the estimation skills for rainfall were smaller than those for temperature (Fig. 5B). By contrast, no significant estimation skills were observed for the other factors (Fig. 5C–E).

Growth chamber experiments to test the effect of temperature on leaf production in dipterocarps

Taking all the results of our EDM of the relationship between leaf production and temperature into consideration, our findings suggested that large numbers of leaves may be produced when temperature decreases and then increases. To test this, we conducted a growth chamber experiment consisting of 20 consecutive temperature treatments, each of which spanned ~3 wk, using 50 *N. heimii* saplings (Appendix S8). The results showed that more leaves tended to be produced at the population level when the temperature was higher (Fig. 6A), confirming the result that higher temperatures have positive effects on leaf production. However, statistical analysis through comparisons with the first low-temperature treatment showed that leaf production significantly increased only when at least two consecutive low-temperature treatments preceded the high-temperature treatments, except for Tr_{1-12} (Fig. 6C and Appendix S9). Furthermore, we also found that more individuals produced leaves if the number of consecutive low-temperature treatments before the high-temperature treatments was increased (Fig. 6H). These results suggest that long-term low temperatures preceding high temperatures also has an important role in the production of significantly higher numbers of leaves during high temperatures.

To understand the relationship between the results at the population level and at the level of individual trees, we compared the total number of leaves produced during a treatment with the percentage of individuals that showed leaf production (Fig. 6A–B), and found a strong positive correlation (Fig. 6G). This suggests
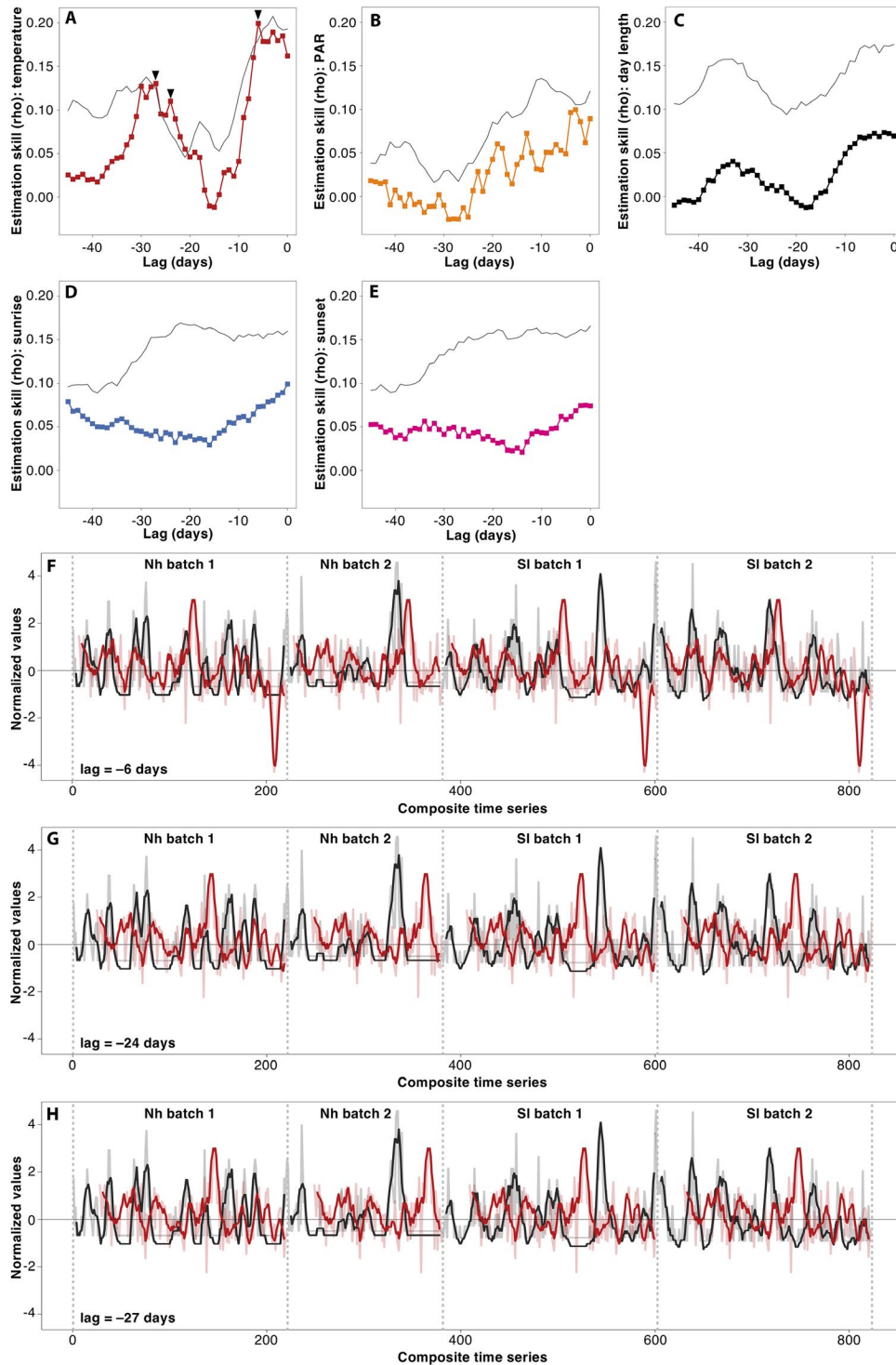
FIGURE 3. Results of convergent cross mapping (CCM) of the composite time series of the daily observation data. The estimation skills (cross-map skills) show the strength of the relationship between the leaf-production data and (A) daily average temperature, (B) daily maximum photosynthetically active radiation (PAR), (C) day length, (D) daily difference in sunrise time, (E) and daily difference in sunset time. Time lags of 0 to −45 d are represented by lines with filled squares. The gray lines in A–E indicate the 95% quantiles of the null distributions of the surrogates, and the colored lines indicate estimation skill. Black arrowheads indicate the positions of peaks with significantly high estimation skills. In CCM analysis, if estimation skill is higher than the null distribution, then the environmental factor is considered to influence leaf production. Also shown are comparisons of normalized values of leaf production with those of temperature at (F) 6 d, (G) 24 d, and (H) 27 d previously. The light and dark gray lines indicate daily data and the 7 d moving average of leaf production, respectively. The lines in light and dark red indicate daily data and the 7 d moving average of daily average temperature, respectively.
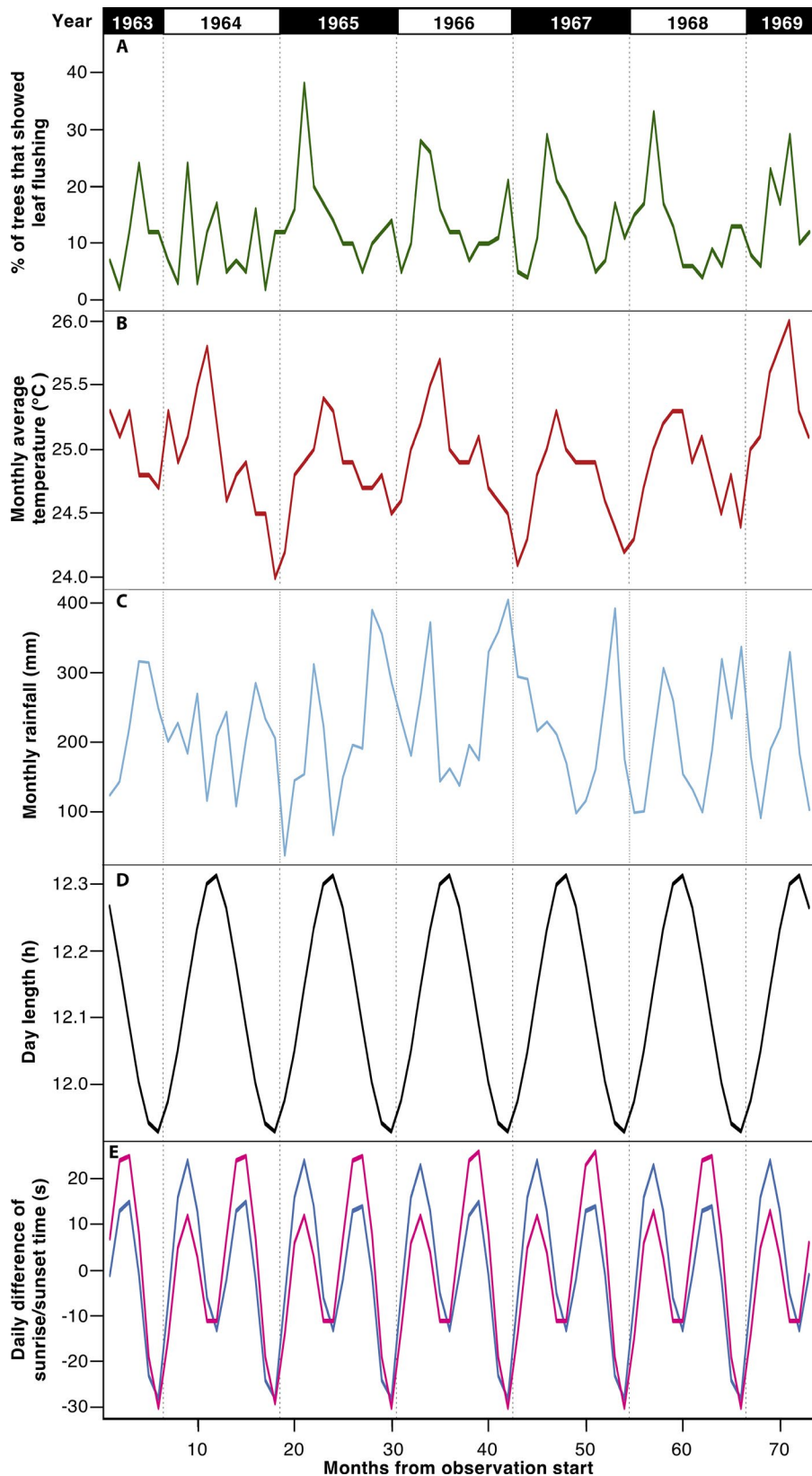
FIGURE 4. Patterns of leaf flushing of adult trees and environmental factors in a hill dipterocarp forest. (A) Patterns of leaf flushing reported by Medway (1972), (B) monthly average temperature, (C) monthly rainfall, (D) day length, and (E) daily differences in sunrise and sunset time in the Ulu Gombak Forest Reserve. Blue and pink lines in E indicate sunrise and sunset, respectively.

that the increment in leaves produced at the population level is explained by an increment in the number of individuals that produced leaves. In fact, at the individual level, the maximum numbers of leaves produced were the same under low- and high-temperature treatments (Fig. 6C). In addition, during consecutive high-temperature treatments, relatively high numbers of leaves were continuously produced at the population level, but leaf production by each individual did not continue throughout these periods. Instead, different individuals produced leaves one after another (Fig. 6C). Furthermore, although the high-temperature treatments increased the percentages of individuals that produced leaves, only a maximum of 62% (31 of 50) of individuals produced new leaves in the same 3 wk period (Fig. 6C and Appendix S9). Because all the individuals produced leaves at least once over the entire experiment (Appendix S9), this cannot simply be explained by the inability of some individuals to produce new leaves under the study conditions.

In our experiment, leaf production tended to become higher if at least the temperature under light conditions was higher. Therefore, we wondered whether temperature could affect leaf production through influencing photosynthesis. However, higher light intensity is also expected to activate photosynthesis, and an increment of light intensity alone did not result in a clear increment of leaf production (Tr_{1-6} in Fig. 6A–C and Appendix S9). Furthermore, a significant increase in leaf production was observed even if only the temperature under dark conditions was increased after four consecutive low-temperature treatments (Fig. 6D–F and Appendix S9). These results suggest that temperature regulates leaf production of dipterocarps by a developmental signal rather than by enhancing the rate of photosynthesis.

DISCUSSION

Regulation of leaf production by small changes of ambient temperature in dipterocarp species

Using field observation and growth chamber experiments, we present evidence that temperature controls the timing of leaf
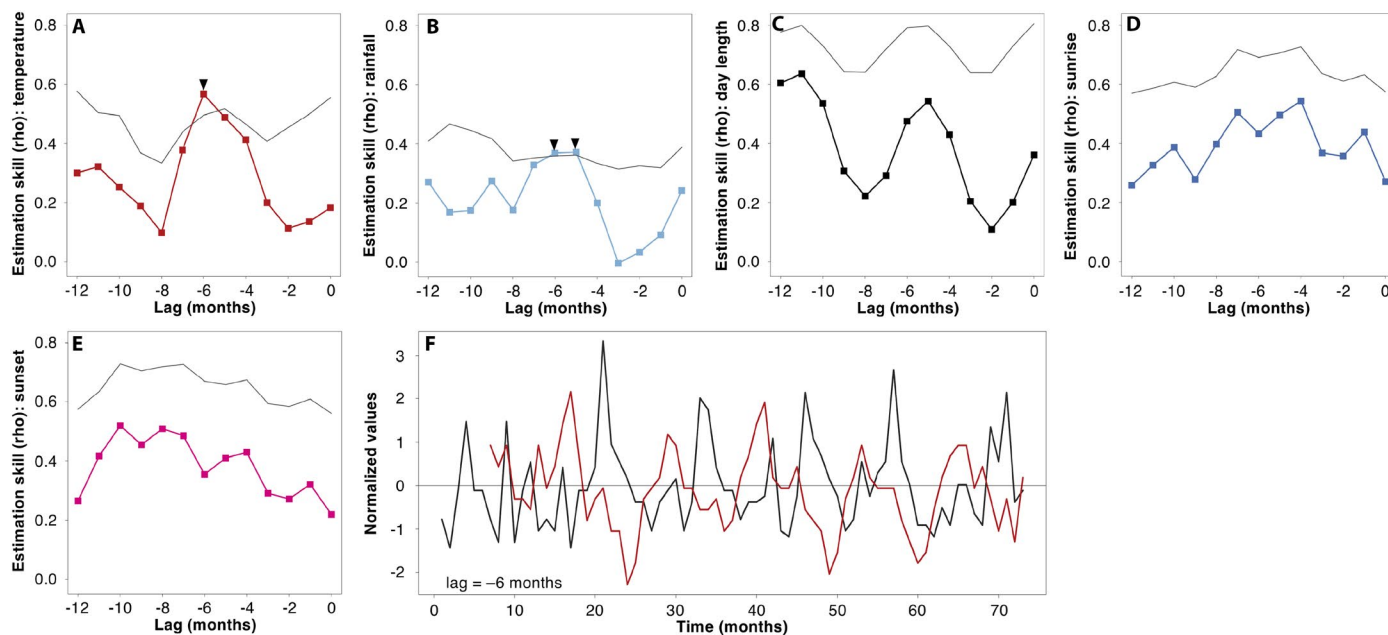
FIGURE 5. Results of convergent cross mapping (CCM) of the monthly data of adult trees. The estimation skills (cross-map skills) show the strength of the relationship between the leaf-flushing data and (A) temperature, (B) rainfall, (C) day length, (D) daily difference in sunrise time, and (E) daily difference in sunset time. Time lags from 0 to −12 mo are represented by lines with filled squares. The gray lines in A–E indicate the 95% quantiles of the null distributions of the surrogates, and the colored lines indicate estimation skill. Black arrowheads indicate the positions of significantly high estimation skills. In CCM analysis, if estimation skill is higher than the null distribution, then the environmental factor is considered to influence leaf production. (F) Comparisons of normalized values of leaf-flushing data with those of temperature at 6 mo previously. The black and red lines indicate leaf flushing and temperature, respectively.

production in dipterocarps. The application of EDM to the daily observation data suggested that changes in the daily average temperature 6, 24, and 27 d before the observation could have significant effects on the pattern of leaf production (Fig. 3A), and that leaf production may became higher (or lower) if temperatures decreased (or increased) around 3–4 wk before the observation and if temperatures increased (or decreased) ~1 wk before observation (Fig. 3F–H). Our growth chamber experiment showed that significantly more leaves were produced only when high-temperature treatments were conducted after ≥6 wk of low-temperature treatment (Fig. 6), which confirmed the results of EDM suggesting the importance of changes in temperature from low to high for inducing dipterocarp saplings to produce high numbers of leaves. As with the previously reported data on leaf flushing of adult trees in the natural environment (Medway, 1972), the results of EDM suggested that changes in the monthly average temperature 6 mo previously could have a significant effect on the observed pattern of leaf flushing (Fig. 5A), and that the percentages of the trees that showed leaf flushing may became higher (or lower) if temperatures around 6 mo previously decreased (or increased) (Fig. 5F). This suggests that a decline in temperature may need to precede leaf production for the amount of leaf production to become high. This finding is similar to those we obtained by daily observation using dipterocarp saplings, although the time scales differed between the two analyses. These results suggest that temperature is an important regulator of the timing of leaf production in both young and adult dipterocarp trees, although we still do not have experimental support for this hypothesis in adult trees.

Our growth chamber experiments showed that increasing temperature only during the dark period promoted leaf production

(Fig. 6D–F). This result indicates that temperature regulates leaf production by a developmental signal rather than by enhancing the rate of photosynthesis. It is well known that in multiple species, ambient temperature regulates multiple developmental changes, such as leaf expansion, germination, and flowering, without changing the photosynthetic rate; this effect is called thermomorphogenesis (Quint et al., 2016; Casal and Balasubramanian, 2019). Thermomorphogenesis is observed in both temperate and tropical species (Parent and Tardieu, 2012). Parent and Tardieu (2012) reported that rates of developmental processes including leaf expansion were regulated by ambient temperature in all 18 species they studied, and that the maximum developmental rates were observed at higher temperatures in tropical species than in temperate species. Therefore, it is possible that temperature regulates leaf production in dipterocarps by a similar mechanism. Furthermore, because the leaf-flushing data obtained by Medway (1972) in the Ulu Gombak Forest Reserve included both dipterocarp and nondipterocarp species, the significantly high estimation skills from leaf to temperature (Fig. 5A) may suggest that temperature regulates leaf production in both nondipterocarp and dipterocarp species. Regulation of leaf production by ambient temperature in spring has been reported in many taxa of temperate tree species (Morin et al., 2009; Vitasse et al., 2009; Hänninen and Tanino, 2011). Considering these similarities, it may not be unexpected that both temperate and tropical tree species commonly utilize ambient temperature as a regulator of the timing of leaf production, even though the temperature ranges of their distribution areas differ and the seasonal temperature changes in the tropics are much smaller. Molecular analyses at the gene level will help us understand whether a common mechanism
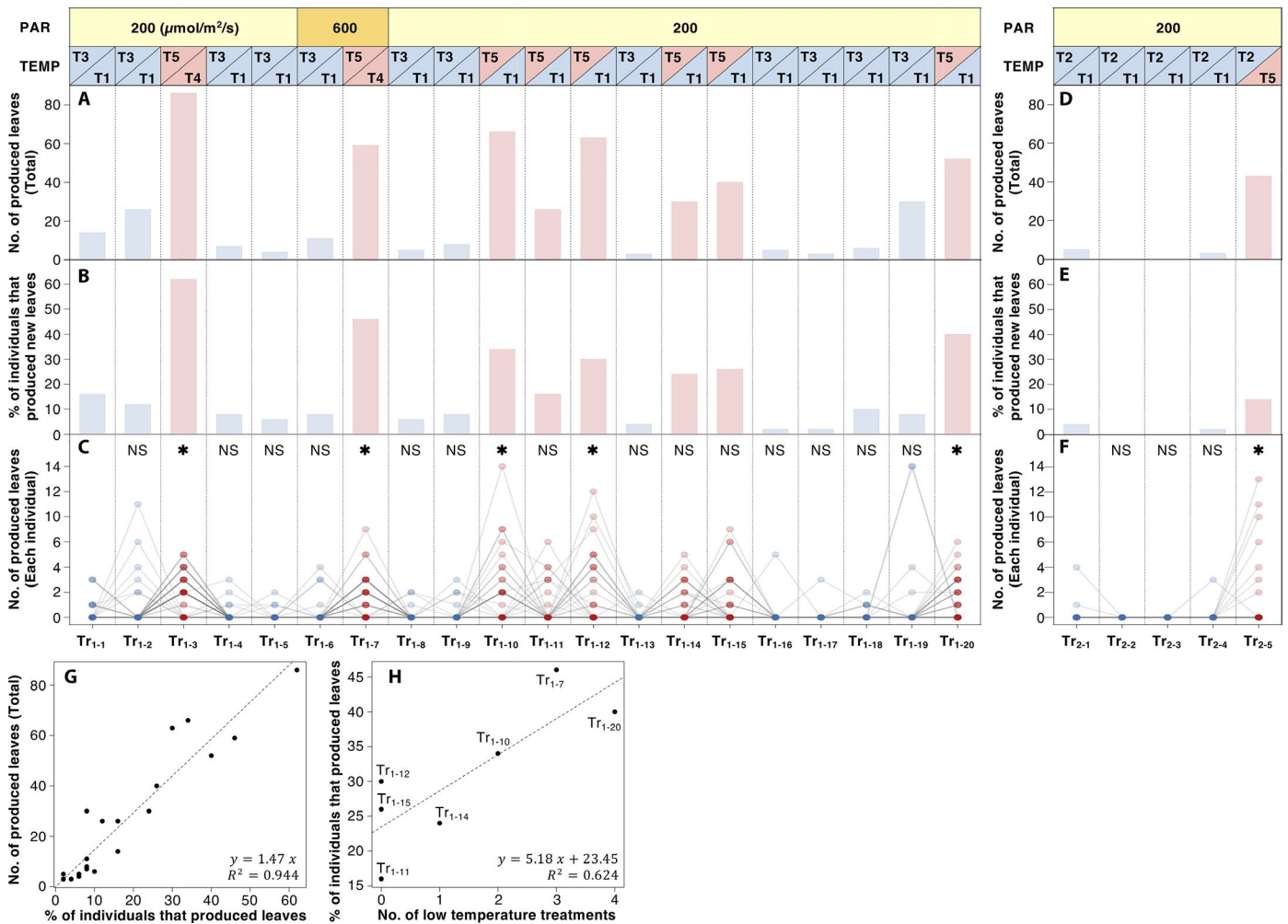
FIGURE 6. Results of two growth chamber experiments to test the effect of temperature on leaf production using 50 saplings of *Neobalanocarpus heimii*. (A) Total number of leaves produced at the population level, (B) percentage of individuals that produced leaves, and (C) number of leaves produced at the individual level during the first growth chamber experiment, which consisted of 20 consecutive treatments from Tr_{1-1} to Tr_{1-20}. (D) Total number of leaves produced at the population level, (E) percentage of individuals that produced leaves, and (F) number of leaves produced at the individual level during the second growth chamber experiment, which consisted of five consecutive treatments from Tr_{2-1} to Tr_{2-5}. In the experiments, two photosynthetically active radiation (PAR) conditions, 200 and 600 μmol m^{-2} s^{-1}, were used, as illustrated in the top panel. Five temperature conditions (T1–T5) used in the experiments are shown in the second top panel, and the top left and bottom right corners of each treatment indicate the temperature conditions under the light and dark periods (T1: 24.0°C; T2: 25.0°C; T3: 27.0°C; T4: 29.0°C; and T5: 32.0°C). In A–F, results under high- and low-temperature conditions are represented in red and blue, respectively. In C and F, darker colors indicate that more individuals showed the same results. The asterisks in C and F indicate a significant increase in leaf production compared with the first treatments (Tr_{1-1} or Tr_{2-1}), and nonsignificant results are indicated by "NS". (G) Comparison of the total number of leaves produced at the population level and the percentage of individuals that showed leaf production. (H) Comparison of the number of consecutive low-temperature treatments before high-temperature treatment and the percentage of individuals that showed leaf production. Tr_{1-3} was not included in the correlation analysis because it was not clear how long plants experienced low temperature before the experiment started.

exists among temperate and tropical trees for regulating leaf production by ambient temperature.

As for thermomorphogenesis, it is known that plants can detect small differences in temperature and use such differences (Quint et al., 2016; Casal and Balasubramanian, 2019). In our growth chamber experiments, we found that an increase of only 2.5°C in daily average temperature can promote leaf production of *N. heimii* saplings (Fig. 6), suggesting that dipterocarps can also detect and utilize such small differences in ambient temperature for the regulation of leaf production. During the daily observations in the nursery

at the FRIM, we observed that differences between the maximum and minimum daily average temperatures were >6°C (Fig. 2 and Appendix S4), which is much larger than the temperature difference that dipterocarps were able to detect. Therefore, although the seasonal variations in temperature in the tropics are much smaller than those in temperate regions, these variations would have nonnegligible effects on the regulation of leaf production. The effects of temperature on leaf production have not been considered seriously in the tropics, where seasonal variations in temperature are smaller than diurnal variation (Medway, 1972; Ng, 1981; Okuda et al., 2003;

Ichie et al., 2004). Because of this preconception, regulation of leaf production by temperature may have been overlooked, and may be an important factor in better understanding the effects of climate change in tropical rainforests.

The growth chamber experiment suggested that a high amount of leaf production at the population level under high temperature is caused by synchronized leaf production among individuals, but not all individuals participated in the synchronized leaf production (Fig. 6 and Appendix S9). In addition, the same individuals did not continuously produce leaves even when high-temperature conditions continued (Fig. 6C). These results suggest that plants need to be in a certain condition, or competence, to respond to high temperature by producing leaves. For example, the nutrient levels of individuals and the development of leaf primordia could be candidate factors in competence.

Considering all these results, we propose a possible mechanism whereby temperature causes fluctuation in leaf production by dipterocarps. First of all, temperatures higher or lower than a threshold act as promoters and suppressors of leaf development, respectively. However, this promotion of leaf development by higher temperature occurs only in plants that have already acquired competence. In a population, a certain proportion of plants acquire competence per day, and plants lose their competence if they produce leaves. Therefore, if the high-temperature conditions continue, different individuals produce leaves one after another, so that at the population level, the number of leaves produced during this period is kept high. Under low-temperature conditions, acquisition of competence continues, but leaf development by each individual is suppressed, so an increasing number of individuals acquire competence. Leaf production at the population level remains low until temperature becomes higher than the threshold and all the accumulated competent individuals start to develop leaves once temperature becomes high, resulting in a peak of leaf production at the population level. This model is consistent with the observed results, but we do not know whether the proposed mechanism is correct, nor whether a threshold temperature and competence really exist. Furthermore, it is also unclear how and where the information about temperature is sensed and utilized for leaf development by dipterocarps. Future studies are needed to answer these questions.

Regulation of leaf production by other environmental factors in dipterocarp species

Although fluctuations in leaf production were observed in the absence of fluctuations in rainfall, and peaks of leaf production were more frequently observed than those of day length and the times of sunrise and sunset, it should be noted that our results do not mean that rainfall, day length, and the times of sunrise and sunset do not induce fluctuations in leaf production. In fact, the CCM analysis using the leaf-flushing data for adult trees showed significant estimation skills from leaf to rainfall (Fig. 5B), suggesting that rainfall may influence the timing of leaf flushing. Furthermore, because the periodicities of day length (12 mo intervals) and daily changes in the times of sunrise and sunset (6 mo intervals) were multiples of the observed periodicities of leaf production (~1.5 mo intervals) (Fig. 2G–H and Appendix S5), it is still possible that they may also partly regulate the fluctuations in leaf production. In a model plant, *Arabidopsis thaliana*, flowering time is known to be regulated by multiple internal and external factors, including age, sugar, plant hormones, day length, and temperature

(Kobayashi and Shimizu, 2013; Bouché et al., 2015). Therefore, leaf production in dipterocarp species is not necessarily regulated by a single environmental factor. However, the CCM analyses using saplings and adult trees did not produce significant results for day length, sunrise, sunset, and PAR (Figs. 3 and 5 and Appendix S7). In the growth chamber experiment, we observed that an increment of light intensity from 200 to 600 μmol m^{-2} s^{-1} did not induce a significant increment in leaf production (Fig. 6A–C and Appendix S9), which is consistent with the results of CCM for PAR. However, we cannot rule out the possibility that the increase in light intensity could not increase leaf production because the light intensities used in the growth chamber experiment were out of the range that regulate the timing of leaf production. Furthermore, it is possible that a significant result could not be observed because the time series was not long enough. In fact, we observed significant results when we analyzed a long composite time series of leaf-production data by concatenating the data from different batches (Fig. 3A), but when we analyzed each time series separately, we were unable to obtain significant results in some cases (Appendix S7). Therefore, it is still difficult to conclude whether these environmental factors regulate leaf production in dipterocarps. Future studies should conduct growth chamber experiments with sets of well-planned treatments to test experimentally the effect of rainfall, day length, sunrise, sunset, and PAR on the regulation of leaf production by dipterocarps.

Importance of conducting phenology census at both long- and short-term intervals using various methods

Because of the size of adult tropical trees, it is not easy to observe leaf production frequently and to conduct experiments to test the effects of candidate environmental factors on leaf production without special facilities such as platforms and cranes (Medway, 1972; Ichie et al., 2004). In addition, it is also difficult to obtain data with many time points because this requires many years of observations (Abernethy et al., 2018). Satellite remote sensing has recently been recognized as a powerful tool to detect long-term seasonal variations in leaf production in tropical rainforests at the landscape level (Myneni et al., 2007; Abernethy et al., 2018). Even with this new technology, there are still limitations and difficulties in understanding short-term temporal variations in leaf production. To address these points, daily observation of leaf production using multiple saplings would be a useful approach. Our daily census of leaf production using digital cameras to monitor multiple dipterocarp saplings showed the feasibility of this method for detecting short-term fluctuations in leaf production (Fig. 2), for experimentally testing the effect of potential triggers of leaf production such as rainfall (Fig. 2), and for applying EDM to understand the effect of environmental factors on leaf production using data from >200 time points (Fig. 3). Although we used these daily observation methods only for dipterocarp species, they can be applied to other tree species in tropical rainforests. We hope that integrating the daily observation method with conventional direct and satellite-based observations at long-term intervals will broaden our understanding of the regulation of leaf production in tropical trees.

CONCLUSIONS

Our daily observations of leaf production revealed that leaf production in dipterocarp species fluctuates as alternating periods of

higher and lower leaf production with variable durations and intervals. Our observations also showed that the fluctuations in leaf production cannot be explained solely by the patterns of rainfall and day length, which have been proposed as triggers of leaf production in dipterocarp species (Medway, 1972; Ng, 1981; Okuda et al., 2003; Ichie et al., 2004; Elliott et al., 2006). Furthermore, analyses using EDM suggested that temperature is responsible for the regulation of the timing of leaf production in both saplings and adult trees of dipterocarps in Southeast Asian tropical rainforests near the equator. Growth chamber experiments confirmed the effect of temperature on leaf production. Because seasonal variations in temperature in this area are much smaller than those in temperate regions, the effect of temperature on leaf phenology in the tropics has not been appreciated (Medway, 1972; Ng, 1981; Okuda et al., 2003; Ichie et al., 2004). Therefore, it had been thought that global climate change does not severely affect leaf phenology in these areas (Reich, 1995; Richardson et al., 2013). However, given that temperature is responsible for regulation of the timing of leaf production in dipterocarp species, leaf phenology in the tropics will be affected by climate change, and in turn may influence global climate in the longer term through photosynthesis and evapotranspiration (Pielke et al., 1998; Pitman, 2003; Bonan, 2008). To evaluate precisely the feedback between vegetation and climate in Southeast Asian tropics, further studies will be required to test the effect of temperature on leaf production in the natural environment.

In this study, we showed that daily observation of saplings is a useful method to understand leaf phenology in tropical trees that enabled us to test experimentally the effects of candidate factors on leaf production and to identify fluctuations in leaf production. In addition to the conventional long-term manual observations and monitoring by satellites, applying daily observation of saplings to many tropical trees will help us understand the regulation of leaf production in tropical rainforests.

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AUTHOR CONTRIBUTIONS

M.J.K., K.K.S.N., S.L.L., N.M., and N.T. designed the research. Monitoring of plants was conducted by M.J.K. with the support of K.K.S.N. Analyses were performed by M.J.K. M.J.K. wrote the article with the help and critique of K.K.S.N., S.L.L., N.M., and N.T.

DATA AVAILABILITY

The monthly leaf-flushing data for the Ulu Gombak Forest Reserve was obtained from Medway (1972: table 6). Monthly average temperature and rainfall data for the Ulu Gombak Forest Reserve for the study period were downloaded from the Climate Change Knowledge Portal (https://climateknowledgeportal.worldbank.org) by specifying latitude 3.35000000 and longitude 101.78333333.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. The saplings of *Neobalanocarpus heimii* and *Shorea leprosula* used for daily observation.

APPENDIX S2. Details of the analysis of the time-lapse digital photography.

APPENDIX S3. Details of the CCM analyses.

APPENDIX S4. Results for daily leaf production obtained using digital cameras.

APPENDIX S5. Autocorrelation analysis.

APPENDIX S6. Results for daily leaf production obtained by manual observation.

APPENDIX S7. Comparisons with the results of daily manual observation.

APPENDIX S8. Representative images of the *Neobalanocarpus heimii* saplings under low (Tr_{1-2}) and high (Tr_{1-3}) temperature conditions during the growth chamber experiment.

APPENDIX S9. Results of growth chamber experiments.

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