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Evolution of Reproductive Plasticity in a Seasonal Tropical Environment

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ABSTRACT

Seasonality can drive the evolution of reproductive plasticity in insects. An extreme form is reproductive diapause, where individuals halt reproduction during unfavourable seasons. How diapause evolves from milder forms of plasticity (e.g., through changes in cue perception and responses) is poorly understood. In the tropics, seasonality is common, but it is unclear how widespread reproductive plasticity is, or how it correlates with dry and wet seasons. Here, we analyse reproductive plasticity in Amazonian butterflies using a four-year monthly time series of reproductive phenotypes. Sampling nine species across four Nymphalidae subfamilies, we observe a widespread occurrence of diverse reproductive plasticity, suggesting repeated evolutionary changes in reproductive plasticity. Detailed analyses of two *Catonephele* species reveal a conserved cue (temperature), with species-specific divergence of cue response (dry season diapause only in *C. acontius*). Together, our data support the ubiquity of reproductive plasticity in the tropics and suggest repeated evolutionary change in diapause.

RESUMEN

La variación estacional puede promover la evolución de plasticidad reproductiva en insectos. Una forma extrema es diapausa reproductiva—la suspensión completa de reproducción durante estaciones desfavorables. Los mecanismos por los que la diapausa evoluciona a partir de formas más leves de plasticidad (por ejemplo, a través de cambios en la percepción y las respuestas a señales) son poco conocidos. El trópico incluye climas estacionales, pero la prevalencia de la plasticidad reproductiva, y su correlación con las estaciones secas y lluviosas son aún desconocidas. Aquí, analizamos plasticidad reproductiva en mariposas amazónicas, aprovechando un registro mensual continuo de cuatro años de fenotipos reproductivos. Muestreando nueve especies de cuatro subfamilias de Nymphalidae, observamos una amplia diversidad de plasticidad reproductiva, lo que sugiere cambios evolutivos repetidos en la plasticidad reproductiva. Un análisis más detallado en dos especies de *Catonephele* reveló que, aunque la señal ambiental (temperatura) está conservada, la respuesta a ella es especie-específica (diapausa en la estación seca exclusivamente en *C. acontius*). En conjunto, nuestros datos apoyan la ubicuidad de la plasticidad reproductiva en los trópicos, y sugieren evolución recurrente de la diapausa.

1 | Introduction

Many organisms experience seasonality and have evolved key adaptations to it. In Lepidoptera (butterflies and moths), two main responses to cyclical environmental variability are migration, a spatial response (Merlin and Liedvogel 2019; Pollard et al. 1998; Sparks et al. 2005), and diapause, a temporal response (Hoffmann and Bridle 2022; Merlin and Liedvogel 2019; Musolin and Saulich 2012). An important form of diapause is adult reproductive diapause, which is particularly important in the tropics (Denlinger 2022; Denlinger et al. 2012; Halali et al. 2024; Koštál 2006; Nylin 2013). This is the halting of oogenesis and mating in females, which allows them to effectively 'wait out' poor environmental circumstances such as dry seasons or winters (Denlinger 2023; Halali et al. 2020; Nadeau et al. 2022). Tuning reproduction in this way to cues that signal changes in environmental quality is key to fitness in seasonal habitats. This requires mechanisms that link sensitivity to relevant environmental signals with tissue-specific responses (van der Burg and Reed 2021). In many temperate regions, photoperiod, humidity, temperature and host plant availability have all been shown to act as cues to drive the onset of seasonal diapause (Goehring and Oberhauser 2002; Klockmann and Fischer 2019). Such seasonal plastic responses have been widely described across insects and especially in butterflies (Jones and Rienks 1987; Kemp 2001; Kopper et al. 2001; Lindestad et al. 2020; Radchuk et al. 2013), moths (Wadsworth and Dopman 2015; Yamashita 1996) and bees (Santos et al. 2018; Treanore et al. 2020).

Many tropical ecosystems exhibit strong seasonality in rainfall, which profoundly affects plant phenology and growth, and consequently imposes constraints on insect reproduction (Feng et al. 2013). However, seasonal diapause is under-studied in the tropics compared to temperate areas (Denlinger 2002, 2023; Halali et al. 2020; Nylin 2013; Ragland et al. 2019; Saunders 1933; Schebeck et al. 2024) (but see Denlinger 1986; Halali et al. 2021; Jones and Rienks 1987; Kemp 2001; Santos et al. 2018), in particular when considering that tropical species form a major proportion of global biodiversity. Studies in temperate areas have revealed that diapause usually occurs in close to 100% of the individuals in a population (Kopper et al. 2001; Hiroyoshi and Reddy 2018) and ends when the climate becomes favourable (Green and Kronforst 2019; von Schmalensee et al. 2024). However, work on diapause in the tropics has found considerable variation in this trait (Halali et al. 2020), with timing, cues and proportion of individuals in a population entering diapause varying between species and location.

Outside of Lepidoptera, variation in reproductive diapause has been quantified in species which have tropical origins. For example, *Drosophila melanogaster* (Meigen, 1830) has rapidly evolved a mild form of reproductive diapause in North America (Erickson et al. 2020), and it is likely that *D. melanogaster* in the African tropics can diapause or show reproductive plasticity as adults (Zonato et al. 2017). While studies in temperate areas have greatly advanced our understanding of the mechanisms and evolution of diapause, it is unknown whether those insights also apply to reproductive plasticity in the tropics (Denlinger 2023). For instance, species across the Australian, Asian and African tropics display remarkable variation in both environmental cues and the proportion of individuals that undergo a reproductive diapause: *Catopsilia pomona* (Fabricius, 1775) (Jones 1987) and *Eurema herla* (WS Macleay, 1826) (Jones and Rienks 1987) show a preparatory and annually consistent cessation of reproduction independent of rainfall, whilst *Euploea core* (Cramer, 1780) (Daglish et al. 1986) and *Eurema laeta* (Boisduval, 1836) (Jones and Rienks 1987) have reproduction linked directly to rainfall. Many species of tropical Mycalesina butterflies living in seasonal environments diapause in the dry season, but there can be large variation in the population with some individuals being reproductively active, depending on the species (Halali et al. 2020, 2021). In sum, diapause seems to be more variable in the tropics compared to temperate areas in terms of the proportion of the population that diapauses, whilst knowledge of the diversity in the use of environmental cues remains relatively limited.

The growing concern is that climate change is making seasonal cues less reliable. The Neotropics are at a large risk of critical transitions in seasonality due to climate change (Nepstad et al. 2008), with models estimating that by 2050 there will be an increase in the number of consecutive dry days by 10–30 days across the Amazon forest system (Flores et al. 2024). Where species rely on consistent and reliable cues for timing of phenology, climate change-induced shifts in these seasonal patterns can lead to maladaptive phenotype-environment mismatches (Ashander et al. 2016; Stenseth et al. 2002; Thackeray et al. 2016; Visser and Both 2005). This imposes strong selection on the extent and architecture of plastic responses. Whether reproductive plasticity will be able to evolve quickly enough to accommodate rapid anthropogenic climate change depends on the amount of genetic variation for plasticity and the alignment of this variation with the main axes of phenotypic and environmental variation (Chevin and Hoffmann 2017; Noble et al. 2019; Riley et al. 2023).

In the tropics, there is a gradient in seasonality from the equator (non-seasonal) to higher latitudes (more seasonal). The southwest Amazon experiences around 4 months a year with less than 60 mm of rain—a 'tropical dry month' as per the Köppen climate classification (Peel et al. 2007). Food and host plants are likely to become less abundant and lower quality during the dry season, making it a less favourable environment (Braby and Jones 1995). Further, extremes of weather during the dry season can also be less favourable for the development of larvae (von Schmalensee et al. 2024), or the unfavourability may link to the seasonality of predators, for example, breeding patterns of tropical birds. The butterfly taxa studied here have diverged across the Amazon, representing around 79 million years of evolutionary history (Chazot et al. 2021; Kumar et al. 2022). This provides an ideal model to study the evolution of reproductive plasticity in a seasonal tropical environment.

Here, we analyse reproductive plasticity in Neotropical butterflies to reveal evolutionary patterns and climatic drivers. We utilise a five-year butterfly monitoring time-series from the southern Amazon, an important biodiversity hotspot and a major geographic origin of butterflies (Kawahara et al. 2023). We quantified reproductive status for 599 individual females of nine species across four subfamilies, focusing specifically on two closely related species from the genus *Catonephele*. We integrated these with local weather station data in order to identify putative environmental cues associated with reproductive phenotypes. This enabled us to quantify how reproductive output varies across the phylogeny and between seasons, providing new insights into the evolution of insect diapause in the tropics.

Our study aims to analyse reproductive plasticity in the tropics and how it varies across the phylogeny, across seasons and across reproductive traits. Specifically, we address the following questions: (1) How has reproductive plasticity evolved in nymphalid butterflies? (2) How does seasonal reproductive plasticity differ between two closely related *Catonephele* species? (3) What are the putative climatic cues of seasonal reproductive plasticity in these species?

We observed a range of patterns of reproduction even within genera, which suggests repeated changes in seasonal reproductive plasticity in multiple clades. Focusing on two closely related species of the subfamily Biblidinae, we observed *Catonephele acontius* (Linnaeus, 1771) to exhibit diapause during the dry season, while *C. numilia* (Cramer, 1775) does not. Despite their differences in reproductive plasticity, finding an association between reproductive plasticity and environmental data reveals that both species share maximum temperature as a likely key driver of reproductive plasticity. Thus, the evolution of diapause combines cue conservation with species-specific divergence of plastic traits, possibly suggesting that different components of reproductive plasticity can evolve independently. Importantly, our time-series data enabled us to establish that the response to temperature is immediate, except for a small anticipatory role for photoperiod and minimum temperatures, which may set up the temporal boundaries of direct temperature responses. Thus, reproductive plasticity in our study species may share features both with direct stress responses and with anticipatory diapause as observed in other taxa. Whilst a formal phylogenetic comparative analysis of tropical diapause would require broad taxon sampling, we demonstrate evolved differences in reproductive plasticity across the phylogeny.

2 | Materials and Methods

2.1 | Study Area and Species

Field collections of butterflies were conducted at Finca Las Piedras (FLP), a biological field station in southeastern Peru (Figure S1) (lat. −12.226348°, lon. −69.112599°; ca. 250 masl) where we lead a long-term study of Lepidoptera diversity and biology. The lowland tropical rainforest site, characterised by pronounced wet and dry seasons, offers an ideal setting for studying butterfly adaptations to a rapidly changing seasonal environment.

We first performed a broad analysis of reproductive dynamics in nine species across the Nymphalidae family, analysing species from the Biblidinae, Charaxinae, Satyrinae and Nymphalinae subfamilies. We then focused on reproductive dynamics in two butterflies *Catonephele acontius* and *C. numilia* (Lepidoptera: Nymphalidae: Biblidinae) with a 9-million-year divergence time (Hedges et al. 2015; Kumar et al. 2022). These species are sympatric across much of the Neotropics, including at the study site in Southeast Peru and are thought to share similar ecological niches, though very limited data are available. For instance, larvae of both species are known to feed on trees in the genus *Alchornea* (Euphorbiaceae) (Beccaloni et al. 2008; Woodson et al. 1967).

2.2 | Specimen Collection

Standardised monthly trapping at FLP was carried out at ten trap stations in intact forest, each station with paired standard butterfly traps placed at ground level and at varying heights in the forest canopy and baited with fermented banana (Freitas et al. 2021). Trapping was conducted during five consecutive days within the first 10 days of each month, with the analyses presented in this paper covering June 2020 to January 2024.

To supplement the specimens collected as part of the long-term trapping study, additional collections were carried out at FLP in the dry season of 2023 (August–October). These included twenty banana-baited traps set up along a ~2 km stretch of forest trail, placed in light gaps near ground level. At the point of collection, geographic coordinates, date, time, sample ID and collector name were noted. Samples were then photographed, identified and stored in glassine envelopes for future dissection.

2.3 | Quantification of Reproductive Phenotypes

To determine reproductive status, we carried out dissections of adult female butterflies caught monthly throughout 2020–2024 (Figure S2). We counted mature eggs (large/yolk-filled) and spermatophores in female butterflies and used these results to assess reproductive activity. The dissections were carried out using a Motic SMZ 160-TLED stereo microscope and standard dissection tools, following the procedure established by Halali et al. (2020). The detailed protocol used in this study can be found in the supporting information S13.

To check for covariance with both body size and wing damage (as a proxy for age), we adopted a quantitative approach similar to that used by Lehnert (2010). We scanned all four wings of 60 new individuals (separate to the 599 samples already reported) using an Epson Scan v600 Perfection and Epson Scan 2 software. To ensure consistent positioning and scaling, a 4 × 4 paper grid was placed on the scanner bed. The resulting images were processed using Fiji version 2.16.0 (Schindelin et al. 2012). Each wing was cropped into a standardised square format to ensure uniformity in subsequent analyses. Using Adobe Photoshop, each wing was isolated and pasted into a new file, leaving only the area with damage visible. For damaged wings, undamaged wing scans were used as references to reconstruct their presumed full shape, creating an "outline wing". These images were then analysed in Scion Image Beta version 4.0.2. To quantify the wing area, the pixel count for both the outline (full) wing and the damaged wing was recorded, and from these values, a % wing damage was calculated. We then quantified their reproductive phenotypes using the same methodology as above.

2.4 | Climate and Weather Data

Contemporary climate and weather data have been collected daily at FLP since July 2017 at chest height in the primary forest (maximum/minimum daily temperature and daily precipitation). This data was used to investigate cues in both *Catonephele* species. Long-term historic climatic data (1901–2021) was downloaded from the Climatic Research Unit Time Series (CRUTS) dataset 4.06 for the grid box −12.5, −69.5, −12.0, −69.0 on the 24th September 2024 (Osborn and Jones 2014). This data was used for the Wavelet analyses and calculating Colwell's Indices to test for climatic seasonality. Wavelet analysis is used for quantifying and identifying periodicity of fluctuations in environmental or other variables (Cazelles et al. 2008). Colwell's indices give information about environmental stability; repeatability of seasonal patterns; and the extent to which seasonality contributes to predictability (Colwell 1974). We define the dry season as June to October, using a measurement of <60 mm of total rainfall in the prior 30 days (Peel et al. 2007; Zeitschrift 1884). This approach provides a stable estimation of the dry season across all four study years. It is important to note that these analyses are based on modelled historical data (CRUTS), covering an area of approximately 5 km^2.

2.5 | Statistical Analyses

All statistical analyses and plotting of figures were carried out using R v4.2.2 (R Core Team 2022), with the packages *WaveletComp v1.1* (Roesch and Schmidbauer 2018), *hydrostats v0.2.9* (Bond 2022), *ggplot2 v3.5.1* (Wickham 2016). For the wavelet analyses, the following functions within *WaveletComp* were used to calculate and then visualise the data: 'analyse. wavelet', 'wt. image' and 'wt.avg'. The following parameters were used: loess. span = 0 (no smoothing), dt = 1 (evenly spaced monthly values), dj = 1/250 (fine scale resolution), lowerPeriod = 2 (months), upperPeriod = 32 (months), n.sim = 10 (simulations). The package *mondate v1.0* ("Mondate: Keep Track of Dates in Terms of Months" 2010) was used to reformat the dates from the original database. *Hydrostats* was used to calculate Colwell's indices (Table S2) using the 'Colwells' function. The following parameters were used: s = 11 (11 bins separate into 12 months), base. binning = 2 (logarithmic base binning), from = 0.5, by = 0.25, base. entropy = 2 (binary logarithm).

To test differences in annual patterns of egg-laying, we used circular statistics with the packages *circular v0.5.1*, and *dplyr v1.1.4* (Lund and Agostinelli 2024; Wickham et al. 2025). Circular statistics relies on the conversion of cyclical data (e.g., months of the year) into angles. This approach returns a rho value which indicates how clustered the data are (0 = nearly uniform across the year, 1 = highly concentrated in a few months). Due to uneven sampling effort, we took 1000 bootstrap resamples of butterflies within each month, which gave us a 95% confidence interval of rho for the egg-laying of each species.

We used two approaches to model (1) the presence/absence of eggs and (2) the non-zero egg counts between the seasons using a binomial generalised linear mixed model (GLMM) (structure: EggPresence ~ season + (1|year), family: binomial) for part 1 and a negative binomial GLMM (structure: EggNumber ~ season + (1|year), family: negative binomial) for part 2 with *lme4 v1.1.38* (Bates et al. 2015) and *MASS v7.3.65* (Venables and Ripley, 2002) respectively. We included year as a random effect to test for inter-year variability.

To test the impact of confounding variables (i.e., body size and age of butterfly), we used wing area as a proxy for body size and wing wear as a proxy for age. We used *t*-tests to compare reproductive and non-reproductive individuals, as well as linear models between wing area and wing damage with egg number (Figures S9–S11).

To test the correlation between our phenotypic and climatic variables, we used both linear regressions and Pearson's product–moment correlation. These correlational analyses used weather data collected and recorded daily at FLP, which was then averaged per month. All raw data and R scripts can be found linked in the supporting repository (Hicks et al. 2025).

3 | Results

Given the range of existing definitions of reproductive diapause, in this paper we compare reproductive activity for all species through multiple measurements, specifically egg number within reproductive females (defined as those holding at least one mature egg), the proportion of individuals without eggs (diapausing) and the proportion of individuals without spermatophores (non-mating). Some butterflies could lack eggs due to other factors (e.g., health/age) which we are unable to quantify.

3.1 | Pronounced Dry-Wet Seasonality of Study Area

Although there are pronounced differences in the climate between the wet and dry season in this area of the Amazon, there is no well-defined boundary. To demonstrate that there is a periodic (i.e., annual) change in climatic variables, we performed a wavelet analysis of two main climatic variables (mean temperature and precipitation) using historical data from 1921 to 2020 (Figure S3). Wavelet analyses showed significant and coordinated annual periodicity for both temperature and precipitation. Furthermore, Colwell's indices (Colwell 1974) (Table S2) suggests that there is medium to high environmental stability at FLP, and that seasonality contributes to predictability, meaning that wet and dry seasons can be classified accurately and reliably. This analysis justifies the use of the Köppen climate classification: <60 mm in the previous 30 days to define a dry season day.

3.2 | Variation in Reproductive Plasticity Across the Nymphalidae Family

We show that changes in reproductive activity during the dry season are common across nine species of Nymphalidae butterflies we investigated (Figure 1). Specifically, *Bia rebeli* (Bryk, 1953), *Amphimacus demophon* (Linnaeus, 1758), *Colobura annulata* (Willmott, Constantino & Hall, 2001), *Nessaea obrinus* (Linnaeus, 1758) and *C. numilia* show similar proportions of mated and unmated females between the wet and the dry season, whilst three others have changes between the

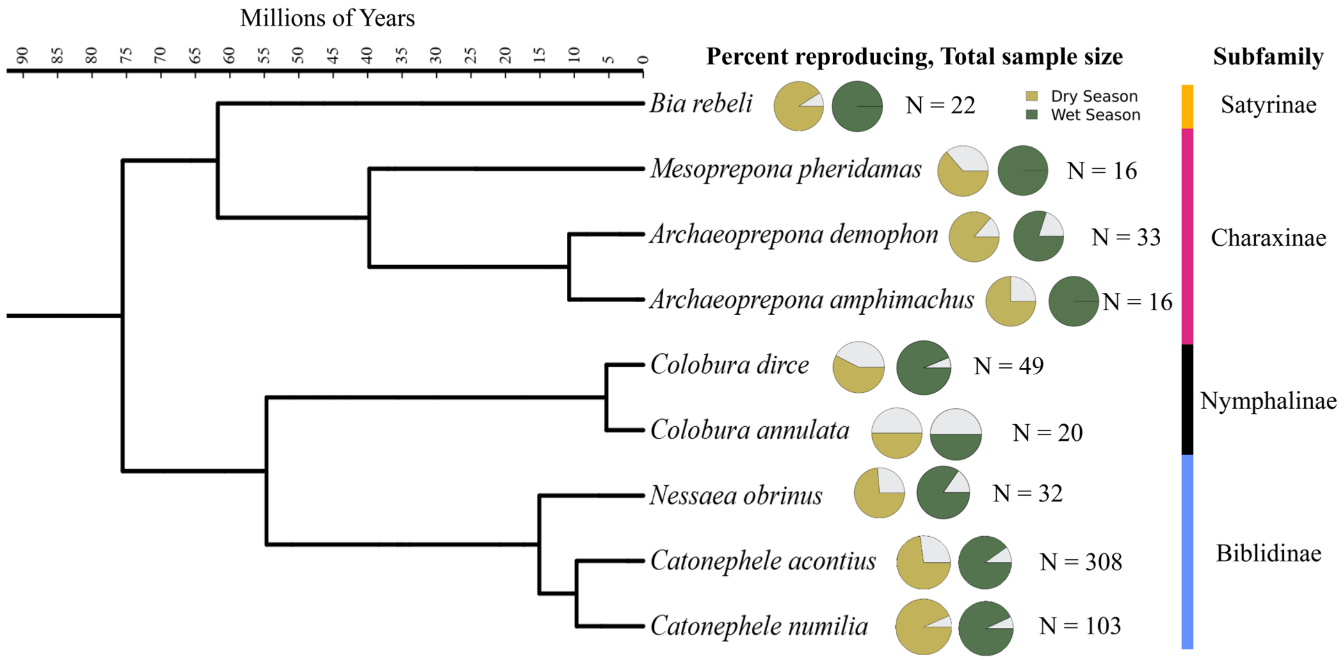

FIGURE 1 | Variation in seasonal reproductive plasticity across 79 million years of Nymphalid butterfly evolution in a neotropical rainforest. We phenotyped reproductive status by counting eggs for 599 individual females of 9 species (in 4 subfamilies) collected in dry and wet seasons over 5 years. The proportion of females diapausing during the dry season and the wet season is represented in pie charts, and the total sample size for that species is noted. Based on phylogeny from (Chazot et al. 2021; Letunic and Bork 2024). A more detailed breakdown of egg number patterns can be found in Table S1.

seasons (*Mesoprepona pheridimas* (Cramer, 1777), *Colobura dirce* (Linnaeus, 1758) and *C. acontius*) (Figure S7).

3.3 | Seasonal Diapause in *C. acontius* but Not *C. numilia*

The two *Catonephele* species showed markedly different seasonal responses in egg production (Figure 2). The egg number distribution for *Catonephele acontius* differs significantly between the wet and the dry season, with a significantly higher proportion of diapausing individuals (individuals holding no eggs) during the dry season (27.1% of 164 individuals) compared to the wet season (9.76% of 144 individuals; X-squared = 14.535, df = 1, p-value < 0.001). In contrast, while a small proportion of *C. numilia* individuals were also diapausing, there was no difference between dry and wet seasons (wet: 7.02%, $n = 57$; dry: 6.52%, $n = 46$; X-squared = < 0.001, df = 1, $p = 1$). These results were both supported by binomial GLMMs that showed the probability of eggs being present in the wet season was significantly higher in *C. acontius* ($\beta = 1.23$, SE = 0.32, $z = 3.819$, $p < 0.001$), but not in *C. numilia* ($\beta = 0.16$, SE = 0.86, $z = 0.182$, $p = 0.856$). Further, including 'year' as a random effect explains negligible variance in the data (*C. acontius*: variance < 0.0001; *C. numilia*: variance < 0.0001). Thus, we observed a species-specific reduction in the proportion of reproductive females.

Next, we analysed the subset of reproductively active females (i.e., those with at least one egg), and observed a reduction in egg number between wet and dry seasons for both *C. acontius* (wet mean = 11.05, dry mean = 8.03, 27.3% reduction) and *C. numilia* (wet mean = 20.38, dry mean = 18.07, 11.3% reduction) (Figure 2). This was supported for both species by a Wilcoxon rank sum test (*C. acontius*: W = 5121, p-value < 0.001 and *C. numilia*: $W = 913.5$, p-value = 0.048). Whilst the result for *C. acontius* is supported by a negative binomial (GLMM) ($\beta = 0.319$, SE = 0.076, $z = 4.213$, $p < 0.001$), it is not for *C. numilia* ($\beta = 0.122$, SE = 0.109, $z = 1.13$, $p = 0.26$). Further, the 'year' explained very little variance (*C. acontius*: variance < 0.001; *C. numilia*: variance < 0.001).

We observed a less pronounced seasonal change in reproductive activity (spermatophore number) in comparison to reproductive output (egg number). Specifically, we observed most individuals of both species holding 1–3 spermatophores, and a small proportion of the population being unmated (Figure 3). *C. acontius* caught in the dry season were just as likely to be carrying spermatophores as those caught in the wet season (wet: 6.10%, $n = 164$; dry: 8.33%, $n = 144$, X-squared = 0.28993, df = 1, p-value = 0.590) with the same pattern found in *C. numilia* (wet: 0%, $n = 57$; dry: 2.17%, $n = 46$, X-squared = 0.01165, df = 1, p-value = 0.914). However, a reduction of 11.2% (wet mean: 1.61, dry mean: 1.43) was seen in average spermatophore number for *C. acontius* in the dry season compared to the wet season (Wilcoxon rank sum test one-sided, $W = 9506$, p-value = 0.040). No such difference was observed in *C. numilia* (Wilcoxon rank sum test one-sided, $W = 1467$, $p = 0.86$) (Figure S5).

Monthly time-series data for egg number throughout the year allow us to examine intra-annual fluctuations in reproductive output in more detail (Figure 4). Both species demonstrate a non-uniform distribution (Rayleigh test, *C. acontius:* $z = 0.19$, $p < 0.0001$, *C. numilia*: $z = 0.064$, $p < 0.001$). However, *C. acontius* has a more distinct seasonal response in egg production,

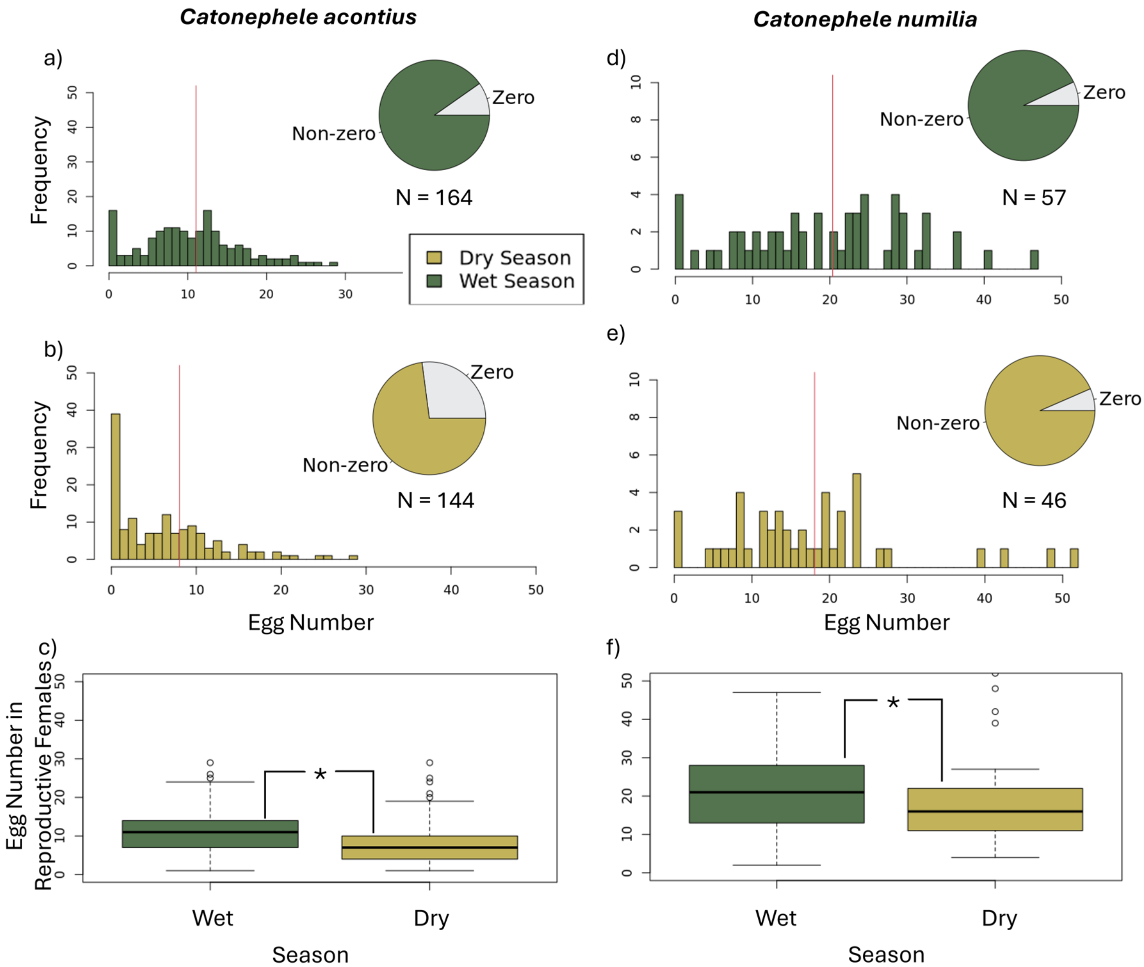

FIGURE 2 | Dry season induces a marked shift in reproductive pattern in *Catonephele acontius* but not in *C. numilia*. Distribution of egg number in (a, b) *C. acontius* and (d,e) *C. numilia* in wet and dry seasons respectively. Histograms show a zero-inflated, positively skewed normal distribution of egg number in both, but the number of diapausing females strongly increases between wet and dry seasons in *C. acontius* and not *C. numilia*. The average number of eggs (red vertical lines in a, b, d, e) for reproductive females reduces significantly from the wet season to the dry season in (c) *C. acontius* (*Wilcoxon rank sum test one-sided, $W = 5121$, p-value < 0.001) and (f) *C. numilia* (*Wilcoxon rank sum test one-sided, $W = 913.5$, p-value $= 0.048$). A comparison with a randomly selected equal sample size demonstrates similar overall patterns (Figure S4).

with egg-laying more clustered in the wet season (median rho = 0.191, 95% CI: 0.145–0.233, mean month = 10.6 (October), bootstrapped $n = 1000$) than in *C. numilia* (median rho = 0.0717, 95% CI: 0.0173–0.147, mean month = 10.8 (October), bootstrapped $n = 1000$). Further, the egg number samples for both species come from different circular distributions (Watson U^2 two-sample test statistic: 2.856, $p < 0.001$) (Figure S12).

3.4 | Relationship Between Weather and Reproduction

To understand how the environment drives seasonal reproductive dynamics of *Catonephele* species, we examined the correlation of each phenotypic variable (proportion of diapausing females; average egg number of reproductive females; proportion of unmated females) with environmental variables (rainfall, temperature, photoperiod), using monthly averages for each variable (Figure 5). For *C. acontius*, there is a strong relationship of maximum temperature with two phenotypic variables compelling an immediate response during the dry season (egg number: Pearson's $r = -0.845$, $t = -4.99$, $p < 0.001$; diapause proportion: Pearson's $r = 0.815$, $t = 4.44$, $p < 0.01$) and a weak and statistically non-significant relationship between unmated proportion and maximum temperature (unmated proportion: Pearson's $r = 0.525$, $t = 1.95$, $p = 0.080$).

In the same analysis for *C. numilia*, maximum temperature was also the best (and only significant) correlate for the average egg number in reproductive females (Pearson's $r = -0.682$, $t = -2.95$, $p = 0.015$).

We also tested time-shifted correlations between phenotypes and climate by 1 or 2 months earlier (testing if and how phenotypic changes precede seasonal shifts in a potential anticipatory response) or 1 or 2 months later (testing if and how potential environmental cues precede phenotypic changes) (Figure 6). The two-month time lag is based on the natural lifespan of the two species (Gonzalez 2024). Across photoperiod, minimum temperature and daily rainfall, there are significant correlations with egg number in reproductive females of *C. acontius* 2 months later (lagging) (minimum temperature: Pearson's

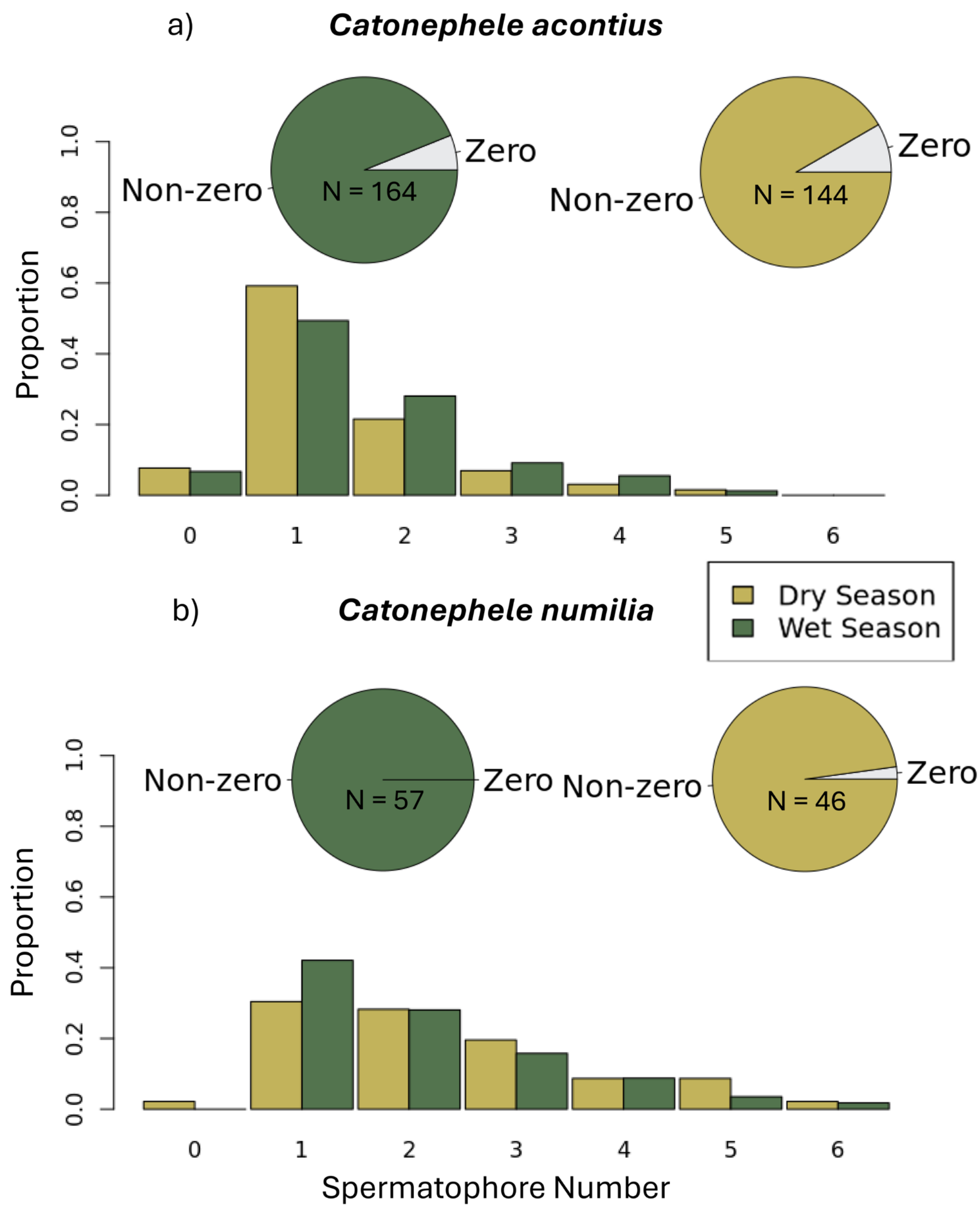

FIGURE 3 | Dry season induces muted change in mating activity in *C. acontius* but not in *C. numilia*. Distribution of spermatophore number in (a) *C. acontius* and (b) *C. numilia* in wet and dry seasons respectively. Pie charts show that the proportion of mated individuals (with spermatophores) varies little between the seasons and between the species, with most individuals holding one to three spermatophore at any time.

$r=0.803$, $t=4.27$, $p=0.002$; photoperiod: Pearson's $r=0.651$, $t=2.71$, $p=0.022$; precipitation: Pearson's $r=0.647$, $t=2.69$, $p=0.023$) and 2 months earlier (leading) (minimum temperature: Pearson's $r=-0.693$, $t=-3.04$, $p=0.013$; photoperiod: Pearson's $r=-0.762$, $t=-3.72$, $p=0.004$; precipitation: Pearson's $r=-0.665$, $t=-2.82$, $p=0.018$). In *C. numilia*, the same is seen with negative correlations of photoperiod (Pearson's $r=-0.691$, $t=-3.02$, $p=0.013$), precipitation (Pearson's $r=-0.621$, $t=-2.50$, $p=0.031$) and minimum daily temperature (Pearson's $r=-0.810$, $t=-4.37$, $p=0.001$) with egg number in reproductive females 2 months earlier (leading) (Figure S8).

In summary, we observe that maximum temperature has a completely different relationship with these life history variables compared to minimum temperature, photoperiod and rainfall. The other climatic variables show associations with the egg number in reproductive females in both the two-month lead and two-month lag scenarios, whilst maximum temperature is only strongly predictive when considered to be moving in tandem with the reproductive phenotypes (Figure 6).

4 | Discussion

Here, we leverage a rare long-term butterfly monitoring dataset from the Neotropics, an important biodiversity hotspot for butterflies and an area with highly seasonal habitats (Kawahara et al. 2023). We use abdomen dissections for 599 individual females of 9 species representing around 79 million years of evolution and combine these with local weather station data. This enabled us to quantify how reproductive output varies across the phylogeny and between seasons. Our data show shared

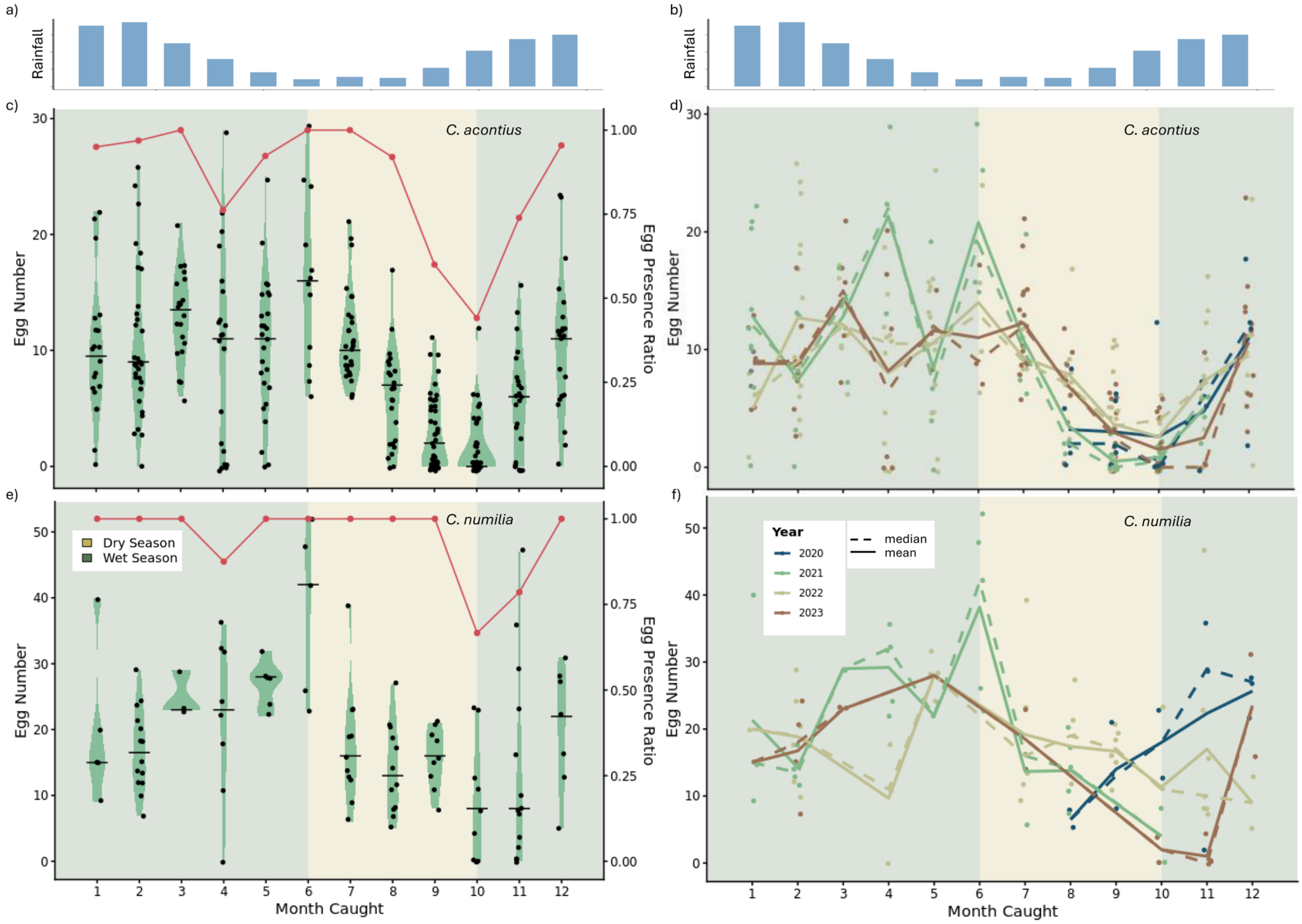

FIGURE 4 | A decrease in average egg number during the dry season in both species, but a seasonal increase in diapausing individuals occurs only in *C. acontius*, and not in *C. numilia*. The seasonality in rainfall is indicated by (a, b) average daily precipitation per month (January to December) and the seasonal background colours in (c, d, e, f). Violin plots are created with data from the dissections of (c) *C. acontius* and (e) *C. numilia* in FLP aggregated over 2020-2024 with the black horizontal line representing the median value for that month. Thickness of the violin represents density of data. The sample size for each month can be found in Table S3. The red line tracks the proportion of reproductive individuals (holding at least one egg), demonstrating the change in diapausing strategists throughout the year. Similar annual patterns are seen across the 4 years of study. Mean and median number of eggs in each *Catonephele* species (d) *acontius*, (f) *numilia* across 4 years of dissections.

environmental cues for reproductive plasticity across species, but also the evolution of trait-specific divergence in developmental response. This may evolve in a modular way, but more experimental work would be needed to understand these evolutionary mechanisms. Our weather analysis adds an important new angle that identifies putative environmental cues, which could inform future studies on how these species may respond to a changing climate. Using long-term field data, we fill a key gap in our understanding of seasonal behaviour in Neotropical insects.

Sampling four subfamilies across the Nymphalidae phylogeny (divergence times spanning 10–79 MYA), we observe phylogenetically widespread occurrence of reproductive plasticity, including seasonal diapause. This suggests repeated changes in reproductive plasticity in multiple clades. This finding is important for two key reasons: firstly, because the Neotropics are likely to experience intense changes under future climate change (Flores et al. 2024). Secondly, these results add the Neotropics to the current geographical range where reproductive diapause has been found, a novel but expected finding given the instances of this trait across other tropical regions (Braby and Jones 1995; Denlinger 1986, 2023; Halali et al. 2021, 2024; Jones and Rienks 1987; Kemp 2001; Tauber and Tauber 1981; Tougeron 2019).

Next, using two *Catonephele* species as a model, we show that *C. acontius* exhibits a type of reproductive diapause during the dry season, while *C. numilia* does not. In *C. acontius* the proportion of diapausing females significantly increases, and the mean number of spermatophores held decreases during the dry season, while we observe no such response in *C. numilia*. This indicates divergent evolution in seasonal reproductive plasticity in this genus that has happened since their last common ancestor 9MYA (Hedges et al. 2015; Kumar et al. 2022). A low percentage of unmated individuals in the dry season suggests that diapause in *C. acontius* is associated with pre-diapause mating. At the same time, we observe seasonal shifts in a separate aspect of reproductive activity, egg number in reproductive females, that is shared between the species, suggesting a conserved

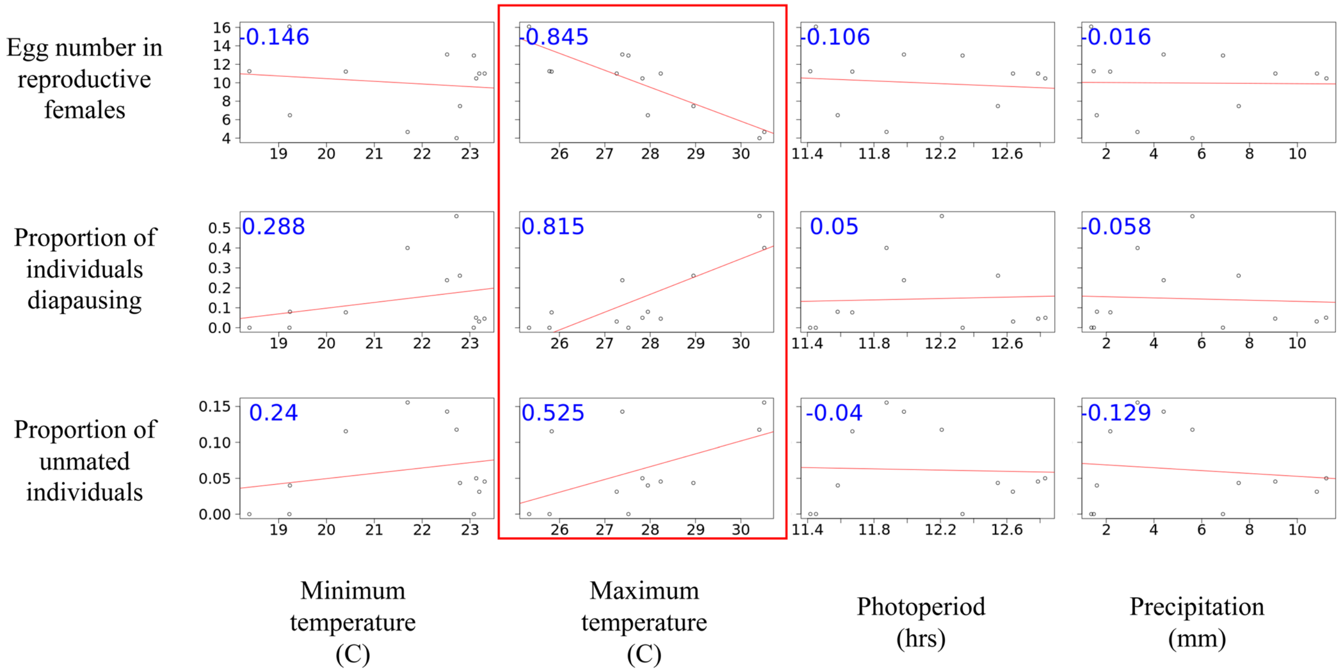

FIGURE 5 | Maximum temperature correlates strongly with phenotypic variables in *C. acontius*. Results from a correlational analysis of monthly values between four climate variables (maximum temperature, minimum temperature, photoperiod and precipitation) and the three phenotypic variables (egg number in reproductive females, proportion of individuals diapausing and proportion of unmated individuals). Blue numbers are Pearson's *r* values, whilst red lines are linear model regressions.

component of reproductive plasticity. Specifically, reproductive (non-diapausing) females of both *Catonephele* species show a modest reduction in egg production during the dry season. Importantly, both the conserved and species-specific seasonal shifts in reproductive plasticity are strongly correlated with predictable seasonal increases in maximum temperature. Under climate change, such a direct response to temperature could lead to rapid induced shifts in reproductive strategy, which could be adaptive as long as climatic correlations stay constant (Walker et al. 2019). Together, our results provide evidence for a shared environmental cue for reproductive plasticity across two *Catonephele* species, but with species-specific developmental consequences of that cue.

Species of tropical savannah-dwelling butterflies have been found to undergo a seasonal reproductive diapause in both Africa and Australia (Braby and Jones 1995; Halali et al. 2020; Jones and Rienks 1987; Kemp 2001); we are highlighting one of the first examples of seasonal diapause in tropical forest species. An adaptation like reproductive diapause may consequently allow these butterfly species to expand their ranges into the Cerrado (Neotropical savannah), like in *Bicyclus* butterflies (Brakefield 2010).

Our climatic analysis revealed that maximum temperature is correlated with reproductive plasticity for both of our study species. In well-studied temperate instances of diapause, it is common to see temperature and photoperiod playing a role in the timing of reproductive diapause (Goehring and Oberhauser 2002; Klockmann and Fischer 2019). Our results suggest that reproductive plasticity—across a suite of correlated traits (egg number, diapause proportion and, though not significantly so, unmated proportion)—is induced by a single and direct response to maximum temperature. Temperature has been linked to seasonal plasticity when coupled with changes in photoperiod (Mallick et al. 2024; Schebeck et al. 2024; Sgrò et al. 2016) and has been well studied in temperate Lepidoptera (Lindestad et al. 2020; von Schmalensee et al. 2024). This response is similar to a heat stress response (Denlinger 2002; Gong et al. 2013; Yocum et al. 1998) where a reduction in environmental quality directly arrests reproduction.

In *C. acontius*, two reproductive traits (egg number and diapausing proportion) are strongly correlated with maximum temperature in an immediate response. However, focusing just on a reduction in egg number across both species, our analysis of a lagging/leading scenario points towards a more complex relationship between local weather and the modulation of egg production (Figure 6; Figure S8). The direct response to maximum temperature, alongside possible prior conditioning via photoperiod and minimum temperature, suggests that this kind of reproductive plasticity exists along a spectrum of reproductive plasticity that includes an immediate stress response. Thus, the putative environmental cue appears to be conserved between species, while the reproductive response to the cue is species-specific (pronounced diapause in *C. acontius* but not in *C. numilia*). Together with the repeated evolution of plasticity across Nymphalidae species (Figure 1), this may suggest that the frequent evolutionary transitions between different forms of plasticity may be facilitated by the independent evolution of cue perception and cue response. Another (non-exclusive) role of temperature may be that it acts as a predictive cue to induce diapause, which could

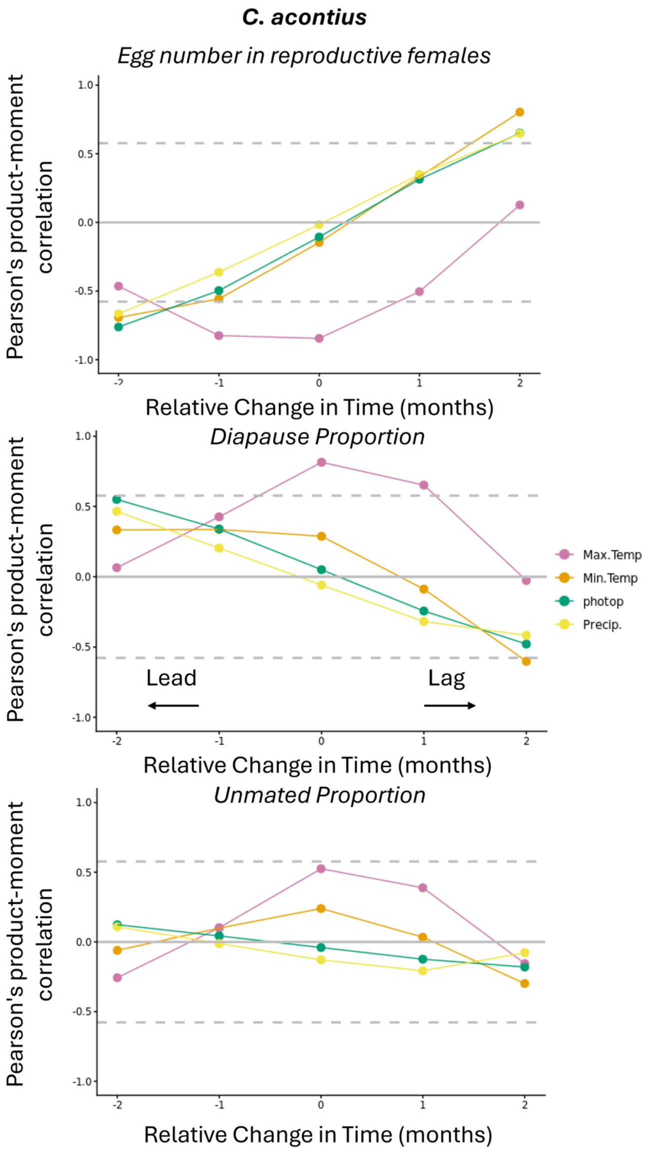

FIGURE 6 | Maximum temperature has a unique relationship with phenotypic variables when considering different sensing and response mechanisms in *C. acontius*. These plots demonstrate the predictive value of environmental variables for reproductive traits (Pearson's *r*) with different relative changes in time (+/- 2 months). This lag refers to how the phenotypic variable has been transformed (e.g., +2 correlates the climatic variable against the phenotypic variable 2 months in the future). The hatched lines represent 95% confidence intervals. Minimum temperature, photoperiod and precipitation all follow similar patterns, having the most predictive value at plus or minus 2 months. Maximum temperature, on the other hand, is unique in having the strongest predictive value with no temporal change, in other words having an immediate effect on the phenotype in question.

have evolved as an adaptation to other harsh aspects of the dry season—for example, a reduction in host plant quality or extremely low rainfall. Within complex climatic conditions, the correlations between climatic variables become important for producing an appropriate phenotype matched to the selective environment. As climate change may rapidly alter the relationships between climatic variables, there is a concern that phenotype-environment mismatches will occur (Walker et al. 2019). However, in the absence of manipulative experiments, it is difficult to disentangle environmental variables as part of either the inductive or selective environment.

The responses of a suite of correlated traits to correlated changes in the environment in both *C. acontius* and *C. numilia* could suggest shared developmental, physiological and genetic pathways, as well as a shared evolutionary history. Diapause may have evolved by integrating new output to existing modules of cue induction and phenotype regulation. Further experimental work would be needed to understand these evolutionary mechanisms in detail; however, this is supported by other models of reproductive diapause in temperate insects, where similar groups of hormones and signalling systems are implicated in creating a diapause response that involves multiple coordinated traits (Green and Kronforst 2019; Denlinger 2002; Sim and Denlinger 2013; Schlichting 1989).

Notably, not all *C. acontius* females go into complete reproductive arrest in the dry season. At the peak of the dry season, 50% of females continue to reproduce, albeit at a reduced rate (Figure 4). This may represent a bet hedging strategy that reduces variance in fitness in variable environments (Overton and Sharkey 2021). Alternatively, if the ability to undergo diapause is genetically determined as it is in other insects (e.g., Erickson et al. 2020), this genetic variation could be maintained through balancing selection.

Both species share similar ranges across South America, with *C. numilia* having a slightly more northerly range into Central America (INaturalist 2024). They share the same group of host plants within the *Alchornea* genus and are likely to both specialise on *Alchornea latifolia* (Swartz, 1788) in the south-eastern ranges of Peru (Alberto Muyshondt 1975). They have similar developmental timings and adult lifespans in captivity (Gonzalez 2024). One difference that we have found is their abundance patterns in FLP (Figure S6), which are consistent with different life-history strategies between the two species.

Reproductive output changes throughout a butterfly's adult lifespan, and therefore age may act as a confounding variable. Without mark and recapture, wild-caught butterflies are difficult to age, but we used wing damage as a proxy (Le Roy et al. 2019; Lehnert 2010; Molleman et al. 2020). Similarly, due to these individuals being wild caught, we cannot experimentally control environmental effects, and we therefore base our inductive cue conclusions on correlational analyses of climate data. Future work should focus on long-term collections of the same species in a Neotropical environment that is seasonal in temperature and photoperiod, but not in rainfall and host-plant quality.

Our work reveals that reproductive plasticity is phylogenetically widespread in the Neotropics. Our analyses also suggest that reproductive plasticity in our study species may share features both with direct stress responses and with anticipatory diapause as observed in other taxa, suggesting that evolutionary transitions between these different forms of plasticity may be gradual and modular. The exact evolutionary outcomes likely depend on the interplay between large-scale gradients in seasonality and local inter-year variability in environmental quality. Ongoing work on ecological, evolutionary and genetic drivers of diapause in tropical species promises to further clarify long-standing questions in the evolution of diapause in complex tropical environments.

Author Contributions

Marcus Hicks, Geoffrey Gallice and Vicencio Oostra conceptualised the study. Jamal Kabir carried out the initial pilot study under the supervision of Geoffrey Gallice. Marcus Hicks collected all phenotypic data. Marcus Hicks, Lizett Retuerto-Silva and Zunilda Escalante-Arteaga carried out the fieldwork, supervised by Geoffrey Gallice and Vicencio Oostra. Tú Minh Au carried out the wing processing and analysis under the supervision of Vicencio Oostra. Data analysis was done by Marcus Hicks, supervised by both Sridhar Halali and Vicencio Oostra, and the writing of the manuscript was done by Marcus Hicks with input and supervision from Sridhar Halali and Vicencio Oostra. All authors contributed to the revision of the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All raw data and metadata supporting the manuscript and R code for all analyses are available on Figshare: https://doi.org/10.6084/m9.figshare.30227872 (Hicks et al. 2025).

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Location of the collection site 'Finca Las Piedras' in Madre de Dios, Peru. **Figure S2:** Female abdomen dissection with eggs and spermatophore labelled. Carried out as described in 'Phenotyping dissection protocol'. **Figure S3:** Clear, coherent annual periodicity in (a) mean temperature, (b) precipitation and (c) the interaction between the two. Also, there is evidence of an interrupted 6-month cycle for temperature. The left panels of section a and b are the wavelet analyses on (a) mean monthly temperature and (b) monthly rainfall. The y-axis is the period (here in months), and the *x*-axis is the calendar year, running from 1901 to 2021. The 'warmer' the temperature plot, the stronger the periodicity. The right-hand side panels are the average wavelet power plots, again with the period on the y-axis. A red line suggests a *p*-value of under 0.05, so there is a significant periodicity of 1 year and of 6 months in mean temperature, whereas precipitation only has a significant periodicity of 1 year. The final part, (c), is a coherence analysis of mean temperature against precipitation. It shows that mean temperature and precipitation are coherent in their annual periodicity (and have been for 120 years), with precipitation lagging slightly behind mean temperature (by less than a week). **Figure S4:** Similar patterns of egg number counts emerge at reduced sample sizes in *Catonephele acontius* as in the full data. Histogram of egg number counts for a random sample of the *C. acontius* with the same sample size as there is for *C. numilia* across the (a) wet and (b) dry season. **Figure S5:** Comparable seasonal trends in the proportion of mated individuals in the population (holding at least one spermatophore) between *C. acontius* and *C. numilia*. The dark purple line represents *C. numilia*, whilst the light blue line represents *C. acontius*. For most of the year, a large majority of females (> 90%) hold at least one spermatophore. However, there is a significant decrease in the number of spermatophore in *C. acontius* during the dry season (see Results section 3.3). The green background represents the wet season, and the yellow background represents the dry season. **Figure S6:** Abundance of a diapausing and non-diapausing species. Abundance of both *Catonephele* species taken from the monthly trapping survey at Finca Las Piedras, from May 2020 to April 2024. *C. acontius* in blue, *C. numilia* in orange. **Figure S7:** Raw annual egg number data for all seven species (whilst *C. acontius* and *C. numilia* are represented in Figure 4), month-by-month. The green background represents the wet season, and the yellow background represents the dry season. **Figure S8:** Relationship between putative environmental cues and reproductive traits for *C. numilia*. Maximum temperature has a different relationship with phenotypic variables than minimum temperature, photoperiod and precipitation. These plots demonstrate the predictive value of environmental variables (represented by different coloured lines) for reproductive traits (Pearson's *r*) with different relative changes in time (±2 months). This lag refers to how the phenotypic variable has been transformed (e.g., +2 correlates the climatic variable against the phenotypic variable 2 months in the future). The hatched lines represent 95% confidence intervals. **Figure S9:** Boxplots comparing wing area (pixels) across reproducing (with eggs) and non-reproducing females (without eggs) across both species. No statistical difference in *C. acontius* (unpaired, two-sided *t*-test, $t = -0.75$, $p = 0.46$) or *C. numilia* (unpaired, two-sided *t*-test, $t = -0.26$, $p = 0.82$). **Figure S10:** Boxplots comparing wing damage (%) across reproducing (with eggs) and non-reproducing (without eggs) and mating (with spermatophore) and non-mating (without spermatophore) females across both species. Statistically significant difference in wing damage between reproducing and non-reproducing *C. acontius* (Wilcoxon, $p < 0.001$), but no difference between mating and non-mating *C. acontius* (Wilcoxon, $p = 0.38$). There was no difference in wing damage between reproducing and non-reproducing *C. numilia* (Wilcoxon, $p = 0.57$), but sample size was small ($N = 8$). **Figure S11:** Egg number and average wing damage across all individuals with wings scanned. No significant linear relationship (linear model, $\beta = 0.105$, SE = 0.090, $t = 1.17$, $p = 0.247$). **Figure S12:** Rose diagram comparing the circular distributions of mean eggs per butterfly for each month between *C. acontius* (blue) and *C. numilia* (red). The months are distributed clockwise around a circular axis, with the *y*-axis representing the mean number of eggs per butterfly per month. **Table S1:** Variation in reproductive plasticity across a phylogeny of neotropical Nymphalidae butterflies. **Table S2:** Colwell's indices for precipitation and temperature variables at study site. **Table S3:** Monthly breakdown of dissection sample sizes at Finca Las Piedras.