Phenological asynchrony in plant–butterfly interactions associated with climate: a community-wide perspective

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Abstract

Although much information has been accumulated on the effects of climate change on particular species worldwide, research aimed at assessing how such change influences biotic interactions from a community-wide perspective is still in its infancy. We contribute to filling in this gap by analyzing a 17-year (1996–2012) dataset that includes records of flower-visitation interactions between 12 butterfly species and 17 plant species in a coastal wetland area in northeastern Iberian Peninsula. We assessed the extent to which temporal asynchronies between plants and adult butterflies are influenced by different climatic variables that affect both plant and insect phenologies. Temperature and degree of aridity at various monthly summaries were used as predictors of the plant–butterfly phenological asynchrony. We identified the seasonal window with the greatest effect on asynchronies for two butterfly generations (spring and summer), and assessed whether the magnitude of asynchrony is associated with the level of butterfly specialization. We used generalized linear mixed models considering a total of 39 plant-butterfly interactions. Average asynchrony was higher in the spring generation and dry conditions during winter lead to decreased temporal overlap with flowers in this butterfly generation, whereas dry conditions in the spring lead to decreased temporal overlap in the summer butterfly generation. The magnitude of the effect was consistently small at the community level (all interactions pooled). Moreover, no clear climatic trend over the study time frame was detected. Finally, specialized and generalized butterflies in their resource use as adults were similarly vulnerable to asynchronies, in contrast to previous predictions of greater mutualistic disruptions in species with narrower niches. We conclude that a least in the Mediterranean region, phenological asynchronies might be more affected by aridity level than by temperature itself, and thus the former can be a key climatic trait to make better predictions in this region.

Introduction

The ongoing climatic change is affecting many ecological processes, among which are interactions such as mutualisms between plants and animals, partly because trophic levels and species within them can respond differently to warming (Walther et al. 2002, Parmesan 2007, Thackeray et al. 2010). One of the most conspicuous responses to climate change is a phenological shift in biological events such as emergence, dispersal, migration, and reproduction. Information is being accumulated from a wide range of ecosystems showing that many species are advancing their phenologies, especially during the spring, although also a good number are experiencing delays, and others simply show no shifts (see review in Peñuelas et al.2013). The different directions and magnitudes of the shifts among taxa is what promotes potential uncoupling (asynchrony) of their interactions, that may translate into disruptions of the ecological functions previously performed by the species interacting, with possible demographic consequences (Miller-Rushing et al. 2010, Kudo and Ida 2013). One of such ecological functions that might be jeopardized by climate change is pollination by animals.

Phenological asynchrony might have detrimental consequences for plant and insect fitness under some specific situations. For instance, (1) when either plants or pollinator species are specialists in the pollination system (Memmott et al. 2007), i.e. a lack of their partners is not easily buffered (compensated) by others present in the area, (2) at the beginning and end of the flowering season, when there might be few alternative species to balance the absence of usual partners (Fitter and Fitter 2002, Thomson 2010, McKinney et al. 2012), or (3) when the period of resource availability (e.g. flowering period for specific pollinators) is too short (Miller-Rushing et al.2010, McKinney et al. 2012, Kudo and Ida 2013). In the last century, climate change has advanced the onset of flowering as well as the flight period of insect pollinators an average of 4 days/ºC/yr in the temperate zones (Memmott et al. 2007, and references

therein). However, both plants and insects show large variation in their responses to abiotic phenological cues and, to date, we still know little about how prevalent the disruption of plant-pollinator interactions due to climate change is (Memmott et al. 2007, Heghland et al. 2009, Miller-Rushing et al. 2010, Bartomeus et al. 2011, Forrest 2014) and what consequences for seed production and pollinator populations such disruption can have (see review in Forrest 2014). A few studies have reported phenological asynchrony in the pollination process to be uncommon in natural ecosystems (Rafferty and Ives 2011; see also Bartomeus et al. 2011, and Forrest and Thomson 2011), whereas others found that high levels of biological diversity can maintain phenological synchrony at the community level by buffering the differential species-specific phenological responses (Bartomeus et al. 2013; Rader et al. 2013). Regarding the ecological and evolutionary implications of plant-pollinator interaction disruptions, information is also scarce, although some studies are advancing in this area (Gilman et al. 2012 and Kudo and Ida 2013).

Elucidating the environmental drivers of plant and pollinator phenologies is necessary to progress in predicting the effect of climate change on pollination, an important ecosystem service. Temperature, in particular, is known to influence the emergence of adult insects in spring (advancing it in warmer springs; Sparks and Yates 1997, Stefanescu et al. 2003, Gordo and Sanz 2006, Ellwood et al. 2012). A few studies have also shown delaying effects of precipitation on insect phenology (Stefanescu et al. 2003, Graham-Taylor et al. 2009), and at high latitudes and altitudes, timing of snow melt appears to be a critical predictor of insect emergence in springtime (Høye and Forchhammer 2008, Iler et al. 2013a,b). For plants, the timing of flowering is known to be sensitive to temperature (Fitter and Fitter 2002, Peñuelas et al.2002, Gordo and Sanz 2006, 2010, Mólnar et al. 2012), precipitation (Peñuelas et al. 2004), and timing of snowmelt in high latitude and altitude plant communities (Høye et al. 2007, Gordo and

Sanz 2010, Lambert et al. 2010, Iler et al. 2013a). In Mediterranean ecosystems, drought is known to influence flowering phenology (for instance, by delaying the flowering of some species; Llorens and Peñuelas 2005) and might as well have an important role in driving plant-insect phenological asynchronies, although this has never been tested. Forrest and Thomson (2011) found that plants are more likely than insects to advance phenology in response to springtime warming, but we need more research to assess whether insects and plants generally respond to different combinations of climatic cues (Forrest 2014). Long-term datasets of species interactions at a given area are scarce, especially plant-pollinator interactions (Hegland et al. 2009). Publications on individual insect species responses to climate change are the most common, and temperature is the most frequently, and often the only, studied factor (Andrew et al. 2013). Nonetheless, other variables may also influence pollinator phenology. In the Mediterranean region, in particular, drought (which combines temperature and precipitation) might be more appropriate than temperature alone when making predictions (Giorgi and Lionello 2008). In addition, delimiting these climatic variables to temporal windows that could affect the interactions might be better than considering annual means for climatic variables.

Butterflies are an ideal group for studying the effects of climatic change because their life cycle is strongly influenced by temperature (e.g. Dennis 1993) and long-term data on their abundance and phenology are currently available (Thomas 2005). They can also act as an important group of pollinators, especially because of their strong flying capacity and ability to move pollen long distances (Bloch et al. 2006). Butterflies can range from highly generalized species to highly specialized in their nectar feeding behaviour, depending upon different factors like length of the flying period and habitat preference (Stefanescu and Traveset 2009).

Here we extend –with five more years - part of the dataset used by Olesen et al. (2011), who studied the temporal dynamics of the plant-butterfly network of 12 years, to investigate the possible asynchrony in plant-pollinator (butterfly) mutualisms in a Mediterranean locality. Long-term (17-yr) observational data recorded while sampling for the Catalan Butterfly Monitoring Scheme (CBMS) allow us to explore climatic cues that can promote either coupling (synchrony) or uncoupling (asynchrony) between butterflies and the flowering plants they visit. A previous study in the same locality provided strong evidence of butterfly phenological advances as a consequence of climate change (Stefanescu et al. 2003). With this basic information we were interested in examining whether such phenological changes result in temporal asynchronies with the plants they visit for nectaring. Specifically, our main objectives were to: (1) test the effect of a number of climatic variables on the possible uncoupling of interactions; we additionally assessed how mean flowering date and mean flight date of butterflies, separately, have changed over time and which climatic cues are associated with such changes, and (2) identify the temporal scale at which climatic variables have a greater effect on asynchronies. In addition, we aimed at testing for differences in the degree of asynchrony between the two butterfly generations (spring vs summer), evaluating whether they respond to the same climatic variables. Finally, we assessed whether plant-butterfly asynchrony is associated with the degree of butterfly specialization (defined here as the number of flowering species each adult butterfly species visits for nectaring).

Since our main goal was to generalize on the effect of climate change on the decoupling of a whole plant-butterfly community, we considered a total of 39 different plant-butterfly interactions, which we used as replicates. However, since some effects may be obscured at the community level (see, for instance, Iler et al. 2013a), we further explored the asynchrony of each particular interaction.

Material and methods

Study site and data collection

All plant-butterfly interactions were recorded at El Cortalet (42º 13'N 3º 05'E), in Aiguamolls de l'Empordà Natural Park, Catalonia, NE Iberian Peninsula. This protected area of 4,784 ha is dominated by meadows, riverine forest and wetlands, and has been monitored for butterflies since 1988 (Stefanescu et al. 2005).

Plant-butterfly interaction data were regularly gathered by an experienced recorder (C. Stefanescu) while sampling for the Catalan Butterfly Monitoring Scheme (CBMS; www.catalanbms.org). Butterfly counts were made once per week (from 1 March until the end of September, a total of 30 weeks per year) while walking at a constant speed a 4-km fixed transect route. All censuses were done under standard weather conditions (i.e. sunny weather, with no strong wind), considering an imaginary area of 2.5 m on each side and of 5 m in front of the recorder. Butterfly counts started in 1988, but here we use the subset from 1996 to 2012, which is the period from which observations on flower visits by butterflies are available. Importantly, a plant-butterfly interaction was recorded only if the butterfly was on the flower probing nectar.

In order to obtain more robust results, we selected only those interactions recorded at least in 12 out of the 17 possible years, which provided a total of 39 plant-butterfly interactions. These interactions involved 17 plant species (most of them perennial) and 12 butterfly species (Table 1). All butterfly species are bivoltine or multivoltine (i.e., have two or more generations per season), except *Melanargia lachesis*, which is univoltine. However, in the analyses, two species (*Colias croceus* and *Leptotes pirithous*) were treated as univoltine because their continuous flying period made it impossible to distinguish between different generations.

Response variables

Phenological data for each butterfly- plant interaction were used to calculate an index of temporal asynchrony, which was defined as the difference in number of calendar days between the mean flowering date of a plant and the mean flight date of a butterfly species. Several studies have used first appearance dates (both for flowering and flight) as indicators of asynchrony; however, this indicator may not correctly reflect shifts in the activity period of a species (Miller-Rushing et al. 2008), being representative for only a subset of the individuals in a population (Hegland et al. 2009). Given that most butterfly species have more than one generation per season, we calculated two asynchrony indices (AI_1, for the first generation, and AI_2 for the rest of the generations which overlap one another, normally from 2 to 4) for each plant-butterfly interaction.

To estimate mean flowering date we assumed that the first day a butterfly species was observed nectaring on a plant species was the flowering onset for that plant species; likewise, its flowering end was assumed to be the last day a butterfly species was found on its flowers. Such assumption is justified because butterfly censusing each year begins on early March, when most plants have not begun flowering yet (Stefanescu, unpub. data). For these calculations we included all observations available for all 20 butterfly species (not only for those considered in this study, i.e. those involved in the 39 interactions) found each year nectaring on each plant species. The mean flowering date was then calculated as the mean of two proxies: (1) the mean day of the year between the first and the last date of flowering, and (2) the date in which 50% of the interactions had occurred, regardless of the butterfly species. To be confident that our estimate of the mean flowering date was adequate, we compared it with the real mean flowering date available from one of the years (1997) in which the flowering phenology was monitored along the entire season. The correlation between both flowering dates was found to be very high (r= 0.93, P <0.001, n = 16) (Supplementary material, Appendix 1).

On the other hand, the mean flight date (MFD, hereafter) was estimated as the date in which 50% of the individuals of each generation have been observed, either on flowers or flying, and hence this variable is independent of flowering phenology (Stefanescu et al. 2003). It was calculated as follows:

$$MFD= \Sigma \ (n \ x \ d) \ / \ N$$

where n is the number of butterflies counted for each of the 30 weeks, d is the ordinal number of the week (from 1 to 30), and N is the total number of butterflies recorded at the end of the season. It includes also estimated values for missing counts (i.e., values estimated as the mean of the preceding and succeeding counts). The date (week) of the mean flight date was then turned into the (Julian) day of the year. We assumed a normal distribution of flying dates, which seems reasonable for univoltine species. For bi- and multivoltine species, we obtained two metrics: MFD_1 (hereafter, spring generation) and MFD_2 (hereafter, summer generations), following Stefanescu et al. (2003).

Predictor variables

Meteorological data on temperature and precipitation were obtained from a meteorological station belonging to Instituto Nacional de Meteorología located precisely in our transect route at El Cortalet. We used the following 10 explanatory variables in linear models to predict asynchronies between plants and butterflies:

a) Annual temperature (T_a) and annual Gaussen index of aridity (GIA_a) from October to September of the following year; that is, the 12 month period that precedes the flight/flowering season. The GIA index (calculated as P/2T, where P and T are precipitation and temperature, respectively) has been widely used as a measure of the degree of drought in a given area (Peñuelas et al. 2007). By definition, when GIA < 1, conditions are considered to be arid; the smaller the value the higher is the level of aridity.

b) Mean temperature (T_{90-w}) and Gaussen index of aridity (GIA_{90-w}) for the 90 days preceding the emergence of the first butterfly generation, corresponding to December, January and February

c) Mean temperature (T_{f-w}) and Gaussen index of aridity (GIA_{f-w}) for the fall-winter season, i.e., for the period September-February (previous to any butterfly emergence).

d) Mean temperature (T_{90-sp}) and Gaussen index of aridity (GIA_{90-sp}) for the 90 days preceding the emergence of second butterfly generations, corresponding to the spring months March, April and May.

e) Mean temperature (T_{s-s}) and Gaussen index of aridity (GIA_{s-s}) for the spring-summer season, specifically for the period March-August.

Data analyses and model selection

We used Generalized Linear Mixed Models (GLMMs; with the library *lme4* and function *lmer* in R v. 3.0.1; R Development Core Team, http://www.r-project.org/) to test for the effects of the predictor variables in the interacting plant-butterfly community. The response variables, AI_1 and AI_2, were treated as count data, thus assumed to come from a Poisson distribution, approached by means of a log link function in the GLMMs. All explanatory variables were standardized prior to analyses and "interaction identity" was included as a random effect in all the models for the analysis at community level in order to prevent pseudoreplication. We tested for interannual variation in the response variable comparing AIC values of a model with just the intercept to a model with year. As the model fitting improved considering the factor year, this was also included as another random variable in all models. Butterfly abundance was used as a continuous covariate in all models.

Effect of climatic variables on the possible uncoupling of interactions and identification of the temporal scale with a greater effect

To evaluate the relative importance of predictor variables in determining the asynchrony within interactions, we constructed a total of 12 models for each asynchrony index (i.e. one for each butterfly generation), corresponding to an equal number of meaningful biological hypotheses which were tested simultaneously. The Akaike's Information Criterion (AIC) was used to rank these models and to select the most parsimonious ones (see Supplementary material Appendix 4 for details).

Asynchrony of each particular interaction

To explore the asynchrony of each particular interaction and to evaluate its contribution to the overall community asynchrony, we built a model which included the predictor variable explaining most variation in asynchrony (T_{s-s}) together with 'interaction' as a main fixed factor, controlling again for year and butterfly abundance; in this case, we ran the analysis only for AI_1, mainly because a similar variation across interactions might be expected for any of the butterfly generations. The model without the intercept was used as baseline to obtain relative measures of asynchrony for each plant-insect interaction.

To discern whether and to what extent the mean flowering date of plants and MFD respond to the same phenological cues, we performed additional separate GLMMs for plants or butterflies. In this case, 'plant' and 'butterfly' species were included as random effects (replicates) together with the variable 'year', and the same 10 climatic variables as predictors; controlling for butterfly abundance in the analyses of MFD for each butterfly generation.

Relationship between the plant-butterfly asynchrony and butterfly specialization

To test for an association between degree of specialization and level of asynchrony for each of the 12 butterfly species, we performed another GLMM, including the butterfly species as a random effect. Degree of specialization was obtained

by averaging the number of interacting plants with each butterfly species during a 12-year period (Stefanescu and Traveset 2009), whereas level of asynchrony for each butterfly was also averaged across the 17 years.

Results

Changes in climate

Although no directional change was evident for any of the climatic variables considered here in all the temporal windows, all of them were highly variable across the 17-year study period, 1996-2012 (see Fig. 1). Mean annual temperature varied from 12.89 ºC (2002) to 16.26 ºC (2003), with an arithmetic mean ($\pm SD$) of 15.36±0.74 ºC and the annual GIA index ranged from 12.05 (2007) to 29.21 (2006), with an arithmetic mean ($\pm SD$) of 19.12±4.88.

Changes in plant and butterfly phenologies over time

The mean flowering date showed high variation in most plant species (10 out of 17) but remained relatively constant in others (e.g. *Inula viscosa*, *Limonium vulgare*, *Lythrum salicaria*, *Prunus spinosa*, and *Rubus ulmifolius*) or varied only moderately across years (e.g. *Lamium hibridum* and *Taraxacum officinale*) (see Supplementary material Appendix 2). Likewise, mean flight date of butterflies also varied in time depending upon species (Supplementary material Appendix 3). *Plebejus argus*, *Ochlodes venata*, and *Coenonympha pamphilus* showed only a slight variation across the 17-yr period; *Pieris napi*, *Pieris rapae* and *Melanargia lachesis* varied moderately, whereas the other six species showed much variability in phenology.

Aridity during the spring months (GIA_{90-sp}) was consistently included in the best models predicting either mean flowering date and mean flight date for the first and the second butterfly generations, i.e., a higher aridity in spring tended to advance flowering and butterfly mean flight date, although butterflies –especially the first generation- were

influenced to a lesser extent than plants by climatic conditions (see Supplementary material Appendix5).

Effect of climate on plant-butterfly asynchrony at the community level

Table 2 shows the results of the best models for each asynchrony index. For AI_1, the two best models included temperature as the most relevant predictor but at two different time periods, the Spring-Summer season and the annual one (T_{s-s} and T_a, respectively). A third model including the aridity during the 90 days preceding the first butterfly generation (GIA_{90-w}) was also equivalent ($\Delta AIC < 2$). Together with the covariate butterfly abundance (the three models 12, 4 and 5, respectively), accounted for almost the 54% of model probability (see Supplementary material Appendix 4, Table A1a). The estimates of the parameters from the best three models selected to predict AI_1 are shown in Table 3a. The slopes of the climatic variables (T_{s-s}, T_a and GIA_{90-w}) were negative and statistically significant in the case of temperature, but not for the aridity index. However, the magnitude of the effect (which would correspond here to the community-level asynchrony) was relatively moderate. All models included 'interaction' and 'year' as random effects, while controlling by butterfly abundance, and showed that 'interaction' by itself explained a larger proportion of the variance (73% against the 0.6 % explained by 'year').

For the summer butterfly generation, the best model predicting asynchrony (AI_2) included the aridity index during spring (i.e. the three months preceding the emergence of the butterflies' second generation (GIA_{90-sp}) (model 9) (Supplementary material Appendix, 4 Table A1b). This variable, together with the covariate of butterfly abundance, accounted for 46% of model probability, assessed by means of Akaike's weights. The estimates of the parameters from the best model predicting AI_2 are shown in Table 3b. A negative relationship was observed between GIA and AI_2, i.e. the higher the aridity (i.e. low values of GIA) the higher the asynchrony index. However, the

magnitude of the effect was again moderate. For this butterfly generation, the proportion of the variance explained by 'interaction' identity was also higher than that explained by year (53% and 0.6%, respectively), but it was lower if compared with the first generation.

The entire range of AI_1 and AI_2 values can be observed in Figure 2. If ranked from highest to lowest values instead of by butterfly species, a small group of interactions was observed that showed relatively high values but a long tail with moderate to low values, especially for AI_2. For each particular interaction, interannual variation in asynchrony was rather low but was higher for AI_1 than for AI_2: ($SD=$ 16.8 and 14.3 on average across the 39 interactions, respectively; results not shown), with values ranging from 5.5 to 59.4 days for the spring butterfly generations and from 3.7 to 24.8 days for the summer ones. The average number of days of asynchrony was 70.43 for the spring generation whereas it was 38.07 for the summer generation.

Figure 3 shows the large variation in the contribution of each particular butterfly-plant interaction to the overall asynchrony considering the best model for the first butterfly generation. Most interactions showed positive values, indicating that they had a greater asynchrony than that of the baseline values, whereas some were negative, corresponding to highly synchronous interactions.

No association was found between degree of specialization and asynchrony ($z=1.02$, p$=$ 0.308, $N=$ 39), suggesting that generalized and specialized butterflies are similarly vulnerable or resistant to disruptions owing to changes in climate (Supplementary material Appendix 6, Fig.A5).

Discussion

Both mean flowering date and mean flight date of butterflies were highly variable across the 17 years (1996-2012) considered in this study. Likewise, temperature and

precipitation varied strongly during this period (as well as during the period 1988-2002; Stefanescu et al. 2003) with no apparent trend over time. A higher aridity during the spring season resulted in an advance of the mean flowering date, which adds to the increasing evidence that flowering periods are initiated earlier under climate warming (e.g. Fitter and Fitter 2002, Menzel et al. 2006, Peñuelas et al. 2013). Our findings that plant flowering respond more strongly than insects to climatic conditions were also consistent with those from two recent studies (Forrest and Thomson 2011, Ellwood et al. 2012). A novel result, however, is that the two insect generations considered were influenced by the same climatic cue. In both cases we found that a higher aridity resulted in advances in mean butterfly flight date.

Our analysis at the community level evidenced an overall climate effect on temporal asynchrony of butterfly-plant interactions, although the magnitude of such an effect was weak. In particular, butterfly-plant asynchronies tended to increase in those years with higher aridity levels from December to February. Previous studies have reported that precipitation is a relevant phenological cue for the emergence of butterflies (Stefanescu et al. 2003) and hoverflies (Graham-Taylor et al. 2009, but see Iler et al. 2013a). Flowering time has also shown to be sensitive to precipitation in a number of ecosystems, including Mediterranean environments (Crimmins et al. 2011, Peñuelas et al. 2004, 2013). Our results showing the importance of aridity in explaining asynchrony are thus not surprising. Temperature also influences insect and plant phenology (see review in Peñuelas et al.2013), and, in fact, mean annual temperature and temperature during spring and summer, showed also to be important in determining temporal asynchrony between plants and the first generation of butterflies. The effects of temperature are, however, highly variable among insect species, with some even showing delayed emergences or no changes at all with climate change (Ellwood et al. 2012). In the study area, Stefanescu et al. (2003) previously showed that phenological

responses to increasing temperatures differed strongly among butterfly species, even among those with similar larval diets.

Interestingly, the level of aridity during spring influenced the temporal asynchrony between flowering plants and the summer generation of butterflies. One possible explanation for this is that a higher aridity might advance butterfly development (while maintaining or also advancing plant mean flowering date) due to an indirect effect of rainfall, as cloudy skies lead to decreases in radiant heat, and thus in temperature, which is known to delay larval and pupal development (Stefanescu et al. 2003). Aridity combines both precipitation and temperature and might somehow amplify their respective effects, mainly if the responses of each species to each of these two variables are idiosyncratic or, at least, differ in magnitude.

Our consistent findings on the effect of aridity on both asynchrony indices actually suggest that this might be the most appropriate climatic trait to make predictions on phenological uncoupling in plant-pollinator systems in the Mediterranean region (Peñuelas et al. 2013). Assessing aridity might be particularly valuable given the expected increase of drought events in this region in the forthcoming years (Giorgi and Lionello 2008). Moreover, in order to reduce the effect of confounding factors, delimiting the climatic variables to small temporal windows might be more appropriate than considering larger windows for climatic variables, such as annual means (Ellwood et al. 2012).

The 39 different butterfly-plant interactions differed much in their contribution to the overall asynchrony found at community level. Moreover, the overall interannual variation in asynchrony level for each specific butterfly-plant interaction was rather low. Two recent studies (Bartomeus et al. 2013, Rader et al. 2013) found that high levels of biodiversity can buffer the negative effects of species-specific phenological shifts, maintaining synchrony at the community level; this is actually predicted by the

biodiversity insurance hypothesis (Lawton and Brown 1993, Loreau et al. 2001). Thus, the weak temporal asynchrony that we found at the community level –although highly variable among interactions- may be due to the differential responses to climatic conditions among butterfly species and among plant species. Such weak temporal asynchrony at the community level, found also in a recent review (see Forrest 2014), can contribute to maintain the pollination interactions between butterflies and plants in the area.

Identifying which species contributed most and which least to the overall community asynchrony, or to determine what traits such contribution was associated with would be quite interesting, though they were not goals of this study. For a powerful analysis, this would require having large sample sizes (i.e. more species) in the different trait categories. For example, it would be interesting to test whether voltinism (i.e. number of generations a butterfly species has in a season) influences asynchrony. In our sample, we only had one truly univoltine species (*Melanargia lachesis*) which showed little contribution to asynchrony (see interaction 39 in Figures 2 and 3). For multivoltine species, strong selection favoring local seasonal adaptations (such as diapause, migration, etc.) has been predicted (Stefanescu et al. 2003); at least in the Mediterranean region, these species might have serious problems to synchronize the second and subsequent generations with the availability of plant resources for larval development, usually affected by summer drought. We rather aimed to test whether the level of adult butterfly specialization can influence the asynchrony with the plants with which they interact. Contrary to expectations, specialist butterflies showed similar asynchrony levels as generalist ones, suggesting that they are as vulnerable to mutualistic disruptions caused by climate as those that interact with many plant species. These findings do not support, thus, predictions of an increased vulnerability of species with narrow niches to climate change (Biesmeijer et al. 2006, Schweiger et al. 2012) or of a

stronger selection for specialized mutualists to maintain phenological synchrony with their partners compared to generalized mutualists (Rafferty et al. 2014). Instead, our results are consistent with other findings (Benadi et al. 2014) suggesting that there are other biotic and abiotic factors that may need to be considered when making such predictions (Ellwood et al. 2012; Gornish and Tylianakis 2013). Regarding the consequences of asynchrony on fitness, they are probably greater for specialized than for generalized butterfly species, even if the level of asynchrony is lower (Biesmeijer et al. 2006). More empirical data from other studies examining mutualistic interactions are certainly necessary to test whether phenological uncoupling due to climate change differs between specialized and generalized species. We must also take into account that given that the larvae of most European butterfly species have a more specialized diet than the adult stage (Altermatt and Pearse 2011), phenological synchrony of larvae with particular host plants could actually be more important for a butterfly species' fitness than adult synchrony with specific flowering plants. Since the timing of occurrence of adults necessarily depends on larval phenology, selection on larval phenology could constrain the phenology of adult butterflies. Again, additional data would be needed to test this idea.

Our findings indicating that the degree of asynchrony differs between particular interactions are in agreement with a recent study by Iler et al. (2013a,b), who reported that the relative importance of phenological cues varies among syrphid taxa, and that these may respond to the same cues but at different rates. Moreover, our results are also consistent with those of two recent studies reporting that species-level phenological responses differ from community-level responses (Gornish and Tylianakis 2013, Iler et al. 2013a). Therefore, we may predict that the varying degree of phenological plasticity will allow certain species, but not others, to undergo adaptive responses, under the new

conditions created by climatic warming, that may prove to be essential for the persistence of those populations subject to severe changes.

Caveats of the study- Although we believe our findings are well supported by the long-term dataset on plant-butterfly interactions and by the robust statistical analyses performed, we need to point out here the possible effects of: measurement errors in estimating plant and butterfly phenologies and non-independence of flowering phenology from butterfly observations, variation in butterfly detectability, and missing values. Since we did not have the 'ideal' data on flowering phenology by directly censusing flowers on plants, except for one year (1997), we had to assume that the first and last day a butterfly species was observed nectaring on a plant species was the flowering onset and end, respectively, for that plant species. We believe, however, that such assumption does not lead to any important bias as butterfly censusing each year begins on early March, when most plants have not bloomed yet. The fact that for one of the years (1997) we found a very high correlation between the estimated mean flowering date and the real mean flowering date (flowers monitored along the entire season) supports also a low bias. On the other hand, butterfly species that are very abundant might be easier to detect and might provide more reliable mean flight date estimates than rare species. This is why, to partly overcome this possible bias, we decided to include butterfly abundance as covariate in all models. Regarding missing values, we missed an average of 3.18 samples (i.e. weeks) out of 30 possible per year (that is, only ca. 10% of the possible counts). Missing counts were distributed throughout the whole season, and never consisted of two successive weeks. Missing counts were estimated as the average mean of the preceding and posterior weeks, a standard procedure in the Butterfly Monitoring Scheme methodology when the number of missing counts is low (see Pollard & Yates, 1993). We are thus confident that missing values had a very small effect, if any, on our results.

Concluding remarks

In order to improve the predictions of ecosystem responses to climate change it is critical that we understand the mechanisms underlying the coupling and uncoupling among interacting species (Parmesan 2007). Our study demonstrates an overall effect of climate on phenological uncoupling in a community of plants and their nectaring butterflies, though such effect is small most likely owing to the differential behavior of each particular plant-butterfly interaction. Our results are concordant with those from other recent studies showing that species-level phenological responses can differ strongly from community-level responses. Importantly, the temporal window of the climatic variable considered in the study can influence the magnitude of the effect, and thus it should be taken into account in future studies. At least in the Mediterranean region, phenological asynchronies might be more affected by aridity levels than by temperature itself, and thus it can be a key climatic trait to make better predictions in this region. Finally, we conclude that, in contrast to predictions, specialized and generalized mutualists were similarly likely to become phenologically asynchronous.

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Supplementary material (Appendix oik-XXXXX at

<www.oikosoffice.lu.se/appendix>). Appendix 1–6.

Table Legends

Table 1.Total number of nectaring interactions considered in this study, corresponding to

12 butterfly species and 17 plant species, during the period 1996-2012. Family/subfamily

(H: *Hesperiidae*, L: *Lycaenidae*, N: *Nymphalinae*, P: *Pieridae*, S: *Satyrinae*). Voltinism (B:

bivoltine species, U: univoltine species, M: multivoltine species, U*: treated as univoltine

in the analyses because of the continuous overlapping of the different generations). The

acronym for each butterfly species is given in parentheses below its scientific name.

Butterfly species	Family/subfamily	Voltinism	Plant species	Interaction
			Ballotanigra	1
			Inulaviscosa	2
			Limonium vulgare	3
			Lythrumsalicaria	4
Ochlodesvenata (HESOVE)	H	B	*Picrisechioides*	5
			Picrishieracioides	6
			Rubusulmifolius	7
			Sonchusmaritimusaquatilis	8
			Trifoliumpratense	9
Celastrinaargiolus (LYCCAR)	L	M	*Lythrumsalicaria*	10
Lycaenaphlaeas (LYCLPH)	L	M	*Trifoliumfragiferum*	11
Plebejusargus (LYCPAR)	L	M	*Lotus corniculatus*	12
			Trifoliumfragiferum	13
Leptotespirithous (LYCLPI)	L	U*	*Lythrumsalicaria*	14
			Lotus corniculatus	15
Polyommatusicarus (LYCPIC)	L	M	*Trifoliumfragiferum*	16
			Trifoliumpratense	17

Table 1 cont.

Cynthia cardui (NYMCCA)	N	M	*Trifoliumpratense*	18
Coliascroceus (PIECCR)	P	U*	*Trifoliumpratense*	19
Pierisnapi (PIEPNA)	P	M	*Ballotanigra*	20
			Brassica nigra	21
			Diplotaxiserucoides	22
			Lamiumhybridum	23
			Lythrumsalicaria	24
			Picrisechioides	25
			Prunusspinosa	26
			Picrishieracioides	27
			Rubusulmifolius	28
			Taraxacumofficinale	29
Pierisrapae (PIEPRA)	P	M	*Ballotanigra*	30
			Brassica nigra	31
			Lythrumsalicaria	32
			Picrisechioides	33
			Rubusulmifolius	34
			Taraxacumofficinale	35
			Trifoliumpratense	36
Coenonymphapamphilus (SATCPA)	S	M	*Ranunculussardous*	37
			Trifoliumfragiferum	38

Table 2. Best models for associations between climate variables and temporal asynchrony between the community of butterflies and the flowering plants they visit, both for the spring generation Asynchrony Index, AI_1 (a) and for the summer generation AI_2 (b). The number of parameters (k), the Akaike´s Information Criterion (AIC) along with the Akaike weights (wAIC) and the log-likelihood (LL) are presented for the models. Models with $\Delta i < 2$ are considered statistically equivalent and Akaike´s weights (wAIC) show an estimate of the probability that the given model is actually the best one among those considered. BAI, Butterfly Abundance Index; T_{s-s}, mean temperature for the Spring-Summer season (from March to August); T_a, mean annual temperature from October to September of the following year; $GIA_{90-w,}$ Gaussen Index of aridity for the 90 days preceding first sampling date and GIA_{90-sp} Gaussen Index of aridity, for the 90 days preceding second butterfly generations. Factors of year and interaction identity were used as random effects.

(a)

Time scale	Hypothesis	k	AIC	wAIC	LL
From March to August	$AI_1 \sim T_{s-s} + BAI$	5	3991.972	0.2632	-1990.986
Annual (Oct-Sept)	$AI_1 \sim T_a + BAI$	5	3993.032	0.1549	-1991.516
90 days preceding the emergence of the first butterfly generation (Dec + Jan + Feb)	$AI_1 \sim GIA_{90-w} + BAI$	5	3993.564	0.1188	-1991.782

(b)

Time scale	Hypothesis	k	AIC	wAIC	LL
90 days preceding the emergence of the second butterfly generation (March + April + May)	$AI_2 \sim GIA_{90-sp} + BAI$	5	3776.873	0.4654	-1883.44

Table 3. Magnitude and direction of the effect of the variables included in the best models selected in Table 2 for each of the asynchrony indices: (a) spring generation (AI_1), and (b) summer generation (AI_2). See Table 2 for the acronyms of the variables

(a)

Model		Estimate	Std. Error	z	p
	Intercept	3.97	0.1386	28.63	<0.001
$AI_1 \sim T_{s-s} + BAI$	T_{s-s}	-0.05	0.0197	-2.35	0.018
	BAI	0.03	0.0078	3.47	<0.001
	Intercept	3.97	0.1387	28.60	<0.001
$AI_1 \sim T_a + BAI$	T_a	-0.04	0.0204	-2.03	0.041
	BAI	0.03	0.0078	3.46	<0.001
	Intercept	3.97	0.1387	28.60	<0.001
$AI_1 \sim GIA_{90-w} + BAI$	GIA_{90-w}	-0.04	0.0207	-1.87	0.061
	BAI	0.03	0.0078	3.58	<0.001

(b)

Model		Estimate	Std. Error	z	p
	Intercept	3.38	0.1232	27.46	<0.001
$AI_2 \sim GIA_{90-sp} + BAI$	GIA_{90-sp}	0.07	0.0202	-3.467	<0.001
	BAI	-0.02	0.0111	-1.431	0.152

Figure Legends

Figure 1. Tendency of the different climatic cues across the 17-year period. Temperature (on the left) and Gaussen Index of aridity (on the right) at different monthly summaries are represented by different colours. Note that for the Gaussen Index, the smaller the value the higher the level of aridity.
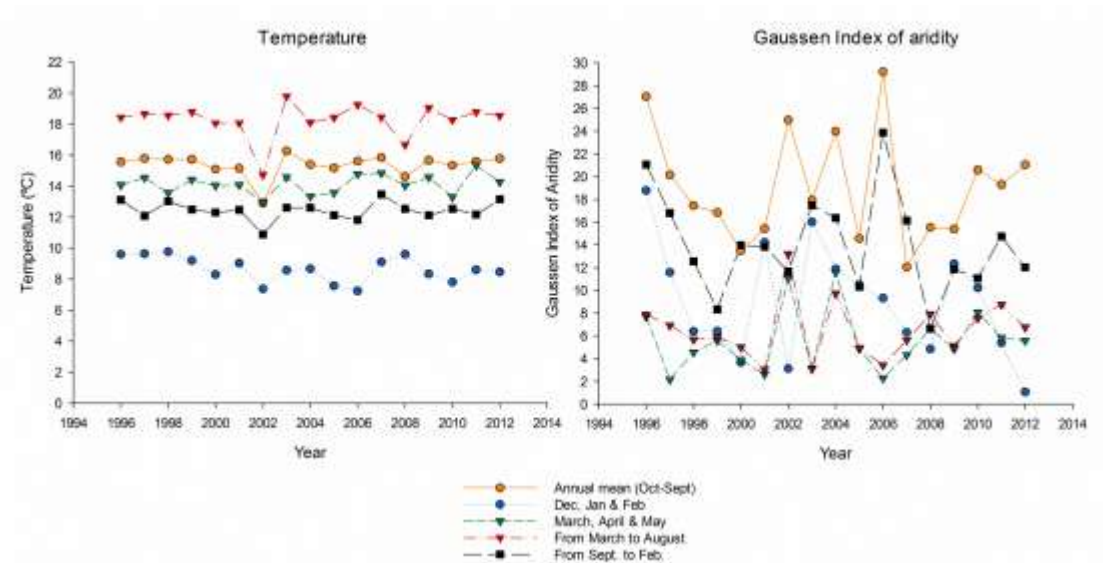
Figure 2. Boxplot representing the index of asynchrony per interaction for the whole time period ranked from highest to lowest median values. Each color represents the interactions corresponding to each butterfly species of this study (see Table 1 in the text for acronyms of butterfly species).
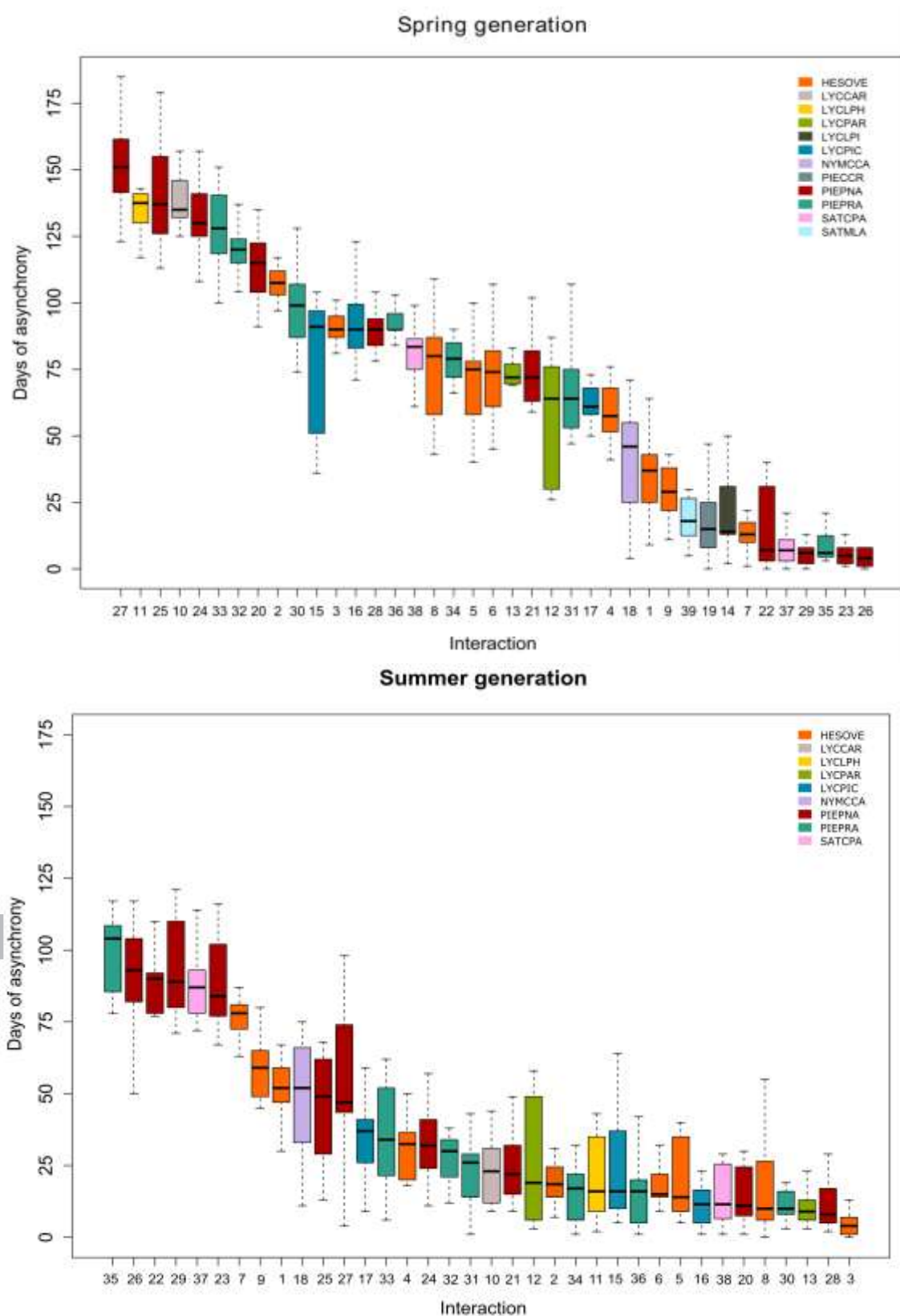
Figure 3. Contribution of each interaction to the overall index of asynchrony of the first generation (AI_1). The number of the interaction in the X label refers to the interaction given in Table 1 and each color represents the interactions corresponding to each butterfly species of this study (see Fig.2 for the identification of each butterfly species according to each color). Positive estimate values correspond to those interactions contributing more than the average whilst the opposite is true for the negative values.