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SEASONAL CHANGE IN NECTAR PREFERENCE FOR A MEDITERRANEAN BUTTERFLY COMMUNITY

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ABSTRACT. A decline in butterfly diversity and abundance has been observed all over Europe, even for rather widespread species. The reasons for this trend are not clear, with one of the possible mechanisms being a decrease in available nectar sources. In the scope of these observations, the nectar sources used by a Mediterranean butterfly community have been analyzed. Clear differences between specialist and generalist species could be observed. However the specialization rate was not stable throughout the year, both considering the butterfly community as a whole and for individual species. When analyzing the temporal variability in nectar use between the seasons, an increasing specialization in nectar use was observed in autumn. While a seasonal trend could be attributed to a change in butterfly species composition, the trend towards more specialization from summer to autumn was related to a change in relative abundance of flowering plants relative to the richness of butterfly species and abundance present. Significantly fewer flowering plants were present in degraded Mediterranean systems during autumn.

Additional key words: Mediterranean, butterfly community, seasonality, flower use, nectar plants

European butterfly species have showed a strong decline over the last several years, with 12% of the species considered threatened and 31 % appearing vulnerable (Van Swaay et al. 2004, Van Swaay & Warren 2006). This problem is not restricted to rare species. Rather, recent evidence suggests that common European species are experiencing a decrease in abundance and distribution (Van Dyck et al. 2009). This does not seem to be a local West-European trend, with similar tendencies observed in NE Spain (Stefanescu et al. 2011) and northern California (Forister et al. 2010). The reasons for this strong decline are not clear but probably have to do with the complex ecology of different butterfly species, making them more sensitive to the current global change (e.g. land-use change, modification in landscape structure, and climate change) (Forister et al. 2010, Munguira et al. 1997, Stefanescu et al. 2011). The reasons for butterfly decline seem to be human induced, as indicated by Konvicka et al. (2006), who found a clear correlation between socio-economic factors and butterfly decline in Europe. Van Swaay and Warren (2006) identified agricultural intensification as the major threat for butterfly conservation in Europe. Similar results were obtained by Stefanescu et al. (2004) and Forister et al. (2010) for Mediterranean regions, where presence of intensive agriculture negatively influenced butterfly species richness.

Previous studies of species decline have emphasized the abundance of host plants, while most other parts of the butterfly ecology were marginally taken into account. More recently, other aspects of the butterfly ecology were investigated such as morphology of host plants, microhabitat, presence of ant communities, habitat structure and fragmentation (e.g. van Swaay et al. 2012). The availability of nectar sources is a factor often neglected in conservation plans, but could play an important role in butterfly conservation (Erhardt & Mevi-Schütz, 2009). It is known that nectar is the main energy source for a large variety of butterfly species, influencing their abundance, fitness and reproductive success (Porter et al. 1992, O'Brien et al. 2004, Ouin et al. 2004, Mevi-Schütz & Erhardt 2005). When nectar sources are limited or lacking during a certain time of the year, adverse effects on the reproductive success of butterfly individuals could be expected. This was already hypothesized to be one of the key factors to explain butterfly decline by Van Dyck et al. (2009). In addition, Ebeling (2008) observed that a reduction in flower diversity caused a decline in pollinator diversity. These studies obviously cannot be extrapolated to the Mediterranean region, but they do indicate the importance of nectar sources on the abundance and distribution of butterflies and other insects, as has been indicated for other regions (Schultz & Dlugosch 1999, Biesmeijer et al. 2006).

Butterfly sensitivity to nectar availability also depends on the level of specialization towards certain plant species. In the past it was generally assumed that butterflies were generalists, nectaring from a wide range of plant species (Shreeve 1992). In recent papers, it has been made clear that a large part of the butterfly communities could be considered specialists nectar feeders (Tudor et al. 2004, Hardy et al. 2007, Stefanescu & Traveset 2009). Furthermore, it was observed that butterfly species of conservation interest were those with the highest degree of specialization on nectar sources and that this specialization is linked to a specialization in host plant as well (Tudor et al. 2004). Due to differences in adult butterfly morphology, different plant species are more suitable for them than others and therefore at least some of the butterfly species are specialized on certain plant species (Corbet 2000, Hardy et al. 2007, Stefanescu et al. 2011). In the scope of conservation, it could be vital to know the nectar plants of the different species considered. This aspect of conservation has already been proved useful by Baz (2002) who observed that the threatened Apollo butterfly only used a limited amount of the nectar plants present in central Spain.

Because the availability of nectar sources could be an important factor in explaining the decreasing trends in butterfly distribution and density, it seems interesting to study the nectar use of butterfly communities in a Mediterranean environment in greater detail. The Mediterranean region is characterized by extremely variable climatic and environmental conditions throughout the year, causing a high inter-annual variability in the presence of flowering plant species. Therefore, we were interested to know how butterfly species cope with this temporal variability in available nectar sources. This study is a first attempt to assess and characterize the temporal trends in flower visits by butterflies in a Mediterranean environment. We studied: 1) The presence of generalist and specialist butterfly species of a Mediterranean butterfly community, 2) The differences in flower use specialization over time at a community and species level, 3) The factors influencing these changes.

MATERIAL AND METHODS

Study area. The study area, "Los Cerros de Alcala", is a protected area located at 40º28'N, 3º20'W at an altitude of 650–700m, just outside the city of Acalá de Henares, province of Madrid, Spain. It is a hilly area, characterized by a high variety in vegetation types, forming a mosaic of different habitats. Due to the large heterogeneity between vegetation types, the area represents parts of the typical central peninsular landscape. The vegetation types present in the area are all shaped by human influence. This has resulted in a low abundance of woody vegetation, except for some *Pinus halepensis* plantations. The climax vegetation of the area would be a closed *Quercus ilex* forest, with presence of *Q. faginea*. No climax vegetation is present anymore, but a garrigue vegetation with a relative high cover of *Q. ilex* and *Q. coccifera* can be found in part of the study area. A vegetation of large graminoids, mainly *Stipa tenacissima*, dominates large parts of the hilly areas where this species was cultivated in the past. Due to sheep grazing, the flatter areas in the region are covered by a pasture like vegetation with a high abundance of *Retama sphaerocarpa*. The valleys, road verges and agricultural field margins are characterized by more disturbed, nitrofile vegetation. All of these vegetation types are a representation of different degraded series from the natural vegetation. Therefore, it is not surprising that part of the native plant species became locally extinct. Recently some efforts were undertaken to re-introduce some of these plant species, which is the case for *Rosmarinus officinalis*.

Data collection. Field surveys were conducted during the year 2009 between March and October with an average two week time interval. A total of 16 field surveys were conducted, covering the major part of the flight period for the present butterfly community. A fixed route of 3.5 km was sampled during the morning and midday (between ±10am and 3pm). The field survey was only conducted under favourable flight conditions for butterflies, with a cloud cover <50%. Therefore some variation is present in the 2 week time span between visits. In midsummer no visit was conducted during the month of August because abundance of flowering plants was extremely low. When butterflies were seen nectaring during the field survey, the number of individuals, the butterfly species, and nectar source used were recorded. A visual check to determine if the individual was truly nectaring was performed to avoid confusion with individuals that were resting on top of flowers.

Analysis. The specialization of the different butterfly species was analyzed by relating species richness to the amount of nectar sources used, excluding species that were observed only once. This approach was first proposed by Gleason (1922) and has been used for similar analysis by Tudor et al. (2004). This relationship typically shows a logarithmic trend, where a deviation from the trend (large residuals) indicates a specialization or generalization in the use of the different nectar sources. Species with residuals >|4| were considered deviating from the trend and thus specialist or generalist species.

To identify temporal differences in nectar use by the butterfly community, the data was split up in three seasonal groups, spring (March–beginning of May) the hibernating and early first generation of spring species, summer (May–June) the moment of maximum species presence and autumn (July–October) with the migratory and multi-generation species present. The Lorenz curve (Gastwirth 1972) was drawn for each of these temporal groups by plotting the cumulative amount of butterfly species (%) observed on each plant species over the cumulative percentage of plant species considered, starting with the plant species where the lowest number of butterfly species has been observed. The same was done for the number of butterfly individuals on each plant species. If a straight line from 0 to 100% would occur, every plant species would have the same percentage of butterfly species visiting it (or number of butterflies for the second case). When some plant species are used by more butterfly species and individuals than others, the result is an upwards curve towards 100%. By drawing such a plot for each season we can identify the temporal differences in flower specialization by the butterfly community studied. However, multiple factors could influence these differences over time. The factors considered in this study are: 1) change in butterfly species composition over time, 2) change in butterfly number over time, 3) change in plant species diversity over time, 4) change in relative abundance of butterfly species/individuals over plant species.

The observed trends will be affected by changes in butterfly species composition over the year. To exclude species composition as an explicatory factor we tested whether we could detect a similar trend towards specialization over the year for individual species. Many indexes that indicate changes in population composition exist. Here we use the Shannon's Equitability Index:

$$E = (-\sum_{i=1}^{S} p_i \ln p_i)/\ln S$$

Where E is the Equitability with values between 0 and 1 with 1 indicating complete evenness, S is the total number of species in the community and p_i is the proportion of the ith species in the population.

This index is an adaptation of the Shannon's Diversity index which is widely used to calculate species diversity (Magurran 1988). However the Shannon's Diversity index is influenced by abundance and evenness of the community. Therefore we used the Equitability index which is just sensitive to changes in evenness of the community. Using the Equitability index, changes in the homogeneity of the nectar sources used can be indicated. The Shannon's Equitability Index for the used nectar sources was calculated for individual butterfly species over the different seasons. Only the butterfly species with the highest number of individuals (>30) and present in more than one season were analyzed. The changes observed in the Equitability index over time indicate a change in the homogeneity/heterogeneity of the nectar sources used over the seasons. If the homogeneity index stays stable between seasons, no temporal variation takes place.

To study the effect of the other factors on the temporal trend in specialization observed we calculated the flower specialization over time with the Shannon's Equitability Index. The index was calculated using the variation in number of butterflies on the different plant species at a daily basis, considering each field visit as a sample. The Pearson correlation analysis was used to relate the Equitability index with the Julian day, the number of butterfly species present, the number of butterfly individuals present, the number of plans species present, the relative abundance of butterfly species over plant species and the relative abundance of butterfly individuals over plant species to study the factors influencing the variation in nectaring specialization over time

RESULTS

A total of 1022 butterfly individuals comprising 39 species were observed nectaring on a total of 57 flowering plant species during this field survey (tables 1, 2). The most common species seen nectaring was *Syrichtus proto*, the most common skipper present in the area. Other species that were seen nectaring frequently are "shade" species (genus *Pyronia*), migratory species (genus *Cynthia*, *Lampides* and *Leptotes*) and the common blues and copper of the area. These represent most of the species present in the area, although some characteristic species which are common in spring, such as *Pseudophilotes panoptes* and *Issoria lathonia*, were not seen nectaring or only few times.

The number of butterfly individuals seen nectaring oscillated between only a few individuals to over more than 200 a day, with a clear peak in May–June and another one in October (Fig 1). The number of species observed changed as well, with relative low numbers in early spring and the same high peak in spring-summer and a lower one in autumn, a similar trend as observed with the number of butterfly individuals.

As expected, a clear differentiation between specialist and generalist butterfly species was found (fig. 2 —for the species codes see table 1). The species are clearly divided in two groups, with some species using a considerable higher variety of nectar sources than others. The species deviating from the tendency are listed in table 3. The species considered "generalists" are all very common species in the area, having multiple generations or a long flight period. The "specialist" species can be divided in two groups. Some are species

TABLE 1. Butterfly Species Seen Nectaring During the Field Survey. Species are arranged in order of declining observed abundance. The species code is also provided.

Species	Number observed	code
Syrichtus proto (Esper, 1808)	141	sypr
Pyronia bathseba (Fabricius, 1793	117	pyba
Lycaena phlaeas (Linnaeus, 1761)	113	lyph
Aricia cramera Eschscholtz, 1821	94	arcr
Lasiommata megera (Linnaeus, 1767)	74	lame
Pyronia cecilia (Vallantin, 1894)	68	pyce
Polyommatus bellargus (Rottemburg, 1775)	52	pobe
Cynthia cardui (Linnaeus, 1758)	46	cyca
Pontia daplidice (Linnaeus, 1758)	44	poda
Colias crocea (Geoffroy, 1785)	38	cocr
Polyommatus icarus (Rottemburg, 1775)	34	poic
Euchloe crameri Butler, 1879	31	eucr
Pieris rapae (Linnaeus, 1758)	28	pira
Lampides boeticus (Linnaeus, 1767)	23	
Leptotes pirithous (Linnaeus, 1767)	18	
Satyrium esculi (Hübner, 1806)	16	saes
Satyrium spini (Denis & Schiffermüller, 1775)	13	
Polyommatus thersites (Cantener, 1834)	10	
Hyponephele lupina (Costa, 1836)	9	
Tomares ballus (Fabricius, 1787)	6	
Plebejus hespericus (Rambur, 1839)	6	
Zerynthia rumina (Linnaeus, 1758)	5	
Carcharodus baeticus (Rambur, 1840)	4	
Melitaea phoebe (Denis & Schiffermüller, 1775)	4	
Papilio machaon Linnaeus, 1758	4	
Melanargia lachesis (Hüner, 1790)	4	
Argynnis pandora (Denis & Schiffermüller, 1775)	3	
Issoria lathonia (Linnaeus, 1758)	3	
Vanessa atalanta (Linnaeus, 1758)	2	
Glaucopsyche alexis (Poda, 1761)	2	
Thymelicus sylvestris (Poda, 1761)	2	
Celastrina argiolus (Linnaeus, 1758)	1	
Callophrys rubi (Linnaeus, 1758)	1	
Pieris brassicae (Linnaeus, 1758)	1	
Nymphalis polychloros (Linnaeus, 1758)	1	
Pieris napi (Linnaeus, 1758)	1	
Polyommatus albicans (Gerhard, 1851)	1	
Glaucopsyche melanops (Boisduval, 1828)	1	
Thymelicus lineola (Ochsenheimer, 1808)	1	

with a large secondary generation during autumn or migratory species, which rely on *Dittrichia viscosa* as their most important nectar source. The second group consists out of univoltine "shade" species such as gatekeepers and hairstreaks, which are typical species from open scrublands/woodlands.

To further characterize the observed differences in nectar use, we split the data in 3 distinct time periods: spring, summer and autumn (table 4). Spring is characterized by a low number of butterfly species and individuals, together with a low availability of flowering plants. The summer generation is characterized by a high number of species and individuals, with a high diversity in flowering plants. The autumn generation is characterized by a moderate number of butterfly species but with a high number of individuals, but the amount of flowering plant species is low.

TABLE 2. Plant species that were significantly used by butterflies as a nectar source. Plant species are listed if they were visited by > 10 individuals or > 5 species.

Plant species	Number of butterfly species	Number of butterfly individuals
Dittrichia viscosa	15	372
Eryngium campestre	10	106
Marrubium vulgare	8	86
Allium sphaerocephalon	8	61
Retama sphaerocarpa	11	52
Scabiosa atropurpurea	9	34
Rosmarinus officinalis	12	32
Chondrilla juncea	6	28
Helichrysum stoechas	8	22
Thymus zygis	10	21
Carduus tenuiflorus	13	20
Cephalaria leucantha	5	14
Echium plantagineum	7	13
Biscutella auriculata	6	12
Jasminum fruticans	3	12
Ruta montana	3	11
Coronilla minima	2	11
Teucrium gnaphalodes	1	10
Ecballium elaterium	5	9
Senecio jacobaea	5	8
Phlomis herba-venti	5	6
Teucrium polium	5	5

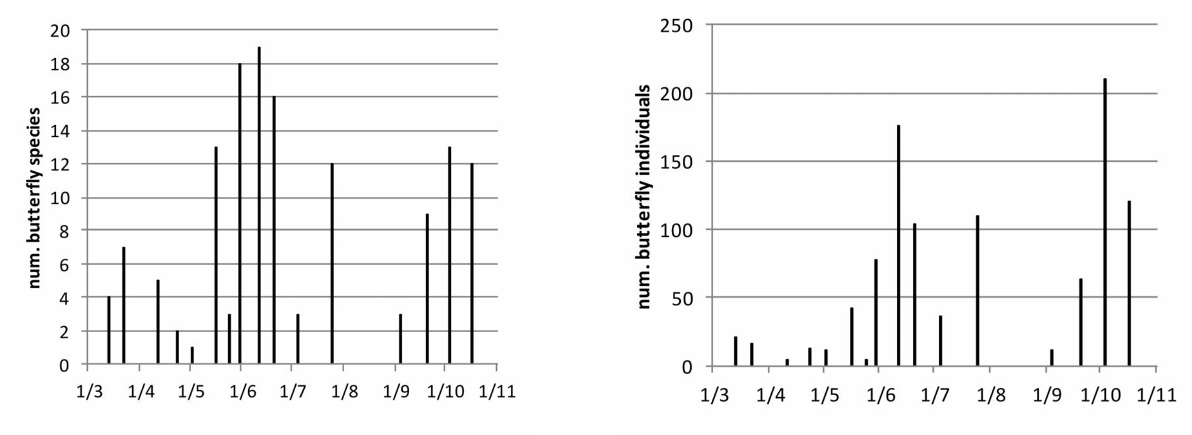

FIG. 1. Number of butterfly species (left) and individuals (right) observed during field surveys.

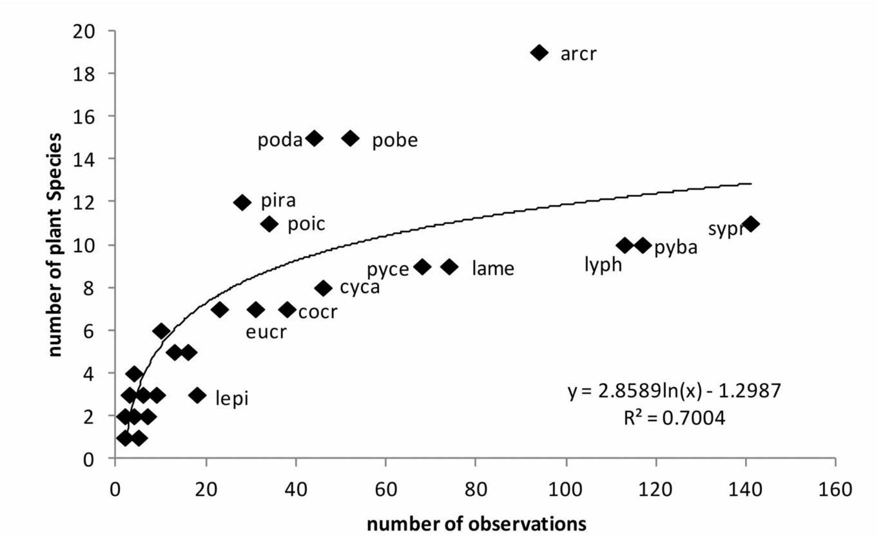

FIG. 2. Number of butterfly observations plotted as a function of the number of plant species visited. The species with only 1 observation were not included.

TABLE 3. Generalists and specialist butterfly species. The main plant species used and % of the main plant species / the total amount of plants used as a nectar source for each specialist species are given.

Generalist	Specialist	Main plant species used	% use of main plant species over total
Polyommatus icarus	*Colias crocea*	*Dittrichia viscosa*	63
Pieris rapae	*Leptotes pirithous*	*Dittrichia viscosa*	61
Pontia daplidice	*Cynthia cardui*	*Dittrichia viscosa*	30
Polyommatus bellargus	*Pyronia cecilia*	*Eryngium campestre*	78
Aricia cramera	*Pyronia bathseba*	*Marrubium vulgare*	56
	Lycaena phlaeas	*Dittrichia viscosa*	49
	Syrichtus proto	*Dittrichia viscosa*	80
	Lasiommata megera	*Dittrichia viscosa*	86
	Euchloe crameri	*Rosmarinus officinalis*	48

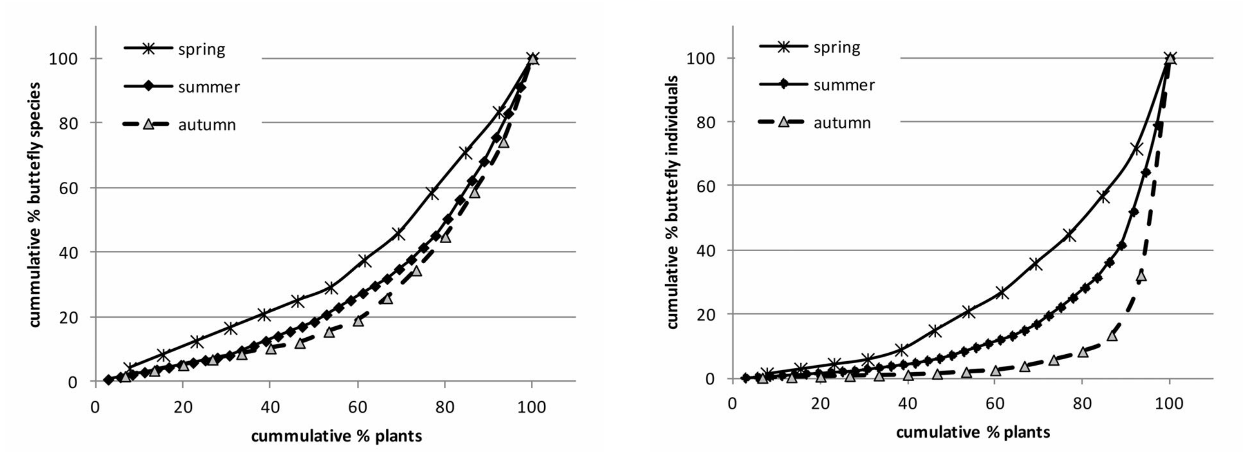

FIG. 3. Cumulative number of butterfly species and individuals per plant species, starting with the plant species with the lowest number, for 3 different time periods.

In Fig 3 we plotted the Lorenz curve, as the cumulative amount of butterfly species observed on each plant species for each season in percentage, starting with the plant species with the lowest number of visits by different butterfly species. The same was done for the number of butterfly individuals on each plant species. A clear tendency over the year can be seen; with a more equalized use of flowering plant species by butterflies in spring, both for species and number of individuals. An increasingly specialized use of certain plant species as nectar source developed over the year. This trend is not related to the sole number of available plant species for each season, as indicated in table 4.

However the results presented in Fig 3 could be influenced by a change in butterfly species present between spring, summer and autumn. Therefore the Shannon's Equitability Index was calculated for individual butterfly species to indicate the variation in number of butterfly visits to the flowering plant species. A value equal to 1 corresponds with a complete evenness in the used nectar sources. This was done for each of the different seasons (table 5). There were almost no butterfly species in large numbers present during both spring and summer, indicating that in this case the specialization trend is correlated with a change in species composition. For the summer and autumn, the same temporal trend was observed as in Fig 3 for almost all species. Furthermore *A. cramera* & *P. bellargus* can easily been recognized as true generalist species, because they both have high index values well into autumn. Other species have high Equitability values during summer but lower ones during autumn, indicating that it is not just the species composition that causes this trend.

To discriminate the other factors possibly influencing the temporal trend found in Fig 3, the Shannon's Equitability Index was calculated for each day of fieldwork separately, indicating the distribution of the number of butterfly individuals over the co-occuring plant species. The Pearson correlation coefficients between the Shannon's Equitability Index and the factors that possibly could influence the temporal trend observed are presented in table 5. The change in Equitability over time is well related with the Julian day, as was observed in Fig 3 at a seasonal scale. However, the number of butterfly species, the number of butterfly individuals and the number of plant species gave lower R^2 results, indicating that they alone are not well related with the temporal trend observed. On the other hand, this trend seems to be related to the relative abundance of butterfly species/numbers over plant species. The Equitability index, the ratio between number of butterfly individuals over number of plant species and the separate number of plant species are plotted over time and presented in figure 4. It can be observed that the number of plant species is not related to the change in the Equitability index, while the ratio follows the inverse trend, with and increasing ratio over the year between numbers of butterfly individuals over plant species.

TABLE 4. Difference in number of plant species, butterfly species and butterfly abundance observed during the different seasons.

	# of nectar plant species	**# of butterfly species**	**# of butterfly individuals**
spring	13	12	67
summer	37	32	405
autumn	15	19	551

TABLE 5. Shannon's Equitability index calculated for number of butterfly individuals present on different flowering plant species during 3 time periods. Values given are for separate species and the mean value for all species together ("total").

	Shannon's Equitability index		
	Spring	Summer	Autumn
Total	0.85	0.75	0.41
Eucloe crameri	0.81	0.72	
Pyronia bathseba		0.64	
Syrichtus proto		1.00	0.37
Lycaena phlaeas		0.82	0.51
Aricia cramera		0.72	0.79
Polyommatus bellargus		0.85	0.53
Colias crocea		0.81	0.24
Pyronia cecilia		0.87	0.39

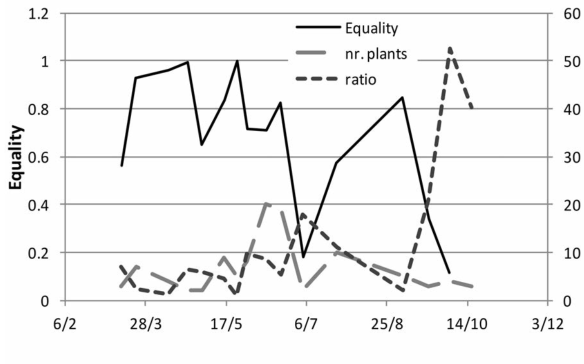

FIG. 4. Trend in Equitability index (left axis), number plants species and the ratio of number of butterfly individuals and number of plant species (right axis) over the year.

DISCUSSION

In this study we characterized the use of different nectar sources over time for a typical Mediterranean butterfly community. The most common species, *Syrichtus proto*, a common skipper in the area, was the most numerous species observed. This is in line with the observation of Tudor et al. (2004) who also observed that skippers are a very active nectaring group of butterflies. However, during spring some of the more common species were not seen nectaring. The reason for this is not clear, although lack of suitable nectar sources could be a possible explanation and should be further investigated.

Although the field survey was only conducted over one year, clear temporal trends could be observed. As expected, butterfly abundance as well as species diversity changed over time in accordance with the available resources, showing peaks in summer and autumn (Fig 1). These two peaks are typical for Mediterranean environments, and also correlate to peaks in vegetation productivity. Species diversity had its peak during May–June, coinciding with the peak of nectar sources available, while the number of individuals observed did not. Abundance reached its highest peak in autumn (table 4). This is a typical trend for species with multiple generations, where the last generation is generally the largest to produce the highest amount of offspring and increase the number of individuals that might survive winter.

As first determined by Tudor et al. (2004), generalist and specialists nectar feeders formed clearly differentiated groups. The specialist species were "shade" species such as gatekeepers. The generalist species were, as expected, the species with multiple generations. This could indicates an adaptation of univoltine species to specialize on common nectar sources during the flight period, while multivoltine species maintain their plasticity to cope with the changing available nectar sources throughout the year.

To our surprise, migratory species were also considered specialist species. Therefore we analyzed the difference in specialization over the year, dividing the data in 3 seasonal groups. The results showed a clear trend to specialization in the use of nectar sources over the season (Fig 3). This trend is partly caused by a

TABLE 6. Coefficients of the Pearson correlation between the Shannon's Equitability index calculated for the number of butterfly individuals present on the different flowering plants for each day separately and the day Julian day of the year, the number of butterfly species recorded, the number of butterfly individuals recorded, the number of plant species recorded and the ratio between number of butterfly individuals over the number of plant species present.

	Julian day	# Butterfly species	# Butterfly individuals	# Plant species	# Butterfly individuals/ plant species	# Butterfly species/ plant species
Slope	-0.003	-0.003	-0.008	0.013	-42.2	-2.54
Intercept	1.12	0.82	0.73	0.58	40.3	3.22
R2	0.41**	0.31*	0.03	0.06	0.69**	0.49**

*p<0.05, **p<0.01

change in butterfly species composition over time, with almost no butterfly species having large broods in both spring and summer. However, this is not the case between summer and autumn. To show that this specialization in autumn is a general trend, not only caused by a change in butterfly species composition over time, the Shannon's Equitability Index was calculated for some of the generalist and specialist species. Although it was observed that some species are true generalists, most species showed an increase in nectar specialization during autumn, coinciding with the previous observations and making it clear that the trend is not only caused by a change in species composition throughout the year.

While the increased specialization in nectaring from spring to summer was expected, the specialization trend in autumn is more related to a lack of flowering plant species compared to the amount of nectaring butterfly species and individuals. This trend can be caused by the numerical dominance of this plant species (*D. viscosa*) or due to the high quality nectar of this nectar source, making it more attractive than other nectar sources. With our dataset it is impossible to separate between the two due to the unavailability of vegetation density measures. The fact that one species is the main nectar source in autumn is an important observation, particularly given that adult food resource distribution plays a key role in determining habitat quality and the suitability of landscapes for butterfly persistence (Erhardt and Mevi-Schütz 2009). This fact also makes the community more vulnerable to temporal and spatial changes and extreme events with negative effects on the occurrence of *D. viscosa*.

One of the reasons for the low number of flowering plants in autumn is the local extinction of typical Mediterranean plant species. Recently, some efforts were undertaken to re-introduce some of these species in our study area. Of these species, *Rosmarinus officinalis* seems especially interesting to use. Although this reintroduced species is only present in very low densities, a high number of different butterfly species used it as a nectar sources during spring (30%) and autumn (42%). This plant species could cover part of the year where low numbers of nectar sources are present. This indicates that for ecosystem restoration of the Mediterranean areas, reintroducing typical plant species can have a positive effect on other species communities, stressing the importance of a "complete" vegetation composition to maintain high butterfly diversity. Further research comparing well conserved and degraded plant communities may further elucidate additional differences.

Currently *D. viscosa* accounts for 70% of the observed butterfly individual's nectaring in our study area. It is a yellow composite, typical for nitrofile and disturbed sites. Our results indicate that different butterfly species could benefit from a high abundance of nectar sources present on small nitrofile zones (e.g. roadsides, field margins) that are present in a nutrient poor environment. Absence of this species in the area would not be a problem for the migratory species, but could negatively affect resident species such as *Syrichtus proto*, *Lycaena phlaeas*, *and Lasiommata megera*.

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