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Article

Management Diversification Increases Habitat Availability for Lepidoptera Papilionoidea in Torretes Biological Station (Spain)

Short Title: Spatial Diversification and Papilionoidea

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Simple Summary: Human intervention has dominated the habitats of the Mediterranean basin since the early Neolithic. Agrosilvopastoral activities have transformed ecosystems, shaping cultural landscapes through human action. This has resulted in a rich spatial mosaic of crop fields and pastures interspersed with fragments of the original forest and native habitats. The abandonment of traditional agrosilvopastoral practices is known to negatively affect biological diversity. The Torretes Biological Station, located in the municipality of Ibi (Alicante, Spain), exemplifies the recovery of traditional management of a former agricultural estate through a program initiated 21 years ago. This area has witnessed the restoration of habitat diversification and the enhancement of plant diversity, which has positively impacted its biodiversity. To assess the impact of this process on the entomofauna, a study of diurnal butterflies, considered bioindicators, was conducted to evaluate the effects of habitat management. Our results highlight that the program of creating and maintaining new habitats at the Biological Station has increased spatial heterogeneity and the availability of trophic resources, leading to a significant increase in the richness and abundance of diurnal butterflies within a short period.

Abstract: Diurnal butterflies (Papilionoidea), considered bioindicators of habitat conservation status, are one of the groups used to assess the impact of habitat management and improvement efforts in the Biological Station of Torretes (Ibi, Alicante, Spain). This area also houses the Botanical Garden of the University of Alicante. The study focused on the period from autumn 2022 to late spring 2023, comparing butterfly diversity and abundance data from the current study with historical data. Our results highlight that open spaces and the availability of trophic resources (nutritional plants) were key factors influencing the richness and abundance of Papilionoidea. The program for the creation and maintenance of new habitats at the Biological Station has increased spatial heterogeneity and the availability of trophic resources, which has led to a significant increase in the richness and abundance of diurnal butterflies in a short period. To support the conservation of Mediterranean ecosystems, entomofauna in general, and Papilionoidea in particular, are proposed as model groups for designing conservation projects based on traditional habitat management. These projects should promote spatial heterogeneity, include programs to enrich plant diversity, and be complemented by environmental education and outreach initiatives.

Keywords: protected natural areas; habitat management; butterfly conservation; entomological reserves; Mediterranean ecosystems

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1. Introduction

The extinction rate of insect species, both locally and globally, has risen significantly in recent decades, becoming a major scientific concern [1]. Highlighting this issue, the Manifesto of Iberian

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Entomologists emerged from the XX Iberian Congress of Entomology in June 2023, urging action to stop this decline and raise public awareness about the vital role of insects in ecosystems (https://www.entomologica.es/manifiesto-xx-congreso-iberico-de-entomologia). Lepidoptera is one of the most diverse and well-studied orders of insects, which has historically aroused both scientific and social interest [3]. The extensive knowledge available about their biology and diversity has fueled numerous studies in ecology, evolution, and conservation [4]. Their key ecological roles and sensitivity to environmental changes make them excellent bioindicators [2–5], which has strengthened their value as a "star group" for promoting biodiversity conservation and education programs [2]. Within Lepidoptera, the Papilionoidea have received special attention due to their captivating beauty, easy observation, and well-known taxonomy. This superfamily encompasses six families in Europe: Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, and Riodinidae [6,7]. Many representatives have been identified as crucial pollinators of natural ecosystems and agricultural crops [8] and bioindicators of environmental health [3,9,10].

The Iberian Peninsula, a biodiversity hotspot in Europe [11,12], boasts a rich mosaic of habitats shaped by a long history of human activity [12–17]. This high spatial heterogeneity contributes to the peninsula's remarkable butterfly diversity. Over 250 butterfly species have been recorded in Spain, rivalling the numbers found in Italy, Greece or Turkey [18,19]. Notably, 21 species are endemic to the Iberian Peninsula [1,20].

However, like many other insect groups, butterflies face numerous threats. Landscape alteration and fragmentation, agricultural intensification, infrastructure development and the introduction of invasive species all contribute to butterfly decline [1,7,17,21–24]. Climate change further exacerbates these threats, particularly in the Mediterranean region, where aridity is increasing, thus altering landscape functioning and displacing species to higher altitudes or towards more northern latitudes [23,25–29].

For this study, the Torretes Biological Station (Ibi, Alicante, Spain) was selected to assess how management diversification programs in protected Mediterranean natural areas may drive and enhance diversity patterns, using the Papilionoidea as a response group. Located in the municipality of Ibi and managed by the CIBIO Research Institute (Centro Iberoamericano de la Biodiversidad) of the University of Alicante, this natural area has become an important space for biodiversity conservation and environmental dissemination through a land stewardship agreement between the Ibi City Council and the CIBIO Institute. In view of its exceptional biodiversity, this enclave was recognized in 2019 as an Entomological Reserve by the Spanish Association of Entomology (https://www.entomologica.es/torretes). In addition, Torretes houses the University of Alicante Botanical Garden. All this is indicative of the uniqueness of this natural space.

The objectives of this study were: a) to assess diversity patterns (richness, ecological diversity and composition) of diurnal butterflies in Torretes, b) to identify temporal changes in the diurnal butterfly community of Torretes through comparisons with previous studies, and c) to propose specific actions to improve the management and conservation of Mediterranean habitats.

2. Materials and Methods

The study was conducted at the Torretes Biological Station (Spain), a former Mediterranean farm that has been transformed into a site for research, conservation and scientific outreach. The area covers 53 ha, 44 of which are forested areas and the remaining 9 are agricultural land. It is located in the north part of the province of Alicante, on the southern slope of the Carrascal de la Font Roja Natural Park, between 900 and 1100 m a.s.l. The climate is Mediterranean, with certain continental influence due to the altitude, characterized by cool winters with occasional frosts and short, warm summers. The average annual temperature of 14.8 ºC and average annual precipitation of 459.5 mm [30,31]. The station is located between the dry mesomediterranean and subhumid supramediterranean levels, with potential vegetation of Kermes oak (*Quercus coccifera* L.) and deciduous formations in the shadiest points.

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For this study, the four types of habitats present at the Torretes Biological Station [32] were considered (Figure 1):

a) Gully. This habitat, characterized by significant depth and substantial vegetation cover, maintains higher humidity and a lower average morning temperature than the other habitats. It is dominated by a holm oak forest (*Quercus rotundifolia* Lam.) with scrub and scattered specimens of Aleppo pines (*Pinus halepensis* Mill.) and junipers (*Juniperus oxycedrus* L.). Two permanent water points along its course support edaphohygrophilous vegetation such as water lilies (*Ninfea alba* L.) and yellow iris (*Iris pseudacorus* L.).

b) Terrace system 1. This habitat is composed of old cultivation terraces of almond trees (*Prunus dulcis* D.A.Webb) and olive trees (*Olea europaea* L.) that are surrounded by typical Mediterranean scrublands with scattered holm oaks and a small patch of Aleppo pines. This terrace system is part of the Botanical Garden and houses an important collection of lilies (*Iris* spp.) and sages (*Salvia* spp.), as well as gymnosperms (firs, pines, cypresses, yews, cedars, sequoias, etc.). This open space is highly exposed to adverse environmental conditions, especially solar radiation and wind.

c) Terrace system 2. This heterogeneous terrace system includes several buildings, greenhouses and a complex system of plantations, which from its origin was designed to house important botanical collections, with emphasis on typically Mediterranean botanical groups such as Lamiaceae. This is an open space that presents two small ponds with aquatic and edaphohygrophilous plants. This habitat is the heart of the Botanical Garden, where most actions are carried out to promote plant biodiversity

d) Pine forest. This area is predominantly dominated by Aleppo pine but includes Mediterranean plant endemism such as Mariola sage (*Salvia blancoana* subsp. *mariolensis* Figuerola), the Alicante lavender (*Thymus moroderi* Pau ex Martínez) or the royal thyme (*Dictamnus albus* L.). Scattered patches of scrubs and some solitary holm oaks are also present.

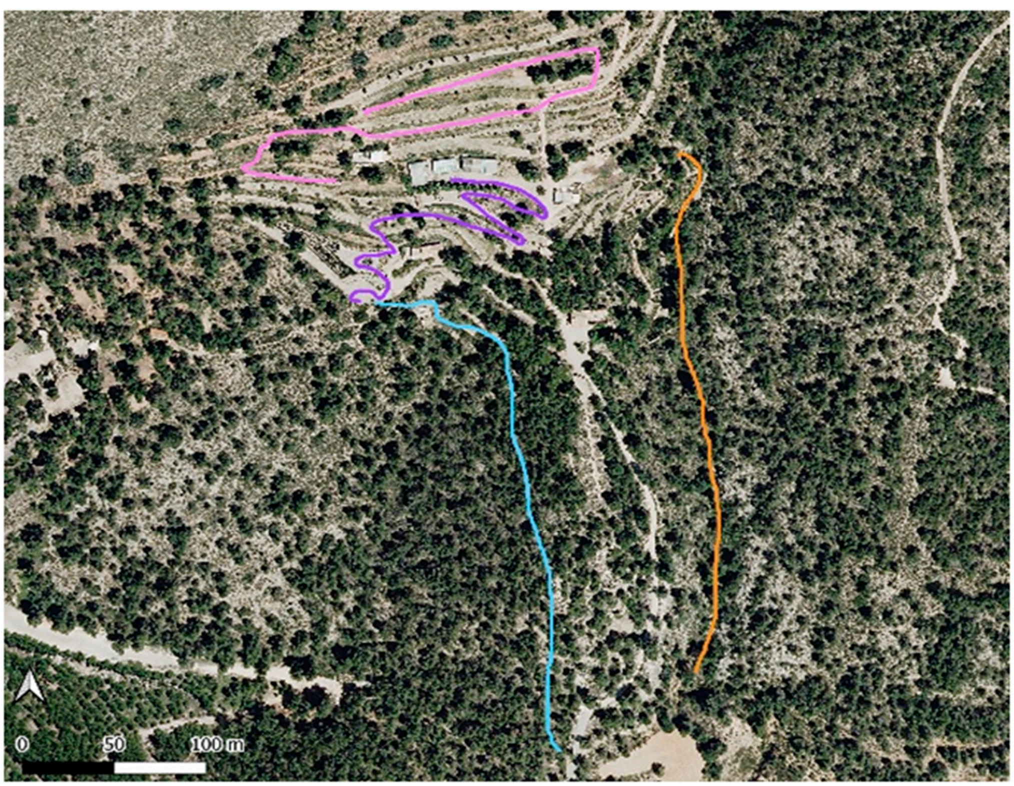

Figure 1. Satellite image of the Torretes Biological Station taken from the National Geographic Information Centre (CNIG), in which the four transects made are delimited with QGIS. pink: Terrace 1; purple: Terrace 2; orange: Gully; blue: Pine forest.

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Species surveys were conducted out according to the method of POLLARD & YATES [33], Transects ranging from 300 and 400 meters were established (Figure 1), with a sampling duration of 30 minutes per transect. Weather permitting, sampling occurred every 10 days over six months, from October 2022 to mid-May 2023, excluding January and February due to low temperatures and limited flight activity. Sampling was carried out on sunny days with minimal wind, between 8:30 AM and 12:00 PM solar time, a period of high activity for diurnal butterflies.

Three different sampling methods were employed to collect data: using an entomological net, photographing individuals, and conducting in situ identification. A total of 11 samplings events were conducted in each habitat.

For statistical analyses, individuals of the genus *Pieris* that could not be identified in flight to the species level were excluded (Table 1) to avoid potential biases in the analysis. Differences in diversity patterns among habitats (spatial variation) were analysed. Sampling coverage was estimated for each habitats ($\hat{C}m$) [34], which ranges from 0 (zero completeness) to 100% (maximum completeness). To assess spatial changes in diversity patterns, Hill numbers (${}^{q}D$) of orders ${}^{0}D$ and ${}^{1}D$ were used [35]. The 95% confidence intervals were used to compare ${}^{q}D$ values [36]. These analyses were performed using iNEXT v2 statistical software [37,38].

Table 1. List of species and number of individuals recorded at the Torretes Biological Station in the study period. **Pieris* sp. refers to the group of individuals recorded in flight of the species *Pieris rapae* and *P. mannii*, as these specimens could not be clearly identified.

Family	Species	Abundance
Hesperiidae	*Carcharodus alceae* (Esper, 1780)	1
	Erynnis tages (Linnaeus, 1758)	1
	Muschampia proto (Ochsenheimer, 1808)	4
Lycaenidae	*Aricia cramera* (Eschscholtz, 1821)	6
	Callophrys rubi (Linnaeus, 1758)	54
	Celastrina argiolus (Linnaeus, 1758)	2
	Glaucopsyche alexis (Poda, 1761)	4
	Glaucopsyche melanops (Boisduval 1828)	8
	Lampides boeticus (Linnaeus, 1767)	65
	Leptotes pirithous (Linnaeus, 1767)	82
	Lycaena phlaeas (Linnaeus, 1761)	1
	Polyommatus bellargus (Rottemburg, 1775)	3
	Polyommatus icarus (Rottemburg, 1775)	2
	Pseudophilotes panoptes (Hübner, 1813)	24
	Satyrium spini (Denis y Schiffermüller, 1775)	2
Nymphalidae	*Hipparchia fidia* (Linnaeus, 1767)	7
	Hipparchia semele (Linnaeus, 1758)	3
	Hipparchia statilinus (Hufnagel, 1766)	3
	Lasiommata maera (Linnaeus, 1758)	8
	Lasiommata megera (Linnaeus, 1767)	83
	Maniola jurtina (Linnaeus, 1758)	2
	Melanargia ines (Hoffmannsegg, 1804)	4
	Melanargia occitanica (Esper, 1793)	1
	Melitaea deione (Geyer, 1832)	6
	Melitaea phoebe (Denis y Schiffermüller, 1775)	27
	Nymphalis polychloros (Linnaeus, 1758)	3
	Pararge aegeria (Linnaeus, 1758)	73
	Pyronia bathseba (Fabricius, 1793)	70
	Vanessa atalanta (Linnaeus, 1758)	1
	Vanessa cardui (Linnaeus, 1758)	29
Papilionidae	*Iphiclides feisthamelii* (Duponchel, 1832)	11

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	Papilio machaon Linnaeus, 1758	8
	Zerynthia rumina (Linnaeus, 1758)	1
Pieridae	*Anthocharis euphenoides* Staudinger, 1869	42
	Colias croceus (Geoffroy, 1785)	55
	Gonepteryx cleopatra (Linnaeus, 1767)	68
	Leptidea sinapis (Linnaeus, 1758)	7
	Pieris brassicae (Linnaeus, 1758)	32
	Pieris mannii (Mayer, 1851)	8
	Pieris rapae (Linnaeus, 1758)	16
	**Pieris* sp. Schrank, 1801	61
	Pontia daplidice (Linnaeus, 1758)	69
	Total	957

Finally, to analyse differences in species composition among habitats, a permutational multivariate analysis of variance (PERMANOVA) was conducted using the Bray-Curtis index and 999 permutations [39]. Multidimensional scaling (MDS) was employed to visualize the relative positions of samples based on their species composition similarity. Bootstrap procedures were used for this analysis and for plotting 95% confidence ellipses. Both PERMANOVA and MDS were performed using PRIMER v7 software [40].

3. Results

A total of 957 individuals were recorded, representing 41 species (Table 1). *Lasiommata megera* and *Leptotes pirithous* were the most abundant species, with a total of 83 and 82 individuals, respectively.

Sampling coverage ($\hat{C}m$) ranged from 0.95 to 0.99 in all habitats studied, indicating adequate sampling effort. Regarding species richness (^0D), significant differences were only found between Ravine-Terrace2 and Pine forest-Terrace2 (Figure 2). In contrast, significant differences in ecological diversity (^1D) were observed in all habitat's combinations): Ravine-Terrace1, Ravine-Terrace2, Pine forest-Terrace1, Pine forest-Terrace2 and Terrace1-Terrace2, except in the case of Ravine-Pine forest (Figure 2).

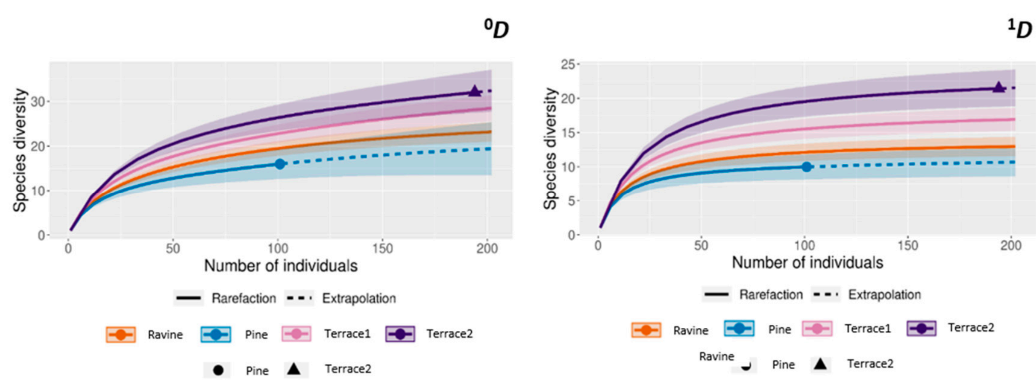

Figure 2. Differences between habitats in species richness (^0D) and ecological diversity (^1D).

PERMANOVA analysis revealed that all studied habitats exhibited statistically differences in species composition (F_{pseudo} = 2.88, df = 3, p < 0.001). No overlap between habitats or ellipses found in any case (Figure 3). This finding reflects a high level of complementarity among the studied habitats in terms of overall Papilionoid diversity.

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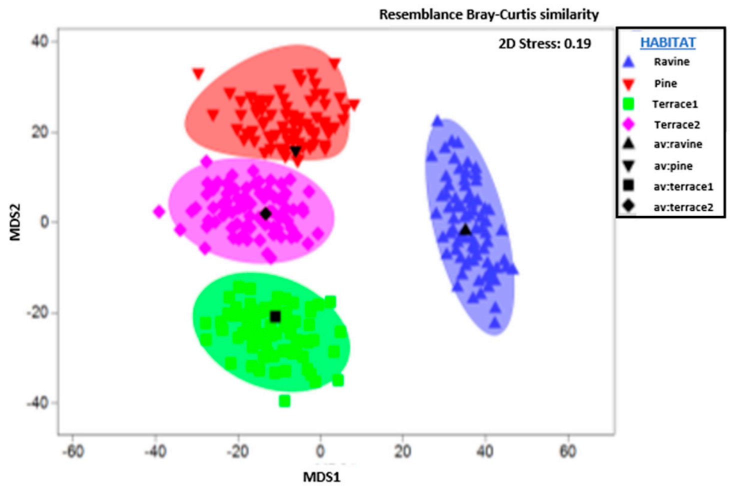

Figure 3. MDS showing the differences in species composition among habitats.

4. Discussion

The Torretes Biological Station revealed a remarkable biodiversity of diurnal butterflies (41 species and 957 individuals) in the period studied between 2022 and 2023. This represents a significant increase in both diversity and abundance compared to a previous study conducted in 2004 using the same methodology and within the same habitats [43], in which recorded 32 species and 563 individuals. This relatively small natural protected area encompasses approximately 60% of the butterfly species known in the inland mountain ranges of the northern part of the Alicante province [41–43]. The richness and abundance of butterflies are strongly influenced by landscape heterogeneity and habitat diversity [45]. These factors provide essential shelter and food resources, including nutritious plants for caterpillars and nectar sources for adults [46,47]. At the Torretes Biological Station, recent habitat management and improvement initiatives, coupled with the establishment of the University of Alicante Botanical Garden, have significantly enhanced habitat diversity and plant richness. Management diversification of habitats inherently promoted greater availability of resources, which seem to benefit the overall biodiversity of butterflies in Torretes in the long term.

The highest values of species richness and ecological diversity were observed in the two assessed terraced systems. These managed habitats are characterized by heterogeneous and abundant trophic resources, and present extensive sunny areas (low vegetation cover) that are used by many butterfly species for thermoregulation [48,49]. In Terrace 2, the most diverse habitat, exhibit a notable presence of butterfly species such as *Pontia daplidice, Colias crocea* and *Leptotes pirithous*, which are known to prefer open and warm environments [50]. Moreover, the presence of water points and the high concentration of aromatic plant species within Terrace 2 could also be contributing to the observed diversity patterns [47,51].

In contrast, the gully and the pine forest habitats presented similar values of richness and ecological diversity. On the one hand, the gully presented a high number of individuals of certain specialist species (rarely found in the other habitats)), such as *Pararge aegeria, Goneopteryx cleopatra* and *Anthocharis euphenoides*, which are associated with more humid environments or the presence of water sources [41,52–55]. On the other hand, in the pine forest habitat, clearly more homogeneous and less suitable for butterflies [56], no remarkable species were found.

The observed continuous rise in both diversity and abundance of butterflies in less than a decade underscores the critical importance of proper management diversification strategies for natural areas [57]. Since the establishment of the Biological Station in 2003, a program of habitat restoration, management and diversification has been developed and progressively implemented. For instance, this strenuous effort resulted in the creation of the University of Alicante Botanical Garden in 2012 and the Entomological Reserve by the Spanish Association of Entomology in 2019. The management initiatives undertaken in this protected natural area have resulted in the improvement of habitats with multiple trophic resources, fostering biodiversity and transforming into a Reserve renowned for its high Mediterranean entomological diversity [33]. Our findings pave the way for the design of new educational programs featuring strategic itineraries with informative butterfly displays, incorporating information on their food plants and habitats. These programs have the potential to spark curiosity among visitors and raise societal awareness about the need to develop and implement insect conservation programs.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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