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# Environmental tuning of an insect ensemble: The tenebrionid beetles inhabiting a Mediterranean coastal dune zonation

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ABSTRACT

Few studies have investigated insect ensembles, i.e. phylogenetically bounded groups of species that use a similar set of resources within a community. The zonation of dune vegetation makes these ecosystems ideal for the study of insect ensembles in a short space. In this study, we investigated if the tenebrionid beetles forming an ensemble on a dune zonation showed variations in community organization (relative abundances and species diversity) in different but spatially associated biotopes defined by different plant communities. Three biotopes (corresponding to European Commission habitat 2110, 2120 and 2230) of a well-preserved Mediterranean dune were sampled using square plots of $2 \times 2$ m at three places. To investigate if there was some association between species and habitat we applied a $\chi^2$ test. Variations in community structure parameters were investigated using Shannon index. The three biotopes host tenebrionid communities with similar species composition and overall abundances, confirming that they form a single ensemble. However, tenebrionid species are differently associated with different biotopes along the zonation, with some species occurring with different proportions among the biotopes. A local selection process can be postulated as a mechanism responsible for these differences.



## 1. Introduction

Multiple species groups can be defined from different perspectives, which led to the introduction of a complex terminology in community ecology [1]. Community is used to simply indicate groups of organisms living in the same place at the same time. A community is therefore not restricted by phylogeny or resource use. Restriction by phylogeny or resources, or both, is described by alternative terms. Guilds are groups of species (without regard to taxonomy position) that exploit the same class of environmental resources in a similar way. A phylogenetically bounded group of species that use a similar set of resources within a community forms an "ensemble" [2–5].

Studies in insect multispecies groups have been largely addressed to investigate communities, and very rarely guilds, because the lack of adequate single species ecological information makes it difficult to group species into guilds [6,7 – as examples regarding beetles]. Even more difficult is to study ensembles, because this implies to study a group of species that: (i) belong to the same taxon (e.g. to a given family); (ii) are synchronically present in the same place; and (iii) are comparable in their ecology. Thus, the study of ensembles has received less attention in comparison with community and guild studies [3,4], and – as far as we know – no study has been

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attempted to investigate if structural parameters (i.e. relative abundances of species and diversity) of a given ensemble can be tuned by small grain spatial variation in environmental characteristics.

The tenebrionid beetles (Coleoptera Tenebrionidae) inhabiting Mediterranean sand dunes offer such an intriguing opportunity. Several aspects make these animals excellent model organisms for ensemble studies. First, tenebrionids belong to a monophyletic taxon, so they form a phylogenetically bounded group. Second, they are abundant and fairly easy to sample on coastal dunes, which makes it possible to obtain sufficiently large samples of individuals in short time even in small sampling plots. A synchronous sampling from small areas is important to ensure that the sampled species belong to the same spatial and temporal community. Also, it is important that sampling of different biotopes is conducted synchronically to avoid that differences among biotopes are in fact a result of temporal differences in insect activity (e.g., day *versus* night or spring *versus* summer). Finally, tenebrionids living on coastal dunes are detritivorous and opportunistic animals, and competition in these insects is considered not an important driver of community organization [see 8 for an extensive discussion]. In general, dune ecosystems include an outstanding ecological diversity in terms of environmental heterogeneity and species composition [9,10]. Furthermore, the dynamic nature of sandy coastal habitats and the strong zonation patterns exhibited by the vegetation make dune communities ideal systems for the study of changes of tenebrionids ensembles in a short space.

In this study, we investigated if the tenebrionid beetles forming an ensemble on a Mediterranean coastal dune zonation showed variations in spring community organization (relative abundances and species diversity) in different, but spatially strictly associated, biotopes [11] defined by different plant communities.

## 2. Material and methods

### 2.1. Study area and data collection

Sampling was done along three vegetation transects on a coastal sand dune system in Central Italy, near Montalto Marina (Latium region) during spring (May 2012). As outlined in previous ecological investigations [12], the study area is characterized by a good conservation status and the three vegetation transects share similar physical settings. Coastal zones are under severe human pressures, which may alter profoundly the structure of insect communities [8]. To minimize the possibility that observed patterns are the result of anthropogenic influence we selected our sampling areas among the less disturbed coastal zones in Latium.

Each vegetation transect included three plots. Each plot belongs to a different biotope as defined by European Commission [13,14] (in parentheses EC codes): Embryonic shifting dunes (2110); Shifting (white) dunes along the shoreline with *Ammophila arenaria* (2120); *Malcolmietalia* dune grasslands (2230). The three biotopes were identified in the field following the dominant and diagnostic plant species as indicated by the Habitat Directive Interpretation Manual [15]. Given that a natural stress gradient develops along the sea-inland profile and gives rise to a vegetation zonation in a short space, these three biotopes are typically disposed perpendicularly to the seashore and virtually contiguous. In particular, the biotope corresponding to EC habitat 2110 is closer to the sea, the biotope corresponding to EC habitat 2120 follows inland and the biotope corresponding to EC habitat 2230 is the most inland [16,17].

For the present study, in each transect, the three biotopes (EC habitats 2110, 2120 and 2230) were sampled using square plots of $2 \times 2$ m placed at distance of about 10 m. Within each plot, beetles were collected by hand for a standard time of 20 minutes. A total of 12 trained students were involved in beetle sampling. Students were randomly divided into three groups of four students. In each transect the three biotopes were sampled simultaneously by four students per plot. Students groups also rotated among transects. In this way, a student group, which sampled one biotope in the first transect, sampled another biotope in the second transect, and another biotope again in the third transect. All three biotopes were therefore sampled by all three groups, albeit in different transects.

In each plot, students were instructed to first collect all beetles that moved on the ground, to reduce the risk that escaping animals abandoned the plot before being sampled. Then, the four students moved from the four angles of the square towards the centre collecting all animals they were able to find. Insect sampling was done by conscientiously searching for beetles on the grounds, under leaves, and sieving sand at the base of the plants. To establish an adequate sampling time, we conducted a preliminary sampling in a $2 \times 2$ m plot placed in a biotope corresponding to the EC 2120 habitat. This preliminary sampling section was conducted by two expert entomologists who collected beetles until no further individual was found. This preliminary sampling required about 10 minutes. Thus, 20 minutes with four persons was considered a sufficient time to collect virtually all tenebrionid individuals occurring in a plot.

To minimize the sacrifice of animals, large sized tenebrionids (*Pimelia*, *Erodius*, and *Tentyria*) were placed into tubes, identified by an expert taxonomist, and released close to the sampling plot after all plots of the same site were sampled. Small sized tenebrionids were instead identified in the laboratory.

A further sampling performed during the summer (July 2012) confirmed that the highest activity and diversity of tenebrionids beetles on coastal dunes are mainly concentrated in spring [8], probably because of more suitable climatic conditions (lower temperature and higher soil moisture during spring).

### 2.2. Data analysis

We used a main effect ANOVA to investigate the first order effects of plant association, site and species identity on tenebrionid abundances. Numbers of individuals were