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CONTENTS

VEGETATION AND LANDSCAPE ON CRYSTALLINE LIMESTONE BEDROCK IN THE VICINITY OF LÁNOV (GIANT MOUNTAINS, CZECH REPUBLIC)

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ABSTRACT

This paper evaluates the structure of the landscape and vegetation in an area of 106.4 ha near the quarry by the village Horní Lánov (4 km east of Vrchlabí) situated in a low part of the Giant Mountains. The bedrock (crystalline limestone), rugged terrain, soil moisture and management affect the biodiversity at this locality. It is botanically well known and a very valuable region because of the high number of nature conservation-important species and habitats that occur there. A total 517 species of vascular plants were recorded there between 2002 and 2010. The whole area was divided into 36 segments each with a relatively homogeneous vegetation cover consisting of particular species of plants. Classification of the segments was done using a numerical classification (Sörensen's similarity index) and Ellenberg's indicator values were used to describe the basic environmental features of the individual segments. The species presence/absence data together with indicator values (light conditions, temperature, water availability, soil reaction and nitrogen activity) were evaluated. The PCA ordination of this data set distinguished three basic types of vegetation cover ("forest", "dry" and "wet") and that the species composition of the vegetation in the area is mostly determined by land-use (deforestation, limestone mining, pasturing and management of forests) and soil moisture.

Keywords: bioindication, classification, landscape structure, ordination, species richness

Introduction

The structure of landscape can be analysed at different scales – large regions displayed at a small scale that covers the whole area of a country or a larger area, or at a medium scale, in which the centre of attention is e.g. a mountain range or medium sized river-basin, or at a large scale of parts of a landscape consisting of a few tens of hectares (Farina 2006). The variability in landscape in the Giant Mountains depends on altitude. The landscape transects (Matějka 2010) can be assigned to a medium scale. This study operates in a large display scale. Results of investigations at a medium scale are suitable for classifying landscape segments based on levels of management in large protected areas (e.g. national parks and protected landscape areas; for example see Matějka 2010; Křenová and Hruška 2012). Analyses carried out at a large scale are not usually published because they rarely produce results suitable for publication in scientific journals.

This paper presents the results of a large scale landscape analysis. The area studied is in the foothills of the Krkonoše Mts. (Giant Mountains, Czech Republic) at the border of the Krkonoše National Park (NP). The landscape is determined by the local geology. Acid, nutrient-poor rocks predominate in the Giant Mountains and the basic rocks that rarely occur there (Faltysová et al. 2002) consist of spatially limited inserts of crystalline limestone. These localities are very important in terms of increasing the biodiversity in the area. Not only do different species of plants (often especially protected or

endangered) occur at these localities but even specific phytocenoses and many species of other organisms that do not occur in the surroundings areas, or only rarely. Since limestone is an important building material it is often quarried at these localities, which often results in the devastation of a substantial part of these localities. One such locality is near Horní Lánov where there is a small quarry that was abandoned a long time ago and an operational quarry that is likely to continue working well into the future. Because in the area to be quarried there are lots of protected species (*Corallorhiza trifida*, *Epipactis purpurata*, *Platanthera bifolia*, *Cephalanthera damasonium*) that are abundant and occur there in representative biotopes (e.g. herbaceous plant rich and calcicolous beech forests, ash-alder alluvial woods), it is important to save these areas for posterity. That is why workers of the Administration of the Krkonoše NP ordered a detailed study of the area near the operational quarry and in the wider surroundings of both quarries. This resulted in unpublished manuscripts by Dřevíkovský (2000) and Málková (2005). The extraordinary scientific value of this area is well established based on historical floristic data (Málková et al. 2004, 2006; Málková 2007).

If the floristic data for only a few segments of landscape are processed it is difficult to evaluate the similarity of such segments only on the basis of species similarity (e.g. Jaccard's similarity index) because the result is highly influenced by the difference in the species richness of these segments. Another method is needed if the objective is to determine the similarity of the segments

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on the base of natural conditions. One possibility is to use the indicator values of the individual species. That is why we used Ellenberg's indicator values (Ellenberg et al. 1992) to describe the characteristics of the landscape. Different forms of this system are widely used. Up to end of 2010, the database ISI Web of Knowledge (www .isiwebofknowledge.com) recorded at least 393 papers on bioindication using indicator values and the first paper was published in 1982 (Degorski 1982).

The aim of this paper is:

– to analyze the landscape in a small area near Lánov based on the floristic structure of the vegetation cover in the biotopes present in the different segments of that landscape;
– to present an analysis of the flora based on Ellenberg's indicator values, which is rarely done compared to evaluation using phytosociological relevés.

This reveals that the current landscape and its vegetation cover is a result of the interaction of natural conditions, current management and other usage.

Material and methods

Location and characteristics of the area of interest

The area of 106.4 ha monitored is situated in the protected zone of the Krkonoše National Park about 4 km east of Vrchlabí (Fig. 1). It is located in the foothills of the Giant Mountains at the border between Horní Lánov and
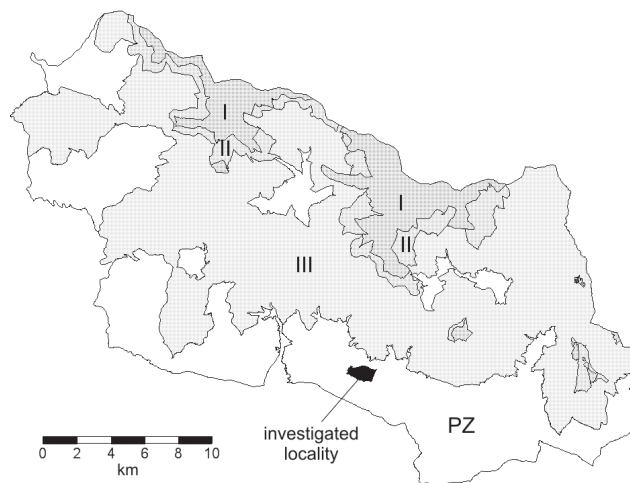
Fig. 1 Location of the area investigated in the protected zone (PZ) of the Krkonoše National Park. Positions of Ist to IIIrd zones of nature protection in the National Park are indicated.

Prostřední Lánov. The northwest boundary is marked by the Pekelský stream and the eastern boudary is the border between Prostřední Lánov and Čistá. The boundary in the south is the road from Horní Lánov through Bíner to Černý Důl (Fig. 2). The altitude varies between 499 and 657 m a.s.l.

The area analyzed is located near the southern part of the Krkonošský region (code 1.68), where in terms of biogeography it gradually becomes the Podkrkonošský region, 1.37) (Culek et al. 1996). This area belongs to Meso-
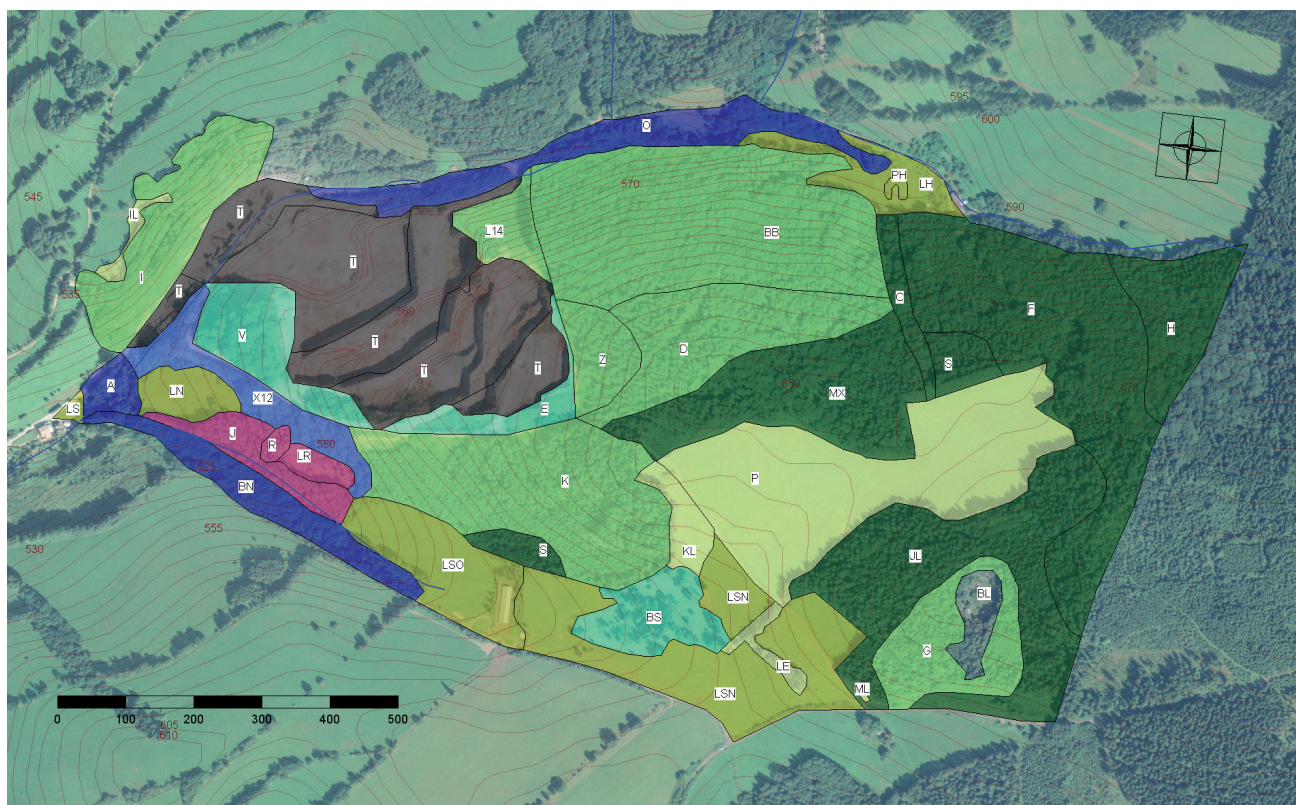
Fig. 2 The study area with the different landscape segments (see Fig. 3 for the colour legend for the classification groups). Ortophoto 2001 is superimposed on the map. The distance between altitudinal contour lines is 5 m.

phyticum, subdistrict 56c (Trutnovské Podkrkonoší; Skalický 1988).

Long slopes, especially those with a northeastern exposure are typical of this area. Palaeozoic and Proterozoic granular limestone and dolomites cover about 2/3 of the area. The southern third of the area is formed by chlorit-sericitic phylits of the same period with a green slate insert in the centre. Especially at the margins there are Pleistocene deluvial and deluviofluvial sediments, near the stream deluvial sediments of a fluvial plain (Holocene). In the west of the area monitored there are sporadic aleuropelites (sandstones) of the upper Paleozoicum.

Soil in the area is predominately of the Kambizem modal, Glej modal in alluvium and Pseudoglej histic type in the area of Bíner, and Kambizem gleyic and dystric in the surrounding areas, and Fluvizem modal occurs in the north-eastern part of the area (terminology follows Kozák et al. 2010).

The area lies on the border between the mild warm zone MT4 and cool zone CH7 according to the updated Quitt's classification (Tolasz 2007).

The area monitored is drained by the Pekelský creek, which flows through the quarry (with two big tributaries on the left side). There is a large area with springs covered by species-rich fen vegetation close to Bíner (segment BS). In terms of the landscape characteristics of altitude, climate and vegetation cover / land-use, the area is transitional between an agricultural and forest landscape (Matějka 2010). According to the data in the land register (data 2003), the KES index (Löw and Míchal 2003, pp. 241–245) varies between 1.46 for the area around Horní Lánov and 0.45 for that around Prostřední Lánov. Values of 0.4–0.8 correspond to an intensively cultivated landscape with a significant level of (agro) industrial elements and values of 0.9–2.9 indicate a common cultural landscape.

In terms of forest typology (Viewegh et al. 2003) the area is located on the border of the 4th and 5th forest alti-tudinal zones. Edaphic series W (limestone) and S (nutrient-medium) predominate here, with the damp areas along the streams categorized as L (alluvial soils on floodplains), U ('unstable' soils in ravines and gulleys) or V (moist to wet) (based on the maps of the Institute for Forest Management (ÚHÚL), Brandýs nad Labem, at 2007; see www.uhul.cz).

Potential vegetation in this area is the association *Dentario enneaphylli–Fagetum* (Neuhäuslová et al. 1998). The geobotanical reconstruction map indicates that most of the area should be covered by forests of sub-alliance *Eu-Fagenion* and of alliance *Alnion incanae* on alluvium.

Vegetation cover is influenced by the long-term effect of human activities. Forest-free areas were created accidentally by human intervention. In the past, large areas were deforested and converted to agricultural land (pastures, meadows and fields). The basic negative effect on the area resulted from the quarrying of limestone, eutrophization and ruderalization (especially near the roads, buildings, car parks, stock pile of quarried limestone), planting of evergreen woody species (especially *Picea abies*) that do not normally grow in this area.

Field survey

Based on detailed floristic surveys the area was divided into 36 segments (Fig. 2, Table 1) each of which includes a characteristic but relatively homogenous complex of environmental factors and vegetation cover. In the BL segment, three parts were analyzed separately because they have significantly different vegetation structures. Lists of species were compiled for each segment separately based on the results of many surveys. From 2002 to 2004, a detailed evaluation of the vegetation was carried out using the methods of mapping biotopes in NATURA 2000 (Chytrý et al. 2001) and the results of 92 phytosociological relevés typical of this valuable association (Málková 2005). The species inventory of the area was carried out from 2005 to 2010.

Table 1 Basic features of the landscape segments.
Classification – classification group according to Fig. 2. L (light conditions), T (temperature), W (water availability), A (soil acidity), N (nitrogen activity) – indices calculated based on the species counts in the Ellenberg's ecoindication classes (%). PCA1 and PCA2 – the PCA ordination score calculated based on the share of species in the ecoindication classes. Cover type: forest – forest prevails in part of the area; dry – partially to fully open woody stand on mesophilous to dry soils; wet – partially to fully open woody stand or forest-free biotopes on moist to wet soils.

Segment	Area (ha)	Number of species	Classification	L $I_{7-9|1-3}$	T $I_{6-9|1-4}$	W $I_{8-12|1-5}$	A $I_{6-9|1-4}$	N $I_{6-9|1-4}$	PCA1	PCA2	Cover type	Short description, comments
T	12.790										bare soils	Operational quarry with buildings, stone crusher and special-purpose built communications
K	7.062	115	B000	28.7	12.5	−71.3	19.4	5.4	3.32	−2.62	forest	Mixed forest (*Betula pendula* and *Picea abies* prevails) with ruderalized fringe phytocoenosis
BB	10.072	112	B000	6.5	14.5	−72.3	50.7	7.3	3.96	−2.60	forest	Strongly-sloping herbaceous plant-rich beech woodland with a large number of protected and endangered species
I	3.653	145	B000	18.2	19.2	−69.3	61.7	0.8	1.49	−2.21	forest	Strongly-sloping calcicolous beech woodland with rocks; large number of species of conservation-importance

Segment	Area (ha)	Number of species	Classification	L $_{7-9\|1-3}$	T $_{6-9\|1-4}$	W $_{8-12\|1-5}$	A $_{6-9\|1-4}$	N $_{6-9\|1-4}$	PCA1	PCA2	Cover type	Short description, comments
MX	5.299	80	B001	1.3	16.7	−67.7	34.8	7.8	4.48	−1.84	forest	Mixed, degraded, species-rich stand
L14	1.083	123	B000	21.6	4.8	−67.3	51.3	10.1	2.57	−1.74	forest	Clear-cut strongly-sloping herbaceous plant rich beech woodland with dense spontaneous tree regeneration; area adjacent to the quarry was quarried in 2010
F	9.345	80	B001	−2.6	10.3	−70.8	51.0	19.7	5.66	−1.31	forest	Degraded herbaceous plant rich beech woodland, partly cut and dramatically opened to light
D	4.142	178	B000	28.1	14.4	−59.9	50.0	16.4	1.63	−1.03	forest	Degraded herbaceous plant rich beech woodland with a high representation of *Picea abies* and *Abies alba*
H	2.474	69	B001	−24.6	−9.4	−72.4	42.3	24.6	7.18	−0.91	forest	Herbaceous plant rich beech woodland on a slope in which sometimes a high proportion of *Abies alba* and *Picea abies* sometimes prevails; elements of scree forest occur on parts of the steep slopes
G	2.607	114	B000	25.0	9.1	−60.6	56.5	18.7	1.69	−0.70	forest	Mixed forest stand with a high proportion of *Picea abies* adjacent to the abandoned limestone quarry; undergrowth is that of a herbaceous plant-rich beech woodland
Z	1.303	137	B000	22.9	4.3	−63.8	45.1	15.5	2.13	−0.59	forest	Degraded herbaceous plant rich beech woodland; high cover of shrubs; many species of conservation-importance; area licensed for quarrying limestone
X12	1.939	59	B01	12.3	33.3	−65.4	50.0	18.4	3.31	−0.37	forest	High dense vegetation with natural tree regeneration and remnants of the original beech woodland
JL	7.312	96	B001	12.0	0.0	−60.8	36.5	22.1	3.41	0.06	forest	Even-aged spruce stand in the pit of the abandoned quarry and original herbaceous plant rich beech woodland with degraded undergrowth
C	0.742	55	B001	−7.8	11.1	−63.6	41.9	34.1	6.07	0.50	forest	Maple stand on an old farm-track; herbaceous plant layer corresponds to that of a degraded herbaceous plant rich beech woodland
S	1.262	82	B001	10.4	−5.9	−56.7	43.8	29.9	4.12	0.84	forest	Originally wet fir beech woodland, now spruce monoculture, sometimes with elements of the original vegetation in the undergrowth
BN	2.053	109	B1	7.8	1.6	−43.2	68.6	54.7	3.43	2.50	forest	Valley with ash-alder alluvial woodland and herbaceous plant rich beech woodland on adjacent slopes; botanically valuable area
BL_D	(0.7661)	118	A1	61.7	4.3	−3.1	42.3	−11.7	−4.11	−0.85	dry	Abandoned limestone quarry with species rich vegetation – bottom of the quarry
BL_P		139	A0	53.3	23.4	−63.5	46.3	−10.1	−2.08	−4.09	dry	Abandoned limestone quarry with species rich vegetation – plateau
BL_S		80	A0	52.5	25.0	−77.6	68.6	−14.9	−3.82	−6.64	dry	Abandoned limestone quarry with species rich vegetation – walls of the quarry
BS	1.883	220	A0	57.3	11.3	−21.4	51.4	0.0	−2.32	−0.16	dry	Species-rich waterlogged swampy meadow and deciduous grove; registered botanical locality

Segment	Area (ha)	Number of species	Classification	L $_{7-9|1-3}$	T $_{6-9|1-4}$	W $_{8-12|1-5}$	A $_{6-9|1-4}$	N $_{6-9|1-4}$	PCA1	PCA2	Cover type	Short description, comments
E	1.062	247	A0	60.6	37.9	−61.2	57.0	5.6	−3.24	−3.11	dry	Xerophilous vegetation in segments in the operational quarry, access road; a high share of synanthropic species
V	2.236	183	A0	46.4	27.6	−72.1	61.7	2.0	−1.20	−2.80	dry	Sparse calcicolous beech woodland around rocks; a substantial part quarried in 2010
IL	0.255	141	C0	66.4	30.0	−69.4	55.8	−15.4	−4.42	−4.17	dry	Species-rich herbaceous plant edge and flowery mesophilous to dry meadow (mown and grazed by sheep)
KL	0.791	88	C0	71.8	27.5	−63.6	45.1	−11.8	−3.54	−1.65	dry	Mesophilous to xerophilous flowery meadow on slope with small rocks; regularly mown
LE	0.384	113	C0	65.7	42.0	−58.7	70.3	8.5	−3.26	−0.36	dry	Mesophilous scrub with a species-rich herbaceous plant undergrowth
ML	0.026	90	C0	69.8	36.6	−61.1	63.0	14.9	−3.74	−2.04	dry	Slightly ruderalized herbaceous plant edge close to the forest
P	8.978	113	C0	75.2	28.1	−57.4	65.6	25.8	−2.81	0.11	dry	Species-poor reclaimed meadow, mown and grazed by cattle; sometimes with remnants of a spring
LSN	5.908	125	C1	74.2	20.8	−27.2	41.1	−7.3	−4.64	−1.40	dry	Reclaimed mesophilous meadow with several springs; mown and grazed by cattle
A	0.573	113	B1	49.0	27.8	−26.9	66.7	56.5	−0.67	4.38	wet	Unmanaged degraded mesophilous to wet lawn in an old orchard with ruins, a timber yard and part of the parking lot (2010)
O	3.162	202	B1	38.3	14.0	−26.1	68.0	42.9	0.11	1.88	wet	Degraded species-rich alluvial ash-alder woodland, locally sparse; high coverage of protected species
LH	1.406	123	C1	64.0	27.3	−32.4	57.8	20.4	−2.42	1.36	wet	Mesophilous to waterlogged meadow with small springs, partly mown and/or grazed by sheep, unmanaged tall herbaceous plants with *Filipendula ulmaria* in parts
LN	0.923	62	C1	60.3	14.3	−36.5	68.8	34.0	−1.63	4.02	wet	Previously reclaimed mesophilous meadow, mown
LS	0.118	67	C1	66.1	22.7	−52.5	65.8	25.0	−1.77	1.77	wet	Fairly representative mesophilous meadow with *Arrhenatherum elatius* along the Pekelský creek; mown several times per year
LSO	2.834	50	C1	59.2	0.0	−33.3	68.4	33.3	−0.63	4.25	wet	Reclaimed meadow with elements of mesophilous *Arrhenatherum elatius* meadow and pastures (cattle grazing; fenced)
PH	0.091	99	C1	64.2	−13.2	7.7	33.9	4.7	−3.45	2.44	wet	Species-rich, slightly ruderalized spring meadow with many species of conservation importance; grazed by sheep
J	1.246	46	D	34.9	33.3	−16.7	81.5	64.1	−0.43	7.75	wet	Hydrophilous young dense maple stand
LR	0.408	32	D	48.1	23.1	−32.0	58.8	37.0	−1.75	4.75	wet	Degraded unmanaged mesophilous to wet meadow
R	0.167	47	D	54.3	4.0	13.6	57.7	40.5	−1.96	6.73	wet	Ruderalized reed bed with tree regeneration in depressions in the terrain between maple stand and unmanaged meadow

Data processing

Only floristic data on the presence of species in each segment were processed in this paper. The question of the floristic similarity of the segments should resolve their classification. In this way the variability in the floristic composition of the whole area monitored will be described. Segments were classified on the basis of presence/absence of all the species recorded using the agglomerative hierarchy method of average distance, with the distance (dissimilarity) measured in terms of the complement of Sörensen's index of similarity (1-S) (e.g. McCune and Grace 2002). Sörensen's coefficient was chosen because the similarity of segments does not depend on the species richness as is the case with other measures (e.g. Euclidean distance).

Species were categorized into the following groups: trees, shrubs (woody species with a height of up to 2 m), indigenous plants (herbaceous species of plants that are not included in the following category) and synanthropic taxa (i.e. species of herbaceous plants that occur mainly in ruderal and weed communities; this was done in the Giant Mountains and their foothills). The list of those species that are considered to be synanthropic is in the appendix. The last two categories are distinguished relatively subjectively. Nevertheless, this classification was used because the ratio of the number of species in both these categories (later called the synanthropization index) can be easily interpreted. While interpreting the level of synanthropization there is a need to remember that it is only a relative representation of synanthropic species, e.g. the invasion by one species of a species rich segment has a lower effect in increasing this index than that it does on a species poor segment.

The environmental conditions were defined in terms of Ellenberg's indicator values (Ellenberg et al. 1992). Species counts in classes defined in terms of light (L), temperature (T), soil wetness (water availability; W), soil reaction (A) and nitrogen activity (N) were evaluated for each landscape segment. In the literature the factor N is described in terms of nitrogen availability but the index indicates the speed of the nitrogen cycle rather than the supply of this element in the ecosystem. This is why it is better to talk about "activity", see (Matějka 1993). Other authors describe this factor using a general term "fertility" (e.g. Hill et al. 2000). Indicator values for continentality were not used for two reasons: most species are indifferent to this factor and the use of this index is only useful when comparing widely separated geographical areas.

Species were separated into classes. Each class contains species with an index equal to a certain value. Classes are marked by letters tagging a particular ecological factor and given value. Species in a particular segment that have a particular value for a particular environmental factor are expressed as a relative frequency, e.g. $L_1 + \ldots + L_9 = 1$. Taxa that do not have an indicator value or for which the value is unknown were not included in the analysis. In the literature mean indicator values are usually arithmetic averages weighted by taking into consideration the species' representation (e.g. coverage). This is possible if data consists of phytosociological relevés (Diekmann 1995, Brunet et al. 2000, Hill et al. 2000), but not if it consists of floristic data (or other data of presence/absence). Some authors also challenge the value of a weighted species presence (Kafer and Witte 2004). As an arithmetic average cannot be calculated using ordinal values such as indicator values, simplified indicator values (indices) are proposed. Such indicator values (generally for factor f) were calculated from simple relative species frequencies in individual classes (f_1 to f_9, resp. f_1 to f_{12} in the case of soil wetness), e.g.

$$I_{6-9|1-4} = f_6 + f_7 + f_8 + f_9 - f_1 - f_2 - f_3 - f_4.$$

Index $I_{7-9|1-3}$ was used analogously, as well as similar indices for water availability.

Data on the relative share of individual classes based on all the Ellenberg's values for all five environmental factors were processed using principal component analysis, PCA. The calculation was based on a correlation matrix of the parameters.

Maps were produced using the programme TopoL xT, version 9.5 (www.topol.cz). Statistical analyses were carried out using Statistica software, version 8 (product of the StatSoft Inc.). The ordination results were plotted using PlotOA software (www.infodatasys.cz/software).

Results and discussion

Species richness

In the whole of the area monitored, 517 species of vascular plants were recorded from 2002 to 2010, 30 of them were trees, 26 shrubs, 351 indigenous plants and 109 synanthropic taxa. The average synanthropization index was 19.7%. The highest indices were recorded in segments P (44%), 4 (37%), O (32%), LR (30%), ML and V (both 29%), the vegetation in which was substantially affected by different factors. For instance, the re-cultivated pasture (segment P) included not only pasture, but also unmanaged ruderal borders and a grassy path. In segment E, there is unmanaged vegetation and no management of the access paths in the functional quarry where invasive species occurred. The high percentage of synanthropic species in segment O is associated with the runoff from the pasture and other sites with buildings and the unmanaged landscape borders of the path (e.g. where the following invasive species occurred: *Reynoutria japonica*, *Solidago canadensis* and *Impatiens parviflora*). In segment LR, the high number of synanthropic species is associated with runoff from the pasture, the sheep-cot and the long time for which it has not been managed.

On the other hand, the lowest synanthropization indices (< 10%) were recorded in segments with closed for-

ests (H, BB and F), where synanthropic species mostly only grow along the paths that go through these stands.

Segment E with 247 species is the richest in terms of species. In this segment there are lots of microclimatically different locations. Xerophilous vegetation predominates in the operational quarry. Synanthropic species are abundant in the surroundings of the access paths from the outbuildings up to the highest levels in the quarry. In this segment, there is locally a high cover of evergreen trees and small depressions in the terrain where shady and humid places occur. Different elements occur close to one another in neighbouring associations, which means there are even species with different indicator values to the chosen ecological factors (especially wetness and light).

Another species-rich segment is segment BS, where 220 species were recorded. In this segment there are two fenced wet fen meadows divided from each other by a small woodlot with a heterogeneous mixture of trees. Heliophilous and hygrophilous species predominate and coexist there along with shade tolerant and mesophilous species. Some xerophilous species also occur sporadically at the margin of this segment.

The richest species forest segment is D (178 species). Compared to the other forest segments, there is an increased level of synanthropization (24%) there. The high number of species is due to the many ruderal species that occur at the borders of the forest and along the paths, as well as the hygrophilous vegetation that occurs in some parts of the segment. Wind damage to the tree stand that occurred in July 2009 has also had a role in determining the species richness there.

The number of species in a given area is not dependent on the size of segment. This may be due to the fact that the segments are relatively heterogeneous in terms of micro-sites, which is largely caused by anthropogeneous influences (e.g. ruderalization in the surroundings of roads and buildings, increased exposure to light in places where trees have been felled or windblown).
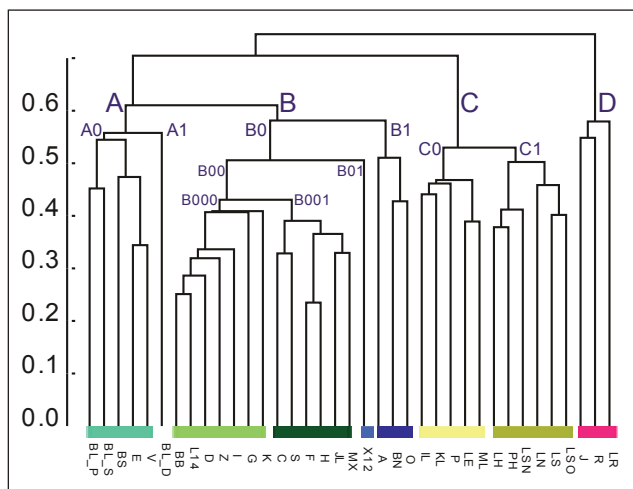
Fig. 3 The average clustering of landscape segments into groups, which was calculated using species presence/absence. Similarities were evaluated using Sörensen's index.

Classification of segments

The classification of the segments (Fig. 3) based on their structure revealed that there are four basic groups: A–D. When viewed in more detail then other subclasses in terms of their ecology can be identified (Tab. 1). The final map of the different groups of segments includes elements of landscape cover, but it is not a vegetation map or a biotope map as defined by the system NATURA 2000 (Härtel et al. 2009).

Bioindication

We assessed 5.4% of all the species recorded as indifferent to light intensity or this indicator value was not set for them. Most of the species (32.7%) are relatively light-demanding with an index of 7. Forest-free area covers less than 40% of the area monitored and these places are richer in species than forest stands. Species with a range of different indicator values for this factor occurred there. Indices $I_{7-9|1-3}$ and $I_{6-9|1-4}$ have similar predictive capabilities (based on correlation coefficient comparison between these indices and indicator values using the arithmetic average; Tab. 2).

Of all the species, 38.1% have wide ecological amplitudes in terms of temperature or this indicator value was not set for them. Most of the species recorded have indicator values of either 5 (25.0%) or 6 (26.7%). Nearly all the species had indices of 3–8 for this environmental factor. The index $I_{6-9|1-4}$ (Tab. 2) appears to be the most suitable for evaluating the temperature conditions.

Of all the species, 13.5% were evaluated as indifferent to the availability of water or did not have indicator values set for this ecological factor. Most of the species present had an indicator value for this factor of 5 (28.0% of all species). Species with the values 2–11 were also recorded there. These results indicate that very different locations can occur there near water (segments O, PH) or very dry places (E). Of the three indices used the index $I_{8-12|1-5}$ (Tab. 2) seems to be the optimal one.

Of all the species, 32.5% were indifferent to soil reaction or the indicator value for this factor for them is not set. Most of the species had an indicator value of 7 (23.4% of all species) for this factor. Species with indicator values for a wide range of values of pH were present. The index

Table 2 Correlation coefficients (r) between bioindication indices and eco-indices calculated as arithmetic means; based on the presence/absence of species in landscape segments selected.

Environmental parameter	$I_{7-9|1-3}$	$I_{6-9|1-4}$
light conditions (L)	0.996	0.993
temperature (T)	0.667	0.991
soil acidity (A)	0.938	0.948
nitrogen activity (N)	0.986	0.990

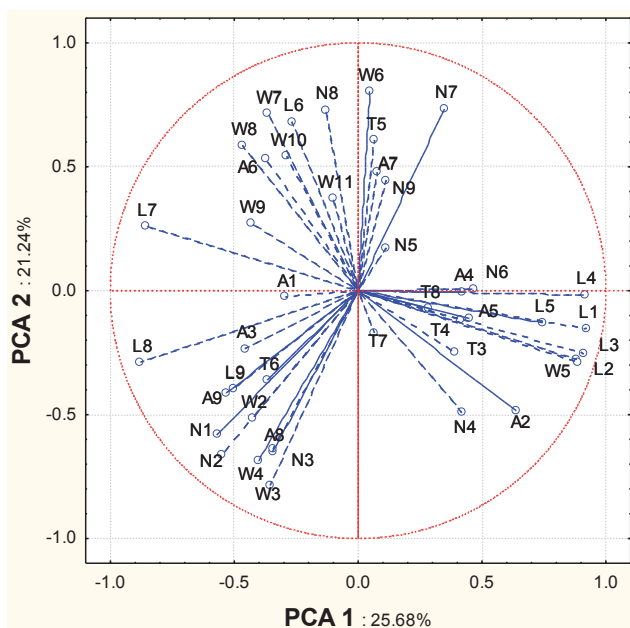| Environmental parameter	$I_{9-12|1-4}$	$I_{8-12|1-5}$	$I_{7-12|1-6}$
water availability (W)	0.885	0.969	0.943

$I_{6-9|1-4}$ (Tab. 2) seems to be slightly more suitable for evaluation of soil acidity.

Of all the species, 14.7% had wide ecological tolerance of nitrogen activity or the indicator value for this factor for them was not set. Species with a wide range of indicator values for this environmental factor occurred with similar incidences (the incidences of species with values of 2–7 were 10–14%). Both count indices $I_{7-9|1-3}$ and $I_{6-9|1-4}$ have a similar predictive capability (Tab. 2).

Ellenberg's indicator system has been used to study landscape structure, for example, Okland et al. (2006) studied the agricultural landscape in Norway based on 1 km² segments. A second example is a study on the effects of landscape on the species composition of the herbaceous plant etage in indigenous forests in Great Britain (Petit et al. 2004).

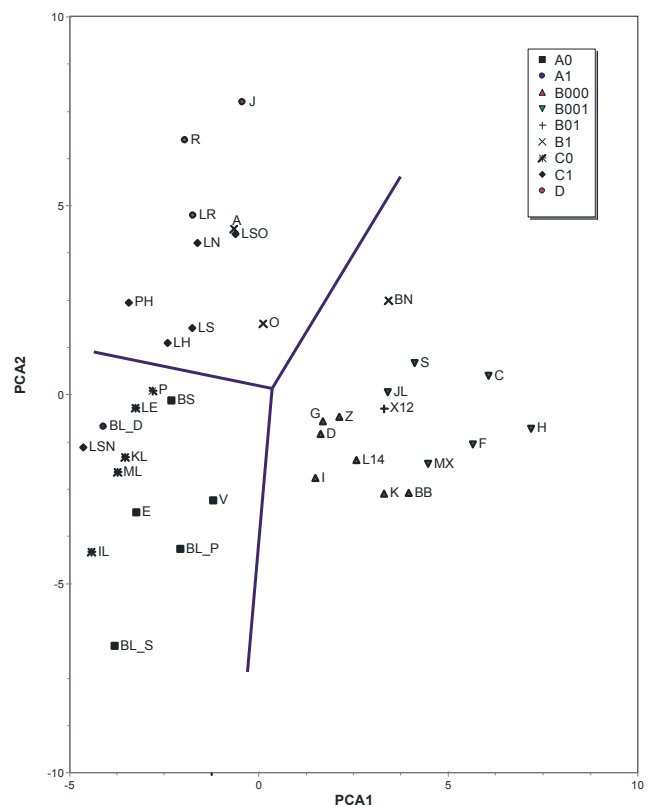### Ordination using the indicator values of the species

Based on the ordination analysis of the relative numbers of species in the particular indicator classes of Ellenberg et al. (1992), there are clearly three basic lines (directions in the ordination space) (Fig. 4). The first line is characterized by L_1-L_5 classes (shade-tolerant species, mostly forest species), W_5 (species with a medium requirement for water) and A_2 (species of acid soil). The second line connects species of wet localities (W_6, W_7) and those occurring in localities with a higher nitrogen activity (N_7, N_8), whose occurrence is related to a higher ordination score along the second axis. The third line is characterized by species with indicator values W_3, W_4 (species of slightly drier localities), N_1, N_2 and N_3 (low nitrogen activity) and A_9 (species growing on basic soil). Simultaneously, this line is more or less closed to the occurrence of species growing in full-sun (L_8).

Location of landscape segments within ordination space (Fig. 5) was used to classify the segments into different groups. The typical forest segments occurred mostly on mesophilous sites (located in right hand sector of the ordination space) and the segments with fens on wet, water-logged soils (located in the upper left hand sector) can have a more or less open evergreen tree layer. The third group includes segments on dry soils with xerothermic elements (lower left third of ordination space). Based on the location of a landscape segment in a particular sector of ordination space it was categorized as either "forest", "wet" or "dry" (Table 1). This classification can in some cases appear to be wrong because of the presence of a species belonging to particular ecological class in a segment that does not include the biotope or micro-site of this species can result in the segment being assigned to the wrong group. In this context, the assignment of segment BS to the "dry" group, although biotopes of fen meadows occur there, is such a case. Nonetheless, there are a lot of microsites with dry soils there where *Carlina acaulis*, *Clinopodium vulgare*, *Euphorbia cyparissias*, *Galium verum* or *Securigera varia* can be found. The great diversity of microsites in this segment accounts for its high species richness.

In the ordination graph (Fig. 5) those groups of segments that were classified on the basis of the occurrence of particular species, are marked. Most clusters occur only

Fig. 4 Space with the first two principal component (PCA) axes localized in terms of the variable – relative share of species of single indicator class according to Ellenberg et al. (1992).

Fig. 5 The position of the segments of landscape in the ordination space of the first two PCA axes calculated on the basis of their relative share of species of single indicator class according to Ellenberg et al. (1992). The segments are classified based on the occurrences of all species (Fig. 2).

in one of three different groups of segments. In the "dry" group there are the classes A0 (5 landscape segments), A1 (1) and C0 (5), in the "forest" group classes B000 (7), B001 (6) and B01 (1) and in the "wet" group uniquely class D (3 segments) and two other classes represented by one segment in the "dry" group (class C) and one in the "forest" group (class B1), which is probably a result of the heterogeneity of particular segments and variability in the vegetation units within individual segments.

Segments categorized in the "forest", "dry" and "wet" groups differ in species richness. The richest segments are on dry soils and the poorest on waterlogged soils.

The level of synanthropization (Fig. 6) and the score along the first indicator axis are significantly correlated ($r = -0.513$; $p < 0.05$), which is because the most synanthropized sites are open and free of forest. The highest synanthropization index was recorded in segment P where a mown reclaimed meadow (used as cattle pasture) is the predominant biotope. Other segments with a high synanthropization index are in the operational quarry and this is connected with limestone excavation (transport, material manipulation). The second ordination axis correlates with species richness in terms of the total number of species ($r = -0.510$), number of species of trees ($r = -0.401$) and shrubs ($r = -0.410$). The highest correlation was with the number of "natural plants" ($r = -0.534$). The segments on dry sites are much richer in species than those on waterlogged soil. This is not the case when there is an increase in variability of (micro)sites within a segment as is the case in segment BS.

Summary

Detailed surveys carried out from 2002 to 2010 revealed that there are 517 vascular species of plants in the wider surroundings of the lime quarries near Horní Lánov, which consists of an area of 106 ha composed of 36 segments of landscape.

The results of the analyses indicate that the species composition of the vegetation cover in the wider area is mostly influenced by two factors, which are evident from the ordination analysis. The first is the way each segment of landscape is used, which is related to the openness of the tree layer or more precisely the occurrence of non-forest sites in the segment. The second is soil wetness.

Nevertheless, the presence of limestone is an important factor for the plant species composition in the whole region. The different bedrock in the southern part of the area monitored seems to be less important, though it does affect the species composition of the segments in this area and their classification (Figs 2 and 3). This can be given by the fact that only phyllites are found in the area and the soils on the phyllites are apparently influenced by the material transfer from the highly located habitats on calcite bedrock.

The variation in the segments, each of which is described by the list of vascular species of plants recorded there can be evaluated using hierarchical agglomerative classification. On this basis, it is possible to draw a map of the different types of vegetation-cover (example in Fig. 2). Ordination analysis based on the relative representation of species (relative species counts) in particular indicator classes according to Elleberg can bring other information about the im-
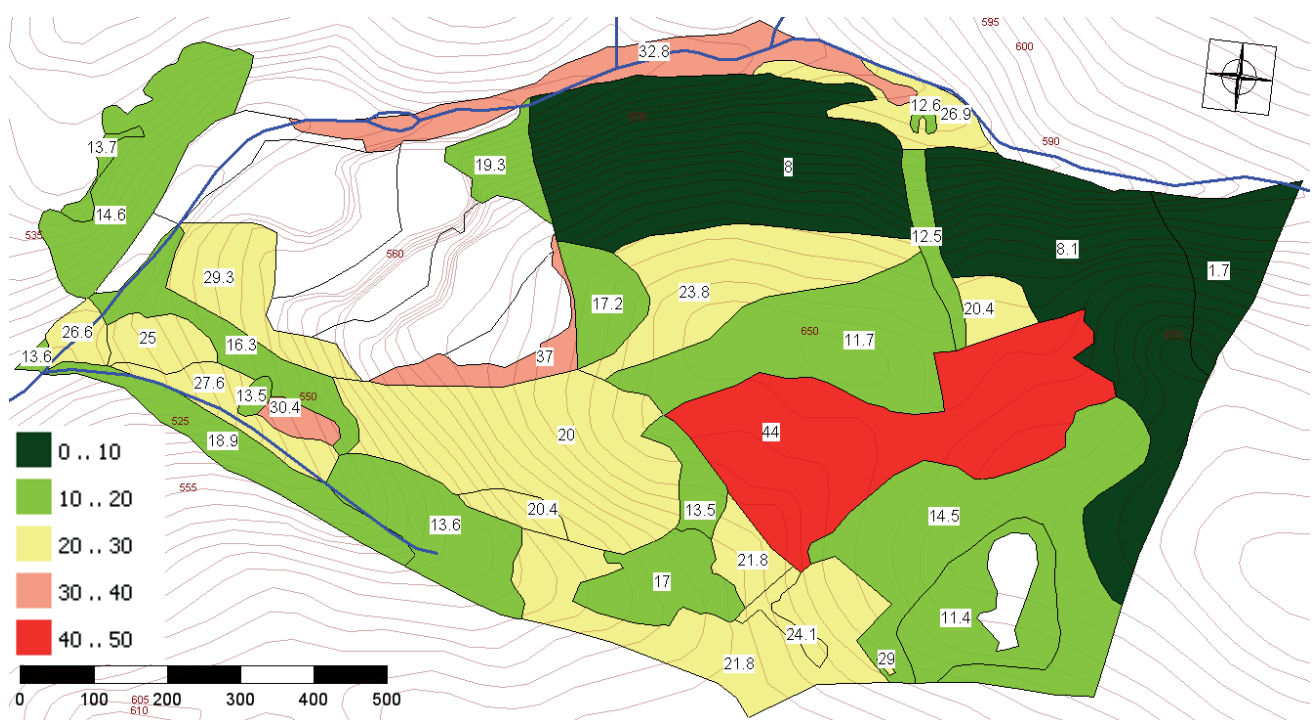

Fig. 6 The landscape segments in the study area classified in terms of their synanthropic index (%).

portance of environmental factors in the differentiation of vegetative cover in differently evaluated segments without considering the species richness of those segments.

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Appendix

List of the synanthropic species recorded in the area studied

Aegopodium podagraria; *Aethusa cynapium*; *Agrostis gigantea*; *Alliaria petiolata*; *Anagallis arvensis*; *Anthriscus sylvestris*; *Arabidopsis thaliana*; *Arctium lappa*; *Arctium tomentosum*; *Artemisia vulgaris*; *Barbarea vulgaris*; *Calamagrostis epigejos*; *Campanula ranunculoides*; *Cannabis sativa*; *Capsella bursa-pastoris*; *Carduus acanthoides*; *Carex hirta*; *C. muricata*; *Cirsium arvense*; *C. vulgare*; *Convolvulus arvensis*; *Conyza canadensis*; *Cuscuta epithymum*; *C. europaea*; *Echium vulgare*; *Elytrigia repens*; *Epilobium ciliatum*; *Equisetum arvense*; *Erigeron acris*; *E. annuus*; *Erodium cicutarium*; *Erophila verna*; *Erysimum cheiranthoides*; *Fallopia convolvulus*; *Galeopsis pubescens*; *G. tetrahit*; *Galinsoga parviflora*; *G. quadriradiata*; *Galium aparine*; *Geranium columbinum*; *G. dissectum*; *G. pusillum*; *G. pyrenaicum*; *G. robertianum*; *Geum urbanum*; *Glechoma hederacea*; *Hypericum humifusum*; *Chaerophyllum aromaticum*; *Chelidonium majus*; *Chenopodium album*; *C. bonus-henricus*; *C. polyspermum*; *Impatiens parviflora*; *Juncus tenuis*; *Lactuca serriola*; *Lamium album*; *L. purpureum*; *Lapsana communis*; *Lolium multiflorum*; *Malva neglecta*; *Matricaria discoidea*; *Medicago falcata*; *M. sativa*; *Melilotus albus*; *M. officinalis*; *Mentha* × *verticillata*; *M. arvensis*; *M. longifolia*; *Microrrhinum minus*; *Myosotis arvensis*; *Myosoton aquaticum*; *Persicaria lapathifolia*; *P. maculosa*; *Pinus mugo*; *Plantago major*; *Poa annua*; *Polygonum aviculare*; *Potentilla anserina*; *P. reptans*; *Reynoutria japonica*; *Rumex crispus*; *R. obtusifolius*; *R. thyrsiflorus*; *Sedum spurium*; *Senecio jacobaea*; *Sherardia arvensis*; *Silene latifolia* subsp. *alba*; *Sinapis arvensis*; *Sisymbrium officinale*; *S. strictissimum*; *Solidago canadensis*; *Sonchus arvensis*; *S. asper*; *S. oleraceus*; *Spergularia rubra*; *Stellaria media*; *Tanacetum vulgare*; *Taraxacum* sect. *Ruderalia*; *Tripleurospermum inodorum*; *Tussilago farfara*; *Urtica dioica*; *Verbascum thapsus*; *Veronica arvensis*; *V. hederifolia*; *Vicia sepium*; *Vicia tetrasperma*; *Vicia villosa* subsp. *villosa*; *Viola arvensis*; *V. tricolor* subsp. *tricolor*.

ESTIMATES OF THE DOSE OF RADON AND ITS PROGENY INHALED INSIDE BUILDINGS

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ABSTRACT

The concentration of radon in the air in buildings ranged from 1.87 ± 3.24 Bq/m³ to 14.27 ± 1.50 Bq/m³ with a mean of 6.31 ± 3.47 Bq/m³ while that of the progeny of radon varied from 0.007 to 0.057 WL (average: 0.025). The mean indoor concentration of radon was considerably less than the lower levels prescribed by EPA (148 Bq/m³), WHO (100 Bq/m³), EEC (400 Bq/m³), ICRP (200–600 Bq/m³) and NRPB (200 Bq/m³). The annual effective equivalent dose of indoor radon (< 0.8 mSv/y) that the bronchial and pulmonary regions of human lungs are exposed to (<0.8 mSv/y) is less than the UNSCEAR and WHO recommended global lower average dose value of 1 mSv/y. The lifetime fatality risk of exposure to the progeny of radon (PAEC) varied from 0.03×10^{-4} to 0.19×10^{-4}, with an average value of $0.08 \pm 0.04 \times 10^{-4}$.

Keywords: radon, indoor, equilibrium-equivalent radon concentration, equivalent dose, lifetime fatality risk

Introduction

The noble radioactive gas radon tends to migrate readily in air or water in spite of the fact that its relatively short half-life (3.82 days) restricts the time for which it can migrate (Choubey and Ramola 1997). The presence of radon in soil, water and rock has greatly facilitated our ability to identify and predict the occurrence of earthquakes, volcanic activity and fault dislocation. Coincidently, its presence at high levels in indoor air could be a health hazard for humans because it is carcinogenic (Karimdoust and Ardebili 2010) and can cause lung cancer (Folger et al. 1994). Radon and its short-lived decay products (^{218}Po, ^{214}Po and ^{214}Bi etc.) in buildings is the major source of public exposure to natural radioactivity, making up almost 50% of the worldwide mean effective dose (UNSCEAR 2000b; Somlai et al. 2007). Two of the α-emitting daughters of ^{222}Rn (^{218}Po and ^{214}Po) contribute over 90% of the total radiation dose attributable to exposure to radon (Gillmore et al. 2001). When radon decays after inhalation or ingestion, it releases energy that can damage DNA in the cells of sensitive organs like lungs and stomach and can cause cancer. Thus, naturally occurring radon in buildings has been identified as a human lung carcinogen (IARC 1988; WHO 2009) and is considered to be the second leading cause of lung cancer after smoking tobacco (Marley et al. 1998; WHO 2005). Recent studies have also provided information on the risks of exposure to lower levels of radon (Lubin and Boice 1997; NRC 1999; EPA 2004). Further, ICRP (1990) recommends that exposure to high levels of radon should be considered to be an occupational hazard and remedial actions need to be initiated in such situations. In recent years, substantial attention has been paid to radon, particularly the problems of exposure to radon and its progeny in building and dwellings. Measurements of levels of radon in the air in dwellings worldwide have been made and reported (Srivastava 2004; Oufnia et al. 2005; Pauloa et al. 2005). In the open air the concentration of radon gas is very low and does not pose a significant health hazard. However, radon is a problem when released into an enclosed or poorly ventilated spaces like dwellings, buildings, caves and mines, where this gas can accumulate and reach relatively high concentrations and become a health hazard. Emanation and migration of radon and its progenies in an indoor environment (in the earth and atmosphere) has been identified as the main source of the radiation from natural radioactive sources that people are exposed to. Indoor concentrations of radon and its short-lived progeny depend mainly on the entry or production rate from various sources and the ventilation rate. However, the level of radon in an indoor environment can also depend on the nature of building materials, soil, water used for drinking and other domestic features (Sohrabi 1998).

Thus, in the present investigations, a RAD7 radon analyzer system was used to study variation in the level of radon in the staff room of the Department of Environmental Science, Bangalore University, Bangalore. The annual effective dose of radon inhaled by the inhabitants was also calculated in order to determine their exposure dosage to radiation.

Material and methods

RAD7 analyzer is an active, high performance, continuous radon-measuring technique (Fig. 1), which is extensively used because it is rugged and simple to use, produces a long-term integrated read out and is highly

sensitive to alpha-particle radiation. In the present study, the surface deposited/airborne/ambient radon activity inside the staff room was recorded by the RAD7 radon analyzer using a continuous 1-day protocol. Grab sampling may not give an accurate value of the radon levels because radon concentrations change significantly and rapidly. The RAD7 detector collects the α-emitters electrostatically and analyses them spectrally.

Ambient air is sucked in by a pump at a rate of 1 l/min, and passes through a drierite/desiccant and filter prior to entering the solid-state detector, which measures the concentration of radon. The RAD7 determines the concentration of radon in air by detecting the alpha decaying radon progeny, ^{218}Po and ^{214}Po, using a solid-state, passivated ion-implanted planar silicon (PIPS) detector. The radon monitor (RAD7) uses a high electric field above a silicon semi-conductor to attract the positively charged polonium daughters, ^{218}Po$^+$ (t1/2 = 3.1 min; alpha energy = 6.00 meV) and ^{214}Po$^+$(t1/2 = 164 ls; alpha energy = 7.67 meV), which are then used as a measure of the concentration of ^{222}Rn in the air. The concentration of radon was measured every hour for 1 day (24 hours), using the continuous monitoring mode. Finally, ^{222}Rn activities are expressed in terms of Bq/m^3 (disintegrations per second per m^3) with 2σ-uncertainties. One Becquerel corresponds to one radioactive disintegration per second and Becquerels per cubic metre (Bq/m^3) is the unit of expression used to define the concentration of radioactive gases, such as radon.
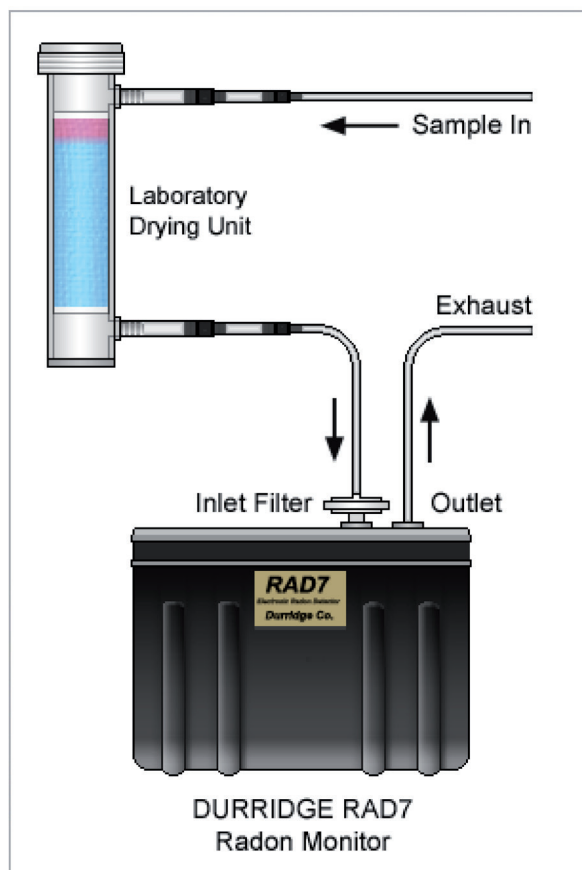

Fig. 1 Experimental set-up for the measurement of radon in air.

There is a desiccant drying tube, containing anhydrous $CaSO_4$, at the interface between the ambient air and the detector, which maintains the relative humidity (RH) of the incoming air below 10% throughout the measurement. If it goes above 10% then desiccant should be replaced, as the sensitivity of the particle detector is lowered significantly when the relative humidity is higher than 10%. Inlet filters at the top of the RAD7 remove the progenies of ^{220}Rn and ^{222}Rn, so that only the concentration of the gas is measured. The detector operates at external relative humidities ranging from 0% to 95% and an internal humidity of 0–10%, with a sensitivity of 4 Bq/m^3 and an upper linear detection limit of 800 kBq/m^3. In other words, the sensitivity of the instrument is 0.8 counts per hour per Bq/m^3 and 0.4 counts per hour per Bq/m^3 in normal and sniff modes, respectively. The dynamic range of the instrument is 4–400,000 Bq/m^3 (0.1–10,000 pCi/l).

Action level for radon inhaled indoors

If the concentration of radon goes above the "Action level" attempts should be made to reduce it because of its adverse effect on human health. In this context, the Environmental Protection Agency (EPA) report that if a person is exposed to an indoor radon level of 4 picocuries per litre (4 pCi/l) or 148 Becquerel per cubic meter (148 Bq/m^3), the probability of developing lung cancer is 13–50 persons per 1000. Furthermore, exposure to a radon level of 20 pCi/L (740 Bq/m^3) is as hazardous as smoking a pack of cigarettes a day. Therefore, EPA recommends a rigid 4 pCi/l (= 148 Bq/m^3) as the action-level for air-borne radon and that a radon action level of lower than this should be maintained in the air in residences (EPA 1986). EPA recommends that it be reduced to a lower level if a home is at or above the threshold value. For conversion, multiply pCi/l (non-SI terminology) by 37 to get Bq/m^3 (SI terminology). WHO suggests that house owners take action when radon levels exceed 100 Bq/m^3, which is a much more conservative figure than the Environmental Protection Agency (EPA) action level of 148 Bq/m^3 (EPA 1991), which has been the USA standard for many years (WHO, 2009). Similarly, the Economic European Community (EEC) has prescribed a level of 400 Bq/m^3 for indoor radon in built dwellings, ICRP (International Commission on Radiological Protection) recommend a range of action levels for radon of 200–600 Bq/m^3 (ICRP 1993b, 1994), NRPB (National Radiological Protection Board, U.K.) a threshold limit value of 200 Bq/m^3 for houses and 400 Bq/m^3 for workplaces (NRPB 1990) and the Irish reference level is 200 Bq/m^3 (ICRP 1993b).

Radon dosimetry / Equivalent dose

Although there are large uncertainties in assessing the dosimetry and epidemiological aspects for converting an exposure to radon to a radon dose (Chen 2005), it is nevertheless essential to be able to estimate the ra-

don dose from the radon concentration because of its harmful effects on the human body. The equilibrium level, time spent indoors and the conversion coefficient (effective dose received by adults per unit ^{222}Rn activity per unit of air volume) of the recorded dose are the major factors determining the level of the dose (ed.) received by bronchial and pulmonary tissues of human lungs. Hence, in the present study we used six different methods to compute the annual effective equivalent dose or inhalation dose attributable to exposure to indoor radon using different radon dose conversion and equilibrium factors.

Method 1:

Concentrations of radon decay products or their equivalent equilibrium concentrations (EECs) can be obtained from radon data using calculation procedure of Kranrod et al., (2009). The concentration of radon recorded indoors (A_{Rn}) in Bq/m^3 can be expressed in terms of equilibrium-equivalent radon concentration (EEC$_{Rn}$) by using relation (1) (Choubey and Ramola 1997) and the equivalent dose received by bronchial and pulmonary tissues in human lungs by using a dose conversion factor (DCF) of 1.0×10^{-5} mSv per Bqh/m^3 (ICRP 1987; Choubey and Ramola 1997) and equilibrium factor (F) of 0.45 in equation (2):

$$EEC_{Rn} = F \times A_{Rn} \quad (1)$$
$$\text{Equivalent dose} = EEC_{Rn} \times DCF \quad (2)$$

Method 2:

Recommendations in the United Nations Scientific Committee's publication on the Effects of Atomic Radiation (UNSCEAR 2000b, 2006; Abd El-Zaher and Fahmi 2008; Abd El-Zaher 2011) can be used to calculate the annual effective dose from exposure to radon. The effective indoor dose (H$_E$) was calculated using a conversion factor of 9.0 nSv/h per Bq/m^3 (UNSCEAR, 2000b), an indoor occupancy factor of 0.8 (Chen and Moir 2010) and an equilibrium factor of ^{222}Rn indoors of 0.4 in equation (3):

$$H_E = C_{Rn} \times F \times T \times DCF \quad (3)$$

where
H_E = effective indoor dose rate in mSv/y,
C_{Rn} = is the arithmetic mean radon concentration in Bq/m^3,
F = 0.4 is the typical recommended equilibrium factor value used for indoor radon (ICRP 1993b, 1994; UNSCEAR 1999),
T = Indoor occupancy time of 7000 h or 80% of office occupancy (0.8 × 24 h × 365.25 = 7012.8 h/y).
DCF = a recommended value of 9 nSv (Bqm^{-3} h)$^{-1}$ or 9 nSv/Bqhm^{-3} or 9.0×10^{-6} mSv/h per Bq/m^3 was used to convert radon equilibrium-equivalent concentration to population effective dose (UNSCEAR 2000b; Chen and Moir 2010) as it lies between the dosimetric and

epidemiological dose conversions (IARC 1988; UNSCEAR 2000b; WHO 2009).

Method 3:

Method 3 involved estimating the annual effective dose (Dy) due to a particular concentration of radon using equation (4) or (5) (Örgün et al. 2008; Ali Asghar Mowlavi et al. 2012):

$$Dy = E_f \times C_f \times O_f \times Q_{Rn} \times T \quad (4)$$

or

$$ED(mSv/y) = A_c \times D_f \times O_f \times E_f \times 24\,\text{h} \times 365 \times 10^{-6} \quad (5)$$

where
E_f = 0.4, indoor radon decay product equilibrium factor,
C_f or D_f = 9.0 nSv/Bq h m^{-3}, radon effective dose coefficient factor,
O_f = 0.8, indoor occupancy factor, which is the fraction of the time people spend indoors – it means, during a year ($T = 365 \times 24$ h) people spend about 7008 h indoors in homes and offices,
Q_{Rn} or A_C = the radon concentration in Bq/m^3.

Method 4:

The effective equivalent dose due to exposure to indoor radon can be expressed in two different ways as indicated by equation (6) and (7) (Martinez et al. 1998). In addition, equation (6) in terms of the equilibrium factor (F) can also be expressed by equation (7) (Farid 1993, 1995):

$$H_E = C_{Rn}d_0 + d_1 C_E \quad (6)$$
$$H_E = C_{Rn}(d_0 + d_1 F) \quad (7)$$

where
C_E is equivalent concentration of radon in Bq/m^3,
F = 0.4, the typical value of the equilibrium factor used for indoor radon,
d_0 and d_1 = the effective dose equivalent conversion factors for radon and radon progeny, respectively.
The recommended values (Mauricio et al. 1985; WHO 1988; Planinic and Faj 1989, 1990; Faj and Planinic, 1991;) are d$_o$ = 0.33 μSv y^{-1}/Bq m^{-3} and d$_1$ = 80 μSv y^{-1}/Bq m^{-3}. In the present study equation (7) was used.

Method 5:

The CEC (1990) recommends that a conversion ratio of 1 Bq/m^3 of ^{222}Rn corresponding to an effective equivalent dose of 0.05 mSv/y can be used to calculate the effective equivalent dose (Maged 2009).

Method 6:

It is possible to estimate the effective dose (mSv/y) due to the inhalation of radon and its progeny by using a conversion coefficient of 9 nSv/Bq h m^{-3}, equilibrium factor of 0.6, outdoor occupancy factor of 1760 h and a dose coefficient for radon dissolved in blood of

0.17 nSv/Bq h m^{-3} in equation (8) (UNSCEAR 2000a; Shashikumar et al. 2009).

$$Dose = (0.17 + 9 \times 0.6)C_R \times 1760 \times 10^{-6} \quad (8)$$

where
C_R = is the arithmetic mean radon concentration in Bq/m^3.

Dosimetry of radon progeny / daughters

The exposure to radon radiation is normally expressed as working level (WL), which is the total energy of α-radiation radiated, when radon and radon progeny reach radioactive equilibrium, provided the radon concentration in the air is 100 pCi/l. In other words, working level is a measure of the concentration of radon progeny, based on the pooled average concentration of radon and can be calculated using the reverse-variance-weighted method for determining the expected exposure to radon in various indoor environments (Bodansky 1989). In the present study, the indoor radon concentration was converted into the equilibrium equivalent concentration (EEC), which was further converted into radon progeny or potential alpha energy concentration (PAEC) using formulae (9) (Shashikumar et al. 2009), (10) (Örgün et al. 2008) or (11) (ICRP 1993a; Upadhyay et al. 2007). Further, the WLM (working level month, WLM/y) is calculated assuming 80% occupancy, which is equivalent to WL times 40:

$$R_N(mWL) = \frac{C_R \times F_R}{3.7} \quad (9)$$

or

$$PAEC(WL) = \frac{A_C \times E_F}{3700} \quad (10)$$

or

$$C_R(Bq/m3) = \frac{PAEC(WL) \times 3700}{F} \quad (11)$$

where
R_N or $PAEC$ = radon progeny concentration (WL or mWL),
F_R or E_f or F = the equilibrium factor between indoor radon and radon progeny,
A_C or C_R = mean concentration of radon gas (Bq m^{-3}).

The annual effective dose due to exposure to radon (^{222}Rn) and its progeny, and average lifetime fatality risk were determined using generic relations (ICRP 1993b; Sannappa et al. 2003). ICRP assumes 80% indoor occupancy (7000 h/year) and an indoor equilibrium factor of 0.4 between radon and its decay products for dwellings, the annual exposure at home to radon progeny per unit radon concentration of 1.56×10^{-2} mJ h m^{-3} per Bq m^{-3} and effective dose per unit exposure at home to radon

progeny of 1.1 mSv (mJ h m^{-3}). Under these circumstances, a radon concentration of 1 Bq m^{-3} corresponds to an annual effective dose of 1.716×10^{-2} mSv. One WLM corresponds to the exposure of an individual to radon progeny of 1 WL concentration (2.08×10^{-2} mJ m^{-3}) for 170 h, which results in 1 WLM being equivalent to 3.54 mJ hm^{-3}. In a home with a PAEC of 1mWL, the annual exposure in WLM is ($365 \times 24 \times 0.8/170 \times 1000$) = 0.0412 WLM. Hence, the dose in terms of working level per month (WLM) can be calculated. The WLM was then converted into annual effective dose by using dose conversion factors: the radon daughter dose conversion factor (ICRP 1993a) for members of the public is 3.88 mSv per WLM (~3.9 mSV per WLM). The lifetime risk associated with exposure to indoor radon was calculated using 1 WLM = 10×10^{-6} cases/year. If the risk persists for 30 years, the lifetime fatality risk is 3×10^{-4} cases/WLM (Nikolaev and Ilic 1999). Hence, the conversion factors of 3×10^{-4} per WLM and 3.88 mSv per WLM (ICRP 1993b) were used to estimate the lifetime fatality risk and the annual effective dose, respectively.

Results and discussion

Temporal variation in the concentration of indoor radon and their respective equivalent dose were obtained using six different methods. The potential alpha energy concentration (PAEC), annual exposure, lifetime fatality risks and annual effective dose due to exposure to radiation from radon progeny are discussed below. Concentration of indoor radon ranged from 1.87 to 14.27 Bq/m^3 of air with an average value of 6.31 ± 3.2 Bq/m^3 over a period of 24 hours. The mean indoor concentration of radon (and in turn short-lived radon progeny) recorded was considerably less than the action levels prescribed by EPA (i.e., 148 Bq/m^3), WHO (i.e., 100 Bq/m^3), EEC (i.e., 400 Bq/m^3), ICRP (200–600 Bq/m^3) and NRPB (200 Bq/m^3).

The equilibrium-equivalent concentration of radon (EEC$_{Rn}$) varied from 0.84 to 6.42 Bqh/m^3 with a mean value of 2.84 ± 1.42 Bqh/m^3. The estimate of equivalent dose using Method 1 yielded an effective equivalent dose ranging from 0.074 to 0.562 mSv/y with an average value of 0.249 ± 0.125 mSv/y, Method 2 an effective equivalent dose that varied from 0.047 to 0.360 mSv/y, with an average value of 0.159 ± 0.080 mSv/y, Method 3 an effective equivalent dose that varied from 0.038 to 0.288 mSv/y, with an average value of 0.127 ± 0.064 mSv/y and Method 4 an effective equivalent dose that ranged from 0.061 to 0.461 mSv/y, with an average value of 0.204 ± 0.102 mSv/y. In contrast, Method 5 gave an effective equivalent dose that ranged between 0.094 and 0.713 mSv/y, with an average value of 0.315 ± 0.158 mSv/y and Method 6 an effective equivalent dose that ranged between 0.018 and 0.140 mSv/y, with an average value of 0.062 ± 0.031 mSv/y (Fig. 2A). It is evident from Fig. 2B that the calculated annual effective equivalent dose due to exposure to indoor
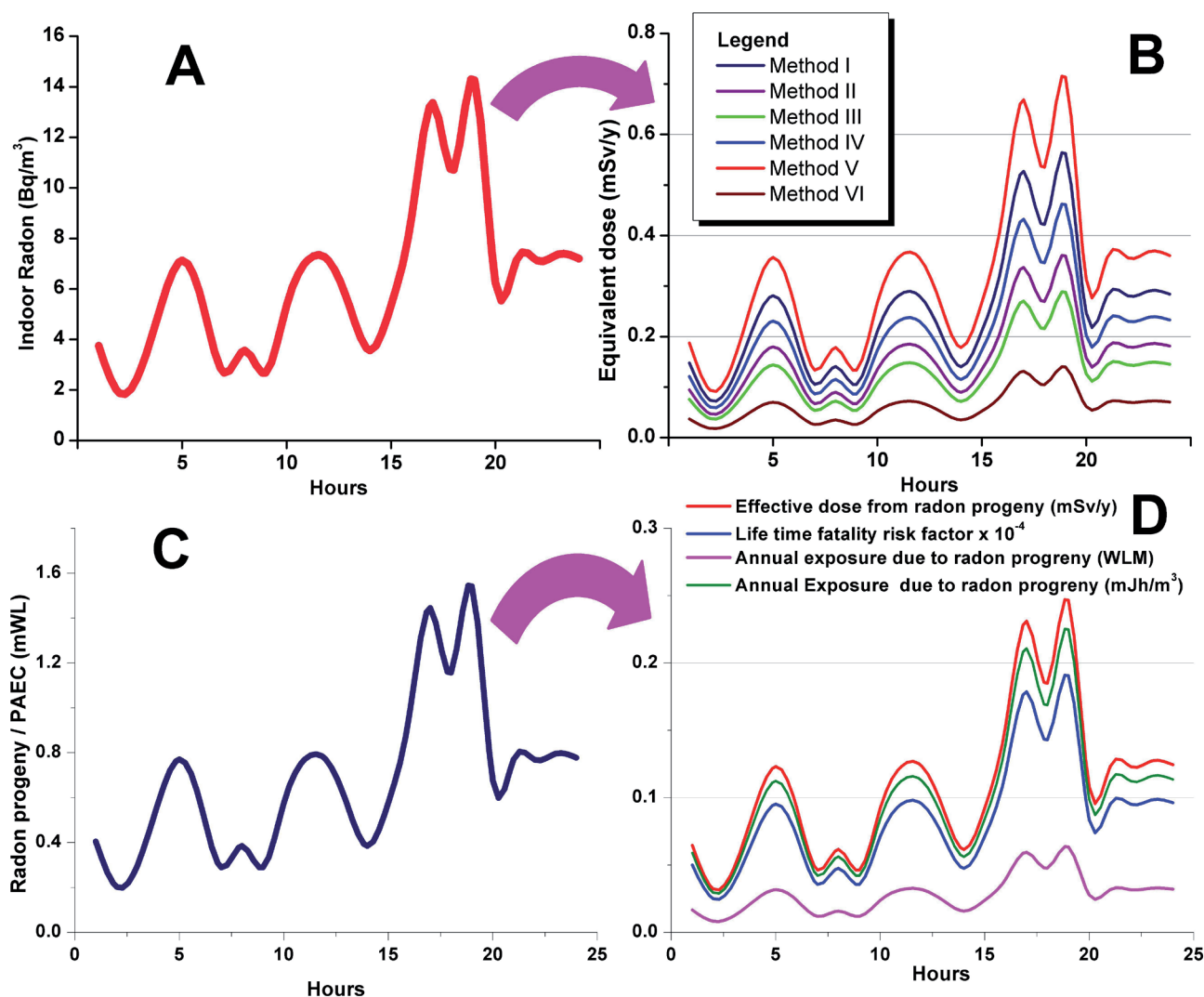
Fig. 2 Temporal variation in (A) the concentration of indoor radon, (B) equivalent dose due to exposure to radon, (C) PAEC or concentration of radon progeny and (D) annual exposure, life time fatality risk and annual effective dose due to exposure to radiation from radon progeny.

radon using all six different methods is below 0.8 mSv/y, which is less than the recommended/estimated global average dose from inhaling radon from all sources, which is approximately 1 mSv/y (WHO 1993; UNSCEAR 2000b;), and is slightly less than half the total natural exposure to radiation of 2.4 mSv/y (UNSCEAR 1988). The estimated annual effective equivalent dose corresponding to the concentration of radon measured was also less than the recommended action level of between 3–10 mSv/y (ICRP 1993b).

Results of this comparative account of the concentration of radon indoors and the corresponding equivalent dose of radiation along with the results obtained by other investigators are presented in Table 2. It is evident that the level of radon recorded indoors and the respective equivalent/inhalation doses are within permissible limits and those recorded in the present study are considerably less than those recorded by other investigators in different parts of the world. Choubey and Ramola (1997) state that radon entering through joints and cracks in buildings increases the indoor concentration of radon.

However, in houses/rooms built of brick and cement, the building materials contain very little uranium and coating the floor with cement reduces the entry of radon into the room from the earth's crust. Abd El-Zaher and Fahmi (2008) conclude that the concentration of radon in kitchens and bathrooms is relatively high compared to that in other rooms in the same dwelling and suggest that it can easily be reduced in these rooms by improving the ventilation. Cheol Min Lee et al. (2012) are of the opinion that the concentrations of radon recorded in these rooms are higher than in other indoor environments, where residents tend to spend most time. Although kitchens and bathrooms are constructed mainly of the same building materials (concrete and cement blocks), the materials used for lining these compartments differ from those used in rooms in the same apartment. Kenawy et al. (2000) state that ceramic materials are a potential source of radon, which mainly results from the decay of thorium and uranium in these materials, which are extensively used instead of the traditional painting materials used in living room and bedrooms (Songül and Güler 1999).

Table 2 A comparison of the concentration of radon and their corresponding equivalent doses recorded indoors on the campus of Bangalore University and those recorded by other investigators in different parts of the world.

Area	^{222}Rn concentration (Bq/m³)			Effective Equivalent dose (mSv/y)				Source
	Min	Max	Mean	Method	Min	Max	Mean	
Bhilangana Valley, Garhwal Himalaya, India	95	208	–	1	3.8	8.1	–	Choubey and Ramola (1997)
Alexandria, Egypt	50.93	105.36	–	2	1.26	2.63	–	Abd El-Zaher and Fahmi (2008)
Dwellings in different areas of Alexandria	36	53	44 ± 16	–	0.61	0.9	0.75	Abd-Elzaher (2012)
Mashhad, Iran	12.3	135.2	–	3	0.25	3.78	–	Ali Asghar Mowlavi et al. (2012)
Ezine (Çanakkale, Turkey)	9	300	67.9	3	0.4	5.2	–	Örgün et al. (2008)
Korea	–	–	50.17 ± 4.08	–	–	–	0.870	Cheol Min Lee et al. (2012)
In a house in Rajshahi, Bangladesh	3.3	37.8	–	4	0.120	1.313	0.516	Farid (1993)
Dwellings in Bangladesh	8.0	46.0	–	4	0.546	1.633	1.195	
Metropolitan zone of Mexico City	0.1*	32.25*	–	4	0.458	0.709	–	Martinez et al. (1998)
Dwellings on the campus of Kuwait University	8.7	23.4	14.8 ± 4.6	5	0.44	1.17	0.74 ± 0.23	Maged (2009)
Mysore, India	16.95	52.85	31.25	6	0.17	0.52	0.31	Shashikumar et al. (2009)
Pune	9.40	28.5	–	6	0.09	0.28	–	Nagaraja et al. (2006)
Kastamonu, Turkey	29	177	–	–	0.73	4.46	–	Kam and Bozkurt (2007)
Campus of Bangalore University	1.87	14.27	6.31 ± 3.16	1 2 3 4 5 6	0.074 0.047 0.038 0.061 0.094 0.018	0.562 0.360 0.288 0.461 0.713 0.140	0.249 0.159 0.127 0.204 0.315 0.062	Present study

Note: * values in pCi/l

Another factor determining the high levels of radon and exhalation rates recorded in these compartments is that the narrow openings into these rooms means they are relatively poorly ventilated. Burning natural gas in houses (Karpinska et al. 2004) and supplying kitchens and bathrooms with water from underground sources are both potential sources of indoor radon (Sujo et al. 2004).

The concentration of radon progeny ranged from 0.20 ± 0.35 to 1.54 ± 0.16 mWL (mean: 0.68 ± 0.34 mWL) (Fig. 2C). The annual exposure of occupants of dwellings in the study area to radiation from radon daughters varied from 0.030 mJh m⁻³ (0.008 WLM) to 0.225 mJh m⁻³ (0.064 WLM) with an average value of 0.099 mJh m⁻³ (0.028 WLM). The life time fatality risk and annual dose radiation from radon progeny (PAEC) varied from 0.03×10^{-4} to 0.19×10^{-4} (average: $0.08 \pm 0.04 \times 10^{-4}$) and 0.032 ± 0.056 to 0.247 ± 0.026 mSv/y (mean 0.109 ± 0.055 mSv/y), respectively (Fig. 2D). The concentration of radon progeny and monthly level of exposure were considerably less than those reported by Upadhyay et al. (2007), Örgün et al. (2008), Shashikumar et al. (2009), Abd-Elzaher (2012) and Oni et al. (2012), etc. Örgün et al. (2008) records PAEC values of 1.6 to 22.5 mWL and monthly exposure values of 0.1 to 0.9 WLM y⁻¹, while Shashikumar et al., (2009) record concentrations of radon progeny varying from 0.09 mWL to 3.92 mWL.

Abd-Elzahfer (2012) reports that the annual exposure of occupants in all areas he studied varied from 0.56 mJ h m⁻³ (0.15 WLM) in the region of Elmandara to 0.82 mJ h m⁻³ (0.23 WLM) in the region of Kingmaryut. The lifetime fatality risk and the annual effective dose of radiation received by occupants of offices range from 0.86×10^{-5} to 1.09×10^{-5} and 0.11 to 0.18 mSv/y, respectively (Oni et al. 2012). Upadhyay et al. (2007) record the concentration of radon daughters varying from 0.84 to 6.38 mWL, the annual exposure to radiation from radon daughters varying from 0.036 to 0.273 WLM, the life time fatality risk varying from 0.11×10^{-4} to 0.82×10^{-4} and the annual effective dose of radiation from radon (PAEC) varying from 0.14 to 1.06 mSv/y.

Conclusion

Radon is a naturally occurring, hazardous, radioactive pollutant that is always present in our surroundings and is one of the causes of lung cancer. It is evident that the indoor radon concentrations we recorded are considerably less than those reported by investigators in other parts of the world. Even the risk of a lifetime exposure to indoor radon in the study area is very low and the occupants of these dwellings are therefore, relatively safe. Proper regulatory standards, like natural and forced ven-

tilation, should be implemented in order to make dwellings more clean and safe. It is also should be kept in mind that only the atmosphere of a single closed staff room was monitored in this study. Therefore, a more extensive survey is required before drawing any definitive conclusions about the general indoor levels of radon and the dosages people are exposed to in buildings on the Bangalore University campus.

Acknowledgements

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WORLD-WIDE DISTRIBUTION OF THE BRYOZOAN *PECTINATELLA MAGNIFICA* (LEIDY 1851)

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ABSTRACT

Pectinatella magnifica (Leidy 1851) is an invasive freshwater colonial animal belonging to the phylum Bryozoa. It is native to the area east of the Mississippi River, from Ontario to Florida. Currently it occurs throughout North America and the first record for it outside that continent was for Bille near Hamburg in 1883. Later, it was found in the Elbe (Havel by Spandau), in Tegeler See, a pond in Wroclaw and in Silesia and Brandenburg. In addition, floatoblasts of *P. magnifica* were found in the upper Elbe in Germany in the 1950s. Then, *P. magnifica* spread to the area of Spandau in Berlin and the Oder, and Wroclaw. It is also recorded in Romania and Turkey. In France, it was recorded occurring in the area called Franche-Comté in 1994. Its occurrence in the Netherlands was first reported in 2003 and then each following year. The newest discoveries are for the Rhine basin in the area between Luxembourg and Germany. Recently, it was also recorded in the Czech Republic and Austria. Besides Europe and North America, it is also recorded in Japan and Korea. The statoblasts of *P. magnifica* are spread by flowing water, zoochory and probably also by anthropochory.

Keywords: Europe, lake, North America, *Pectinatella magnifica*, river

Characteristics of *Pectinatella magnifica*

Pectinatella magnifica (Leidy 1851) is an invasive organism belonging to the phylum Bryozoa (Carroget et al. 2005). Bryozoans (syn. Ectoprocta, Polyzoa) are a group of tiny colonial invertebrate animals. They often occur in epibenthos and littoral bioceonoses (Rogick 1934; Rogick 1957; Bushnell et al. 1987; Ricciardi and Lewis 1991). Most species live in marine environments. According to the literature there are 94 freshwater species (Massard and Geimer 2008).

A colony is formed by zooids, each consisting of two parts: a body wall called cystid and polypide, wherein the viscera are located. Body cavities of the zooids in a colony interconnect with one another and individuality is suppressed. The exterior of the cystid consists of a layer of secretion, which can be e.g. chitin or a jelly-like substance. Colonies grow by budding and can reach sizes of from a few millimetres to several kilograms (Wood 1989). They are filter feeders, which feed mainly on plankton and detritus (Wood 2001) that they collect by means of a retractile ring of ciliated tentacles called a lophophore (Riisgård et al. 2004).

Leidy found and described *Pectinatella magnifica* in 1851 near Philadelphia. He placed it in the genus *Cristatella* and called this new species *Cristatella magnifica*. However, he soon discovered that this new species differs from others in the genus *Cristatella* and established the new genus for this species: *Pectinatella* (Opravilová 2005).

Origin of *Pectinatella magnifica*

Research on the freshwater bryozoan *Pectinatella magnifica* started in the early 20th century, (e.g. Davenport 1904; Wilcox 1906; Braem 1911). Jullien (1885), who recorded the occurrence of *P. magnifica* in America in the Mississippi River in 1885, ignored the fact that it occurred in the Hamburg area and described it as species endemic to America. Loppens (1908; 1910) records *P. magnifica* occurring in America and Europe, but did not comment on this fact. Davenport (1904) also records its occurrence outside America, e.g. in Germany (Borodin 1928). Most authors claim that *P. magnifica* comes from North America (e.g. Jullien 1885), although Borg (1930) does not exclude the possibility that it might have a cosmopolitan distribution. Lacourt (1968) thinks that its assumed import into Europe and Asia is uncertain.

Kraepelin (1884; 1887) and Kafka (1887) did not doubt that *Pectinatella magnifica* originated from North America. They describe it as an unarctic species, which was accidentally introduced into Europe. At first it occurred only in the area of Hamburg. Hartmeyer (1909), who recorded its spread out of this area and records it in the Havel and Oder rivers, shared this opinion. Historical analyses of the records of this species in Europe indicate that the hypothesis of accidental introduction is the most probable and coherent, followed by spreading along navigable watercourses. Massard and Geimer (2002) also record the occurrence of the family *Pectinatellidae* in four zoogeographic areas – Palaearctic, Unarctic, Neotropic and Oriental (*i.e. Pectinatella magnifica* – Palaearctic: European part and part of Asia, Nearctic, Neotropic and Oriental). It has not been recorded in tropical Africa, Australasia or on Oceanic Islands in the Pacific (Rogick and Schalie 1950).

Worldwide distribution

According to many authors *Pectinatella magnifica* is an autochthonous species characteristic of lentic areas, such as sluggish rivers and outflows from lakes in the eastern and partly also the central part of North America, roughly east of the Mississippi River, from Ontario to Florida. Occurrence outside this area is very likely only a result of it being introduced. It now appears to be well established all across North America, except in areas with a cold climate (Rogick and Schalie 1950; Lacourt 1968; Everitt 1975; Rodriguez and Vergon 2002).

There is a single record of statoblasts of *Pectinatella magnifica* in Guatemala (Lacourt 1968) and colonies in Japan (Mawatari 1973; Oda 1974; Rodriguez and Vergon 2002) and Korea (Seo 1998; Rodriguez and Vergon 2002).

Occurrence in the Southern Hemisphere is so far unknown.

P. magnifica was recorded in Rattlesnake Creek, a few miles from Buffalo (Kellicott 1882), the Mississippi River in 1885 (Jullien 1885) and in other states of the USA on the north-eastern coast of the Atlantic Ocean: Massachusetts, Maine and Mississippi (Kraepelin 1887). It was recorded in lakes near Coldspring Harbor in New York State in 1898 (Davenport 1898). Subsequently it was found in a relatively wide area in eastern USA. According to Davenport (1904) it now occurs from the Great Lakes on the border with Canada to Florida and from the Mississippi to the Atlantic Ocean.

Dendy (1963) states that *P. magnifica* is occasionally an abundant species in the Alabama ponds area and other studies indicate it also occurs in Ohio (Wood 1989), Michigan (Bushnell 1965), north-western Louisiana (Everitt 1975), Texas and the north-western Pacific area (Neck and Fullington 1983; Wood 2001). It is also recorded in north–western Indiana (Barnes and Lauer 2003).

Currently (from the 1990s), this bryozoan is reported from lakes along the north-western coast of USA in the states Michigan, Massachusetts, Pennsylvania, and Canada (Opravilová 2005).

Occurrence in Europe

The first record of this species outside North America was from Bille near Hamburg in 1883 (Zimmer 1906; Bernauer and Jansen 2006). Later it was recorded in the Elbe (Havel by Spandau) and the river basin of the Elbe (Tegeler See by Berlin) (Hrabě 1935).

It is also recorded as occurring in a pond of the zoological institute in Wroclaw, which is connected with the Oder River and in Silesia and Brandenburg (Hrabě 1935).

Floatoblasts of *P. magnifica* were recorded in the upper Elbe in Germany in the 1950s by Kothé (1961). That is, this species occurs in the Elbe and Havel in the area of Spandau in Berlin and the Oder near Wroclaw in Poland.

It is not easy to explain its occurrence in Romania and Turkey but it might indicate that this species is spreading into central and south-eastern Europe. It is interesting that it does not appear to be spreading in the opposite direction, into Western Europe. It is clear that the Mittelland canal, which connects the Elbe, Wesser, Ems and Rhine, does not provide a system of rivers and canals flowing to the east (Massard and Geimer 2002). Studies of d'Hondt and Condé (1996) and Notteghem (1999) did not explain how *Pectinatella magnifica* got to France. It probably first occurred in the canal de l'Est (Territoire de Belfort) and spread there from the east (d'Hondt and Condé 1996). Its presence in this area was confirmed in 1994 (Haute-Saone canal-Territoire Belfore) (Notteghem 1999). This canal follows the Rhone-Rhine canal, connecting the Rhine and the Elbe. Colonies of *P. magnifica* were discovered in an area called Franche-Comté in 1994. Gradual spread was later recorded in Franche-Comté and surrounding regions (Rodriguez and Vergon 2002). It was found in a pond situated in the valley of the Moselle in France in 1995, first near Nomexy and later near Chatel-sur-Moselle in a slowly flowing part of the Moselle (d'Hondt and Condé 1996). It was recorded in the Oder between Küstrin and Hohensaaten in 1996 (Tittizer et al. 2000), by Bru in 1997 in the Meurthe basin, in a pond situated in the Monseigneur (a tributary of Mortagne) and in a pond situated in another tributary of the Mortagne in 1999 (Notteghem 1999). This author also records many colonies in Lake Sorme (Burgundy) in 1998. There are other reports of it occurring in four different hydro geographic areas, the Rhone, Saone and Rhone, Loire and Siena. Its occurrence near Nennig probably relates to previous occurrences in France, from where it could have been transported by the Mosselle (Germany-Luxembourg part). This canal was opened in 1964 (Massard and Geimer 2002).

Occurrence of *P. magnifica* in the Netherlands was reported first in April 2003 (Massard and Geimer 2002) and then each following year. However, it is not recorded as occurring in Luxembourg and neighbouring regions of Belgium, France and Germany (Geimer and Massard 1986) or in the Rhine (Franz 1992). Tittizer et al. (2000) also concludes that it is unlikely that the Rhine was the route via which *P. magnifica* spread into France (Massard and Geimer 2002). Instead, these authors hypothesize that its introduction into this area was via commercial fishing or ship transport. If this is correct, *Pectinatella* was introduced into the Rhine via French canals (Rhone-Rhine, Marne-Rhine (the upper Rhine) or the Moselle (the middle Rhine).

The most recent records of this species of bryozoan are for the Rhine basin, in the area between Luxembourg and Germany (Massard and Geimer 2002) and in France. It is reported in the following areas of France: Vosges in north-eastern France in 1996 and Belfort in the east and upper Saona (Rodriguez and Vergon 2002). Grabow 2005 records it occurring in the summer of 2003 in a former

gravel pit connected to the Rhine, in Baden-Wuertemberg (Germany).

In summary, apart from its occurrence in USA and Canada *P. magnifica* is recorded occurring in Western and Central Europe (France, Luxembourg, Germany, Poland, Czech Republic and Austria (Kraepelin 1887; Lacourt 1968; Rodriguez and Vergon 2002; Devin et al. 2005; Bauer et al. 2010; Balounová et al. 2011), Romania (Lacourt 1968), Corsica (Notteghem 2009) and Asia Minor (Lacourt 1968).

Currently *P. magnifica* is spreading in France, Germany and the Czech Republic (Rodriguez and Vergon 2002; Bauer et al. 2010; Balounová et al. 2011).

Mode of dispersal

There is little information on the mode of dispersal of *P. magnifica*. The spread in slowly flowing streams is certainly significantly determined by the flow of the water (Rodriguez and Vergon 2002). According to Oda (1974) this species is also spread by zoochory (dispersal of statoblasts on feathers of birds). Dispersal by water animals and transport with fish (fingerling) in aquaculture is also probable (Osburn 1921; Seo 1998).

This species could have been spread from North America into Europe by shipping (Lacourt 1968). Another means of anthropochory might be the transport of fishing equipment (Seo 1998).

However, *P. magnifica* is mainly thought to be dispersed by transport along rivers on boats and between water reservoirs by water birds. The big anchors on the statoblasts enable them to attach to different objects in the water, such as ships and row-boats, and the feathers of birds. It is also possible that statoblasts of this species are spread in the droppings of birds because their viability is not changed by digestion (Opravilová 2005). It is recorded that 38% of the statoblasts of this species passed through the digestive system of *Anas platyrhynchos* unharmed (Brown 1933).

Statoblasts have been recorded in the stomach contents of fish, in particular young fish of *Micropterus salmoides*, *Pomoxis annularis*, *Lepomis pallidus* and *Dorosoma capedianum* (Osburn 1921; Brown 1933). Notteghem (1999) states that it is likely that *P. magnifica* is spread by introducing young fish into rivers and ponds. The spread of this species towards the west from western Texas occurred when water canals were constructed (Neck and Fullington 1983). (This spread was probably facilitated by a combination of transport by water birds and man.)

Conclusion

Pectinatella magnifica is a native of the area east of the Mississippi River, from Ontario to Florida. Currently it occurs throughout Northern America. It was first recorded outside North America in the River Bille near Hamburg in 1883. In Europe it currently occurs mainly in the basins of the Elbe, Oder and Rhine rivers in Germany, Poland, France (including Corsica), Czech Republic, Netherlands, Austria and Rumania. It is currently spreading in France, Germany and the Czech Republic. In addition to Europe it is also in Japan and Korea.

The above summary indicates that the spread of *P. magnifica* is not a local phenomenon, but has occurred in several areas simultaneously. The records indicate it is spreading mainly towards the east. It occurs regularly in Western Europe where its spread is restricted to local occupation of other suitable locations. Central Europe can be considered to be the area where this species first colonized Europe and the boundaries of its current distribution there are the basins of the Elbe and Vltava rivers with local intrusions into the Morava River basin. East and South Eastern Europe from Slovakia are considered to be potential areas for further spread.

It is assumed that anthropogenic factors such as shipping, construction of dams, connecting channels, exploitation of resources (e.g. gravel and sand) in swampy areas and the associated transport of mining equipment, tourism, recreation, water sports, etc. have contributed considerably to the spread of this species. A certain proportion of the spread of *Pectinatella magnifica* can be attributed to the natural dispersal of statoblasts, particularly by water birds. This may also be facilitated by human activities such as the construction of water works, which provide habitats for aquatic birds, including resting places for migratory birds. Due to the occurrence of *Pectinatella magnifica* in mainly relatively oligotrophic to mesotrophic waters, often used in different ways for recreational purposes, it is also likely that statoblasts are dispersed by these activities.

Acknowledgements

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ASSOCIATION BETWEEN PATTERNS IN AGRICULTURAL LANDSCAPES AND THE ABUNDANCE OF WHEAT APHIDS AND THEIR NATURAL ENEMIES

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ABSTRACT

Effect of different landscape patterns on insect distribution and diversity was determined by studying wheat fields in complex and simple agricultural landscapes. We studied the influence of simple and complex agricultural landscapes on wheat aphids and their natural enemies in terms of the time of migration, abundance, population growth rate of the aphids and parasitoid abundance. The results indicate that the diversity of natural enemies is greater in the complex agricultural landscape and the effect of natural enemies on the abundance of wheat aphids was greater in the complex non-crop habitat. Wheat aphid hyperparasitoid populations differed in different agricultural landscapes with a greater number of parasites in complex agricultural landscapes. Resident times of predatory natural enemies differ in simple and complex agricultural landscapes. The number and types of predatory natural enemies are higher in complex than simple agricultural landscapes. Aphid population growth rates and the maximum population densities of wheat aphids differed significantly in simple and complex landscapes. Maximum population densities of different wheat aphids were very different in simple and complex landscape structures. The population growth rates and maximum population densities of the different predatory natural enemies and hyperparasitoids differed greatly.

Keywords: landscape pattern, natural enemy, population dynamics, wheat aphid

Introduction

Landscapes are composed of various types of habitats that could potentially determine the abundance of natural enemies (Bianchi et al. 2008). Natural enemies have the potential to control many insect pests and prevent outbreaks of insects in forests and agro-ecosystems (DeBach and Rosen 1991) and ultimately contribute to a reduction in the use of pesticides and their adverse effects on the environment (Naylor and Ehrlich 1997). In all agricultural landscapes, there are habitats that provide alternative food sources, hibernation sites and hosts or prey for natural enemies (Landis et al. 2000) and therefore the composition of landscapes has strong effect on the numerical responses of natural enemies (predators and parasitoids) (Elliott et al. 2001). The mechanism that results in species responding differently to landscapes composition is not the same for all species. In order to understand the ecology of landscape one needs to understand the spatial and temporal dynamics of organism (Wu and Hobbs 2002). Activity of insect pests can be affected by many aspects of landscape composition (Tscharntke and Brandl 2004; Hassan et al. 2012) and movement of species in landscapes is determined by habitat specificity and dispersal capacity (Roland and Taylor 1997; Wanger and Edwards 2001). Thus, the composition of landscapes is important in determining the movement of insect pests and natural enemies in a landscape.

The pattern of agricultural landscapes greatly influences insects and their relationship to predators and parasites. There is little research on the effect landscape patterns on insect communities and interspecies relationships (Vollhardt et al. 2008) but recently it has become an important topic for ecologists (Andrén 1994; Boutin et al. 2002; Haila 2002; Baguette et al. 2003; Dauber et al. 2003). This study included both heterogeneous and homogeneous landscapes with a gradient from simple agricultural landscapes in which most of the land was cultivated (89.3% arable) to complex agricultural landscapes in which a high percentage of the land was covered with non-crop habitats (39.6% arable). In order to determine the relationships between wheat aphids and their natural enemies in simple and complex agricultural landscapes on the Yinchuang plain, we studied the distribution of the natural enemies of wheat aphids in different agricultural landscapes (Table 1). We propose to test the following hypotheses: 1) the complexity of the structure of a landscape and diversity of natural enemies of wheat aphids are positively correlated – the more complex the structure of the landscape, the greater the diversity of natural enemies. 2) structure of the landscape affects migration of winged wheat aphids and the numbers of parasites and predators recorded in different agricultural landscapes. This paper explores the effect of landscape on the numbers of natural enemies of wheat aphids.

Table 1 Species composition of wheat aphids, parasitoids, hyperparasitoids and predators collected in spring wheat fields and reared in the laboratory.

Pest group	Species	Complex agricultural landscape (CAL)	Simple agricultural landscape (SAL)
Insects	*Macrosiphum avenae*	1757 ± 235	2376 ± 428
	Schizaphis graminum	1318 ± 152	1790 ± 192
	Rhopalosiphum padii	416 ± 75	598 ± 83
Parasitoids	*Aphidius avenae*	311 ± 36	447 ± 51
	A. gifuensis	147 ± 35	214 ± 44
	A. sichuanensis	15 ± 4	8 ± 3
	Trioxys asiaticus	7 ± 2	8 ± 3
	Lysiphlebus confusus	4 ± 2	9 ± 3
	Praon volucre	6 ± 3	9 ± 4
	P. rhopalosiphum	3 ± 1	6 ± 2
	Tetrastichus sp.	2 ± 1	8 ± 3
Hyperparasitoids	*Asaphes vulgaris*	49 ± 17	35 ± 13
	Asaphes suspensus	78 ± 28	68 ± 23
	Pachyneuron aphidis	170 ± 39	119 ± 34
	Aphidencyrtus aphidivorus	13 ± 2	7 ± 3
	Dendrocecrus carpenter	9 ± 3	8 ± 3
	Alloxysta sp. 1	175 ± 42	131 ± 36
Predators	*Hippodamia tredecimpunctata*	8 ± 3	5 ± 2
	Hippodamia variegata	6 ± 2	11 ± 4
	Coccinella septempunctata	2 ± 1	5 ± 2
	Harmonia axyridis	3 ± 1	4 ± 2
	Propylea japonica	12 ± 4	7 ± 3
	Chrysopa sinica	7 ± 3	8 ± 3
	C. formosa	4 ± 1	6 ± 2
	Sympetrum croceolum	3 ± 1	2 ± 1
	Chlaenius pallipes	19 ± 8	59 ± 15
	Pterostichus gebleri	4 ± 1	13 ± 3
	Cymindis binotata	3 ± 1	21 ± 6
	Cymindis daimio	4 ± 1	19 ± 5
	Calosma maderae	5 ± 2	13 ± 4
	Scarites terricola	6 ± 1	18 ± 3
	Harpalus crates	9 ± 2	6 ± 4
	Harpalus salinus	7 ± 1	4 ± 2
	Staphylinus maxillosus	17 ± 5	21 ± 7
	Erigonidium graminicolum	19 ± 6	27 ± 8
	Pardosa astrigera	23 ± 8	39 ± 12
	Lycisa coelestris	10 ± 3	13 ± 4
	Theridionocto macutatum	5 ± 2	9 ± 3
	Misumenops tricuspidatus	11 ± 3	19 ± 5
	Pardosa laura	8 ± 3	5 ± 2
	Tetragnatha shikokiana	9 ± 3	7 ± 2
	Xysticus ephippiatus	18 ± 5	12 ± 4
	Erigone prominens	14 ± 5	11 ± 2
	Agelena opulenta	8 ± 2	3 ± 1
	Scaeva selenitica	3 ± 1	5 ± 2
	Syrphus corollae	9 ± 3	21 ± 4
	Syrphus nitens	8 ± 3	19 ± 5

Materials and Methods

Study region and experimental design

The study was conducted in Yinchuan (38°26′05N, 106°22′04E) in Ningxia Province, PR China. The areas studied were assigned to the following categories of landscape: (1) complex agriculture (CAL hereafter) or highly heterogeneous agricultural areas, (2) simple agriculture (SAL hereafter) or homogenous agricultural areas (Zhao Zihua 2010). There were three study areas: I – Xixia army horse ranch in Yinchuan, Ningxia (complex landscape); II – Zhangzheng Bridge in Xingqing district (complex landscape); III – Zhangzheng town in Xingqing district (simple landscape). In all the regions studied no pesticides were applied and they were managed according to the recommended production technology for that area and crop. They were studied from May to July in 2009, 2010 and 2011. Wheat aphids generally tend to stay and increase in numbers in a field before flying away. A five-point (East, South, West, North and Center) sampling grid was established, depending on the local characteristics. A total of 70 wheat fields of different sizes in different landscapes were studied.

Wheat aphid populations recorded in different periods

Three important factors in the population dynamics of aphids were recorded on different dates throughout the sampling period in 2009, 2010 and 2011: (1) appearance in the fields (10 April – 15 May), (2) period of population growth (16–30 May), and (3) peak numbers (30 May –

20 June). The aphids and natural enemies were counted at intervals of 10 days from 15th April to 20th June.

Monitoring of arthropods / Insect Sampling

Method used to survey wheat aphids: At each point on the grid 100 representative wheat plants were randomly selected. Each of these plants was examined over a period of 15–20 min and any *M. avenae*, *S. graminum* or *R. padi* and wingless aphids present on the plants were recorded thereby generating five sets of data for the grid (Table 2).

Method used to survey parasitoids: Using the grid described above, 100 wheat plants were randomly selected and visually examined for 15–20 min. The number of mummified aphids, *S. graminum* and *R. padi* and wingless aphids were counted. Mummified aphids were taken to the laboratory and placed in a Petri dish labeled with the date of collection and a sample code number and kept under the following conditions (16 : 8 L : D, 20 ± 1 °C, RH = 65 ± 3%) in an incubator. Over a period of more than 40 days we recorded whether parasitoids had emerged from mummies every day at 5:00 PM. These parasitoids and the aphid mummies were stored in 90% alcohol prior to identification.

Net method: Predators were surveyed using the same checkerboard 5 point random sampling grid described above. Each position on the grid was swept 10 times. Ten adult insects from each sweep of the net were collected and together with debris placed in a poison bottle, with a total of five bottles collected at each position. All adult

Table 2 Sampling parameters used in agricultural landscape patterns.

Sample parameters	Order	Family	Species
Aphids	Homoptera	Aphidinea	*Macrosiphum avenae* (F.)
	Homoptera	Aphidinea	*Schizaphis graminum* (Rond)
	Homoptera	Aphidinea	*Rhopalosiphum padi* (L.)
Primary Parasitoids	Hymenoptera	Aphididae	*Aphidius avenae*
	Hymenoptera	Aphididae	*A. sichuanensis*
	Hymenoptera	Braconidae	*A. gifuensis*
	Hymenoptera	Aphididae	*Lysiphlebus confusus*
	Hymenoptera	Aphididae	*Praon volucre*
Secondary Parasitoids	Hymenoptera	Charipidae	*Alloxysta* sp.
	Hymenoptera	Pteromalidae	*Pachyneuron aphidis* (Bouche)
	Hymenoptera	Pteromalidae	*Asaphes suspensus* Nees
	Hymenoptera	Pteromalidae	*Asaphes vulgaris* Walker
	Hymenoptera	Megaspilidae	*Dendrocerus carpenteri* (Curtis)
Predators	Coleoptera	Coccinellidae	*Hippodamia tredecimpunctata* (Say)
	Coleoptera	Coccinellidae	*Hippodamia variegata* (Goeze)
	Diptera	Syrphidae	*Metasyrphus corollae* Matsumura
	Coleoptera	Carabidae	*Chlaenius pallipes* Gebler
	Neuroptera	Chrysopidae	*Chrysopa intima*
	Araneae	Lycosidae	*Pardosa astrigera* Koch
	Hemiptera	Miridae	*Deraeocoris punctulatus* (Fallen)

specimens brought back to the laboratory were identified to species. Any nymphs collected were taken back to the laboratory and reared to the adult stage and identified, and the numbers of each of the species noted.

Trap method: Beetles and spiders in the wheat fields were captured on the ground and in the soil. Disposable plastic cups (height 9 cm diameter 7.5 cm) were used as traps. Five sampling points were established in each plot and 5 cups placed at each sampling point so that in each plot there were 25 cups. The attractant placed in the cups was a 2 : 1 : 1 : 20 mixture of vinegar, sugar, methanol and water by weight. Each cup contained 40~60 ml of this mixture. The cups were inspected every six days when all the arthropods were removed and taken back to the laboratory for identification and the attractant in the cup was replaced and the trap reset.

The large soil animals were hand-sorted and placed in 75% alcohol. Small soil animals were extracted from the soil using a Tullgren funnel (2 mm standard sieve) and the specimens collected and sorted using a zoom microscope and preserved in 75% alcohol prior to identification.

Statistical analysis

The numbers of cereal aphids and their natural enemies were subjected to one way analysis of variance (ANOVA) using statistical software (SAS institute Inc., 2006). Means of numbers of cereal aphids and their natural enemies were compared using LSD and a 5% level of significance.

Results

The effect of agricultural landscape on the time of the colonization of wheat field by aphids and their natural enemies

Parasitoids: The parasitoids were first recorded in the different agricultural landscape at about the same time.

The first record of *A. avenae* in fields in the complex agricultural landscape was 26 April and 10 days later in the simple agricultural landscape (Table 3). The other parasitic wasps also occurred later in the simple landscape except for *L. confusus,* which occurred there earlier. Significantly more parasitic wasps were recorded in the complex agricultural landscape and only *L. confusus* was more abundant in the simple landscape. The total number of parasitoids in the complex landscape was significantly greater than in the simple landscape (Table 3).

Hyperparasitoids: All species of hyperparasitoids were recorded a few days earlier in the complex landscape (Table 4). The number of individuals of each hyperparasitoid recorded varied. The numbers of *P. aphidis*, *A. suspensus* and *A. vulgaris* were significantly greater in the complex landscape. But the number of *D. carpenteri* was significantly higher in the simple landscape and that of *Alloxysta* sp was not significantly different in the two landscapes (Table 4). The total number of hyperparasitoids was significantly higher in the complex landscape.

Predators: The first predators were recorded at different times in the two agricultural landscapes. The predators were all first recorded during the period when the aphids first arrived in the fields (10 April to 15 May). Predators were first recorded later in the simple landscape, except for *H. tredecimpunctata* (Table 5). *C. pallipes*, *P. astrigera* and aphids were all recorded on the first day the fields were sampled in both the complex and simple landscapes and it is possible that they overwintered in the wheat fields.

The numbers of all the species of predators were significantly greater in the complex landscape (Table 5). The total number of species of predators was significantly greater in the complex landscape.

Table 3 Effects of different agricultural landscapes on the time of the appearance and numbers of parasitoids in wheat fields. The aphids were first recorded 10 April – 15 May.

Parasitoid species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Aphidius avenae	Time of appearance	26 April	16 April
	Numbers	0.46 ± 0.28a	0.32 ± 0.22b
A. gifuensis	Time of appearance	10 May	5 May
	Numbers	0.56 ± 0.27a	0.36 ± 0.18b
A. sichuanensis	Time of appearance	13 May	10 May
	Numbers	0.23 ± 0.11a	0.16 ± 0.17b
Lysiphlebus confusus	Time of appearance	30 April	8 May
	Numbers	0.08 ± 0.05a	0.11 ± 0.08b
Praon volucre	Time of appearance	13 May	8 May
	Numbers	0.14 ± 0.07a	0.12 ± 0.14b
Total parasitoids	Time of appearance	26 April	16 April
	Numbers	1.47 ± 0.53a	1.07 ± 0.42b

Table 4 Effects of different agricultural landscapes on the time of the appearance and numbers of hyperparasitoids in wheat fields. Hyperparasitoids were first recorded 10 April – 15 May.

Hyperparasitoid species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Alloxysta sp.	Time of appearance	8 May	15 May
	Numbers	0.59 ± 0.24a	0.33 ± 0.18a
Pachyneuron aphidis	Time of appearance	2 May	10 May
	Numbers	0.88 ± 0.25a	0.46 ± 0.16b
Asaphes suspensus	Time of appearance	2 May	10 May
	Numbers	0.51 ± 0.13a	0.29 ± 0.07b
Asaphes vulgaris	Time of appearance	10 May	15 May
	Numbers	0.26 ± 0.11a	0.19 ± 0.07b
Dendrocerus carpenteri	Time of appearance	15 May	20 May
	Numbers	0.11 ± 0.06a	0.28 ± 0.10b
Total hyperparasitoids	Time of appearance	2 May	10 May
	Numbers	2.35 ± 0.41a	1.55 ± 0.32b

Effects of the structure of the landscape on the population growth rate and maximum population density of wheat aphids and their natural enemies

Aphids: The population growth rate and maximum population density of *M. avenae* was 6 and 2 times greater, respectively, in the simple than in the complex landscape (Table 6). For *S. graminum* the population growth rates were significantly greater in the complex landscape, but the maximum population density they achieved was significantly greater in the simple landscape. *R. padi* achieved significantly higher population growth rates and maximum population densities in the complex landscape. The averages of the population growth rates and maximum population densities of all the species of aphids were significantly higher in the complex landscape.

Parasitoids: There were no significant differences in population growth rates or maximum population densities of parasitoids in the complex and simple landscapes (Table 7) except for *A. avenae*, which achieved significantly higher population densities ($F = 36.26$, df = 14, $p = 0.0001$) in the complex landscape and *P. volucre* with significantly higher population growth rates ($F = 28.86$, the df = 14, $p = 0.0001$) in the simple landscape. Total population growth rates were not significantly different in the two landscapes but the maximum population density was significantly greater ($F = 18.62$, df = 14, $p = 0.026$) in the complex landscape.

Table 5 Effects of different agricultural landscapes on the time of the appearance time and numbers of predators in wheat fields. Aphids first recorded, 10 April – 15 May.

Predator species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Hippodamia tredecimpunctata	Time of appearance	26 April	16 April
	Numbers	0.93 ± 0.41a	0.41 ± 0.26b
Hippodamia variegata	Time of appearance	2 May	10 May
	Numbers	0.41 ± 0.19a	0.33 ± 0.19a
Metasyrphus corollae	Time of appearance	2 May	10 May
	Numbers	0.79 ± 0.26a	0.31 ± 0.14b
Chlaenius pallipes	Time of appearance	10 April	10 April
	Numbers	5.62 ± 3.19a	1.62 ± 1.27b
Chrysopa intima	Time of appearance	30 April	8 May
	Numbers	0.52 ± 0.37a	0.23 ± 0.12a
Pardosa astrigera	Time of appearance	10 April	10 April
	Numbers	3.18 ± 0.93a	1.86 ± 0.53b
Deraeocoris punctulatus	Time of appearance	26 April	5 May
	Numbers	1.09 ± 0.61a	0.88 ± 0.37a
Total predators	Time of appearance	10 May	10 May
	Numbers	11.54 ± 3.62a	5.64 ± 1.21b

Table 6 Effects of the structure of the landscape on the population growth rate and maximum population density of wheat aphids.

Aphid species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Macrosiphum avenae	Growth rate	29.13 ± 4.76b	4.845 ± 0.95a
	Max population density	286.42 ± 49.32b	144.82 ± 26.33a
Schizaphis graminum	Growth rate	53.25 ± 15.32b	30.21 ± 16.42a
	Max population density	319.32 ± 57.32a	396.54 ± 36.91b
Rhopalosiphum padi	Growth rate	43.43 ± 25.32a	25.62 ± 9.35a
	Max population density	252.83 ± 45.32b	136.56 ± 28.32a
Total wheat aphids	Growth rate	39.43 ± 11.84b	13.73 ± 7.49a
	Max population density	821.65 ± 66.56b	677.81 ± 32.98a

Table 7 Effects of different agricultural landscapes on the population growth rate and maximum population density of parasitoids in wheat fields.

Parasitoid species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Aphidius avenae	Growth rate	18.63 ± 3.62	21.65 ± 6.93
	Max population density	139.62 ± 32.63a	96.83 ± 21.83b
A. gifuensis	Growth rate	11.26 ± 2.93	13.52 ± 4.83
	Max population density	62.53 ± 18.63	46.83 ± 15.81
A. sichuanensis	Growth rate	9.63 ± 2.83	7.82 ± 1.99
	Max population density	1.26 ± 0.28	1.13 ± 0.19
Lysiphlebus confusus	Growth rate	7.26 ± 2.92	9.36 ± 3.62
	Max population density	0.92 ± 0.19	0.83 ± 0.21
Praon volucre	Growth rate	3.25 ± 1.16b	5.36 ± 1.83a
	Max population density	1.08 ± 0.31	1.24 ± 0.418
Total parasitoids	Growth rate	10.01 ± 3.63	11.71 ± 4.82
	Max population density	205.41 ± 43.26a	146.86 ± 31.63b

Table 8 Effects of different agricultural landscapes on the population growth rate and maximum population density of hyperparasitoids in wheat fields.

Hyperparasitoid species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Alloxysta sp.	Growth rate	21.38 ± 6.83	16.89 ± 5.88
	Max population density	42.29 ± 15.62	35.09 ± 11.24
Pachyneuron aphidis	Growth rate	13.62 ± 4.26	9.39 ± 3.19
	Max population density	39.95 ± 14.59	35.26 ± 16.93
Asaphes suspensus	Growth rate	11.22 ± 3.13	8.92 ± 2.12
	Max population density	26.38 ± 8.69	22.83 ± 7.93
Asaphes vulgaris	Growth rate	7.26 ± 2.92	9.36 ± 3.62
	Max population density	18.62 ± 5.93	26.83 ± 7.63
Dendrocerus carpenteri	Growth rate	9.63 ± 3.61	8.92 ± 4.62
	Max population density	4.68 ± 1.36	3.26 ± 1.06
Total hyperparasitoids	Growth rate	12.62 ± 4.12	10.68 ± 3.99
	Max population density	132.92 ± 49.83	122.27 ± 38.89

Hyperparasitoids: There were no significant differences in population growth rates or maximum population densities of hyperparasitoids in the complex and simple landscapes (Table 8). In total there were five main species of hyper–parasitoids the population growth rates and the maximum population densities of which were greater in the complex than the simple agricultural landscape. They were: *Alloxysta sp.* (21.38 ± 6.83 vs. 16.89 ± 5.88, 42.29 ± 15.62 vs. 35.09 ± 11.24), *Pachyneuron aphidis* (13.62 ± 4.26 vs. 9.39 ± 3.19, 39.95 ± 14.59 vs. 35.26 ± 16.93), *Asaphes suspensu*s (11.22 ± 3.13 vs. 8.92 ± 2.12, 26.38 ± 8.69 vs. 22.83 ± 7.93), *Dendrocerus carpenteri* (9.63 ± 3.61 vs. 8.92 ± 4.62, 4.68 ± 1.36 vs. 3.26 ± 1.06), but the differences are not significant. Only the population growth rates (7.26 ± 2.92 vs. 9.36 ± 3.62) and max population density (18.62 ± 5.93 vs. 26.83 ± 7.63) of *Pachyneuron* were greater in the complex landscape, but the differences ($F = 5.19$, df = 14, $p = 0.062$, table 9) are not significant.

Table 9 Effects of different agricultural landscapes on the population growth rate and maximum population density of predators in wheat fields.

Predator species		Complex agricultural landscape (CAL) ± SE	Simple agricultural landscape(SAL) ± SE
Hippodamia tredecimpunctata	Growth rate	7.26 ± 2.39	5.36 ± 2.03
	Max population density	10.67 ± 3.60	6.91 ± 2.38
Hippodamia varie-gata	Growth rate	6.45 ± 2.31	5.26 ± 1.96
	Max population density	13.83 ± 3.66	11.22 ± 4.06
Metasyrphus corollae	Growth rate	8.63 ± 4.26a	3.19 ± 1.01b
	Max population density	1.31 ± 0.34a	0.68 ± 0.14b
Chlaenius pallipes	Growth rate	0.87 ± 0.22a	0.43 ± 0.11b
	Max population density	4.38 ± 1.09a	1.01 ± 0.16b
Chrysopa intima	Growth rate	14.09 ± 5.21a	8.67 ± 2.34b
	Max population density	7.63 ± 2.26a	5.29 ± 3.01b
Pardosa astrigera	Growth rate	1.26 ± 0.41	1.52 ± 0.71
	Max population density	5.66 ± 2.36	6.29 ± 3.21
Deraeocoris punctulatus	Growth rate	3.62 ± 1.21	2.38 ± 0.92
	Max population density	2.69 ± 0.88	2.37 ± 0.79
Total predators	Growth rate	5.88 ± 1.88a	3.83 ± 1.36b
	Max population density	46.17 ± 15.26	33.77 ± 10.89

Predators: The population growth rates and maximum population densities of the 7 species of predators differed in the complex and simple landscapes (Table 9). In the cases of *H. tredecimpunctata*, *H. variegata*, *P. astrigera* and *D. punctulatus*, however, the differences were not significant, but for the other three species both the population growth rates and maximum population densities differed significantly. For *M. corollae*, *C. pallipes* and *C. intima* the population growth rates ($F = 19.69$, df = 14, $p = 0.029$; $F = 16.82$, df = 14, $p = 0.034$; $F = 13.62$, df = 14, $p = 0.038$) and population densities ($F = 23.92$ df = 14, $p = 0.021$; $F = 39.95$, df = 14, $p = 0.001$; $F = 12.66$, df = 14, $p = 0.041$) were both significantly greater in the complex landscape.. Both the total population growth rate and the maximum population density of predators was significantly higher in the complex than in the simple landscape ($F = 22.69$, df = 14, $p = 0.23$; $F = 28.91$, df = 14, $p = 0.018$).

Discussion

Population dynamics of wheat aphids in different landscapes

There is no need to control wheat aphids in northern China, because the wheat aphid populations that occur there are relatively low. The number of alatae recorded in the two landscapes differed. Although the numbers of alatae recorded in the complex landscape was significantly lower than the simple landscape, the population growth rate of the aphids was considerably different possibly because of the high degree of fragmentation of the complex landscape, which makes it more difficult for natural enemies to find prey. But wheat aphids need to be controlled in the south of China.

Wheat aphid abundance was not affected by the structure of the landscape, which is consistent with the findings of Costamagna et al.'s (2004) study on *Pseudaletia unipuncta*. Here we think it is likely that wheat aphid abundance is not affected by the complexity of the structure of the landscape but by the degree of fragmentation of host plant habitats in the landscape.

Percentage parasitism

The difference in percentage parasitism recorded in complex and simple landscapes was very slight. This may be attributable to the following: (1) landscape structure affects the richness and diversity of parasitic wasps but not in the area studied. (2) non-crop habitats limits the richness and transfer of parasitic wasps. (3) difference in the population dynamics of the parasitic wasps and aphids. The little difference in the percentage parasitism in the two landscapes may because the population density of the host in the two landscapes is as Costamagna et al. (2004) and Zhao et al. (2012) have shown very similar and host density is usually the main factor determining percentage parasitism. The richness and diversity of parasitic wasps increased with host density with a higher diversity in the simple landscape, which is what theory predicts. A high percentage of grassland in a landscape is associated with an increased number of predatory natural enemies and increase in the control of the wheat aphid population. An increase in the complexity of the structure of the landscape is also associated with an increase in the diversity of predatory natural enemies. Therefore, the design of agricultural landscapes should take into consideration the need to maintain species diversity. At the landscape scale, increasing the proportion of grassland in an agricultural landscape can also strengthen the role of the natural enemies

in biological control by maintaining a higher diversity of natural enemies. Designing landscapes that facilitate biological control by increasing the efficiency of natural enemies and reducing the colonization of crops by pests is ultimately a reasonable use of resources. Mosaic landscapes should include grassland, woodland, wetland, buildings, roads etc. We should make full use of these resources to enhance biological control and the value of ecosystem services. Landscape planning may be the most effective means of increasing the numbers of natural enemies, especially in agricultural landscapes.

The diversity of parasitoids and variation in percentage parasitism

The diversity parasitoids and percentage parasitism of aphids in a complex landscape were lower than those in a simple landscape. In the complex landscape the habitat fragmentation index was 1.54 times greater than in the simple landscape. Habitat fragmentation reduces the effectiveness of the foraging behaviour of natural enemies of pests (Landis et al. 2000). It also affects the searching behaviour of the pests and determines to some extent when and the numbers of aphids that colonize the fields. There are many predators and parasitoids of aphids in wheat fields. In the complex landscape the rapid growth of the wheat aphid populations may be because the habitat fragmentation there reduced the efficiency with which the natural enemies were able to find and consume aphids. In the complex landscape studied the population growth rates of the three species of wheat aphids were higher than in the simple landscape.

Conclusion

In order to determine the effect of landscape structure on the population dynamics of wheat aphids we need to study in greater detail the following aspects: (1) the relationship between the complexity of the landscape and the ability of wheat aphids to locate their host plants and their subsequent population growth rate, (2) the question, whether the greater habitat fragmentation of complex landscapes adversely affects the foraging for aphids of predators and parasitoids and (3) which structures of complex landscapes affect the foraging for aphids by predators and parasitoids.

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Disclosure

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MATING PATTERNS IN THE APHIDOPHAGOUS LADYBIRD, *HIPPODAMIA VARIEGATA*, DEPEND ON BODY SIZE

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ABSTRACT

Body size dependent mating patterns were investigated in an aphidophagous ladybird, *Hippodamia variegata* (Goeze), which was reared on mustard aphid, *Lipaphis erysimi* (Kalt.). Both males and females of varying body sizes were used to test the hypothesis that *bigger is better*. The costs of copulation on the life history traits of the mating partners were determined. Pairs of virgin beetles were allowed to mate once under controlled conditions and the duration of copulation of heavy beetles (504.00 ± 45.93 min) lasted significantly longer for than that of light beetles (270.00 ± 26.67 min). Body size was significantly positively correlated with duration of oviposition, fecundity and egg viability. When light individuals mated with heavy partners, copulation lasted longer between heavy males and light females (483.00 ± 54.73 min) than between light males and heavy females (378.20 ± 83.03 min). These results support the hypothesis that males determine the duration of copulation and that the reproductive success of large males is greater than that of small males. The longevity of heavy males was significantly shorter (37.40 ± 1.50 days) than that of light males (53.10 ± 2.84 days). This difference in the longevity of beetles of different sizes could contribute to the significant variation body size in *H. variegata* recorded both in the field and stock cultures, where light males outnumber heavy ones. Thus, although large males have a reproductive advantage over small males, other factors, such as reduced longevity, may constrain the evolution of even larger males.

Key words: reproduction, fecundity, mating duration, aphid, evolution, copulation

Introduction

Body size affects behaviour and performance of individuals in most animal species (Filin and Ovadia 2007). This could be true of insects that vary greatly in body size, especially in the same gender. Individual variation in body size can be an indicator of individual differences in competitive ability (Filin and Ovadia 2007). Large body size in females in most insects could be a result of natural selection maximizing fecundity, which results in female-biased sexual size dimorphism (Honek 1993; Head 1995). Such females are competitively superior, acquire more resources and are more fecund (Wall and Begon 1987; Belovsky et al. 1996). It is interesting to note that the frequency of small males is greater than that of large males in wild populations (Pervez, unpubl. data). Thus there is a significant variation in the body sizes of both genders. In predaceous ladybirds (Coleoptera: Coccinellidae) body size dependent sexual dimorphism is recorded in many species (Kawauchi 1979; Hodek and Honěk 1996).

In life-history theory body size is directly associated with reproductive effort and performance (Roff 1992). This theory predicts that reproductive effort should increase as life expectancy decreases (Roff 1992; Stearns 1992). However, there is little empirical evidence in support of this hypothesis. Thus, a study is needed that conclusively shows that life expectancy decreases with increase in reproductive activity. The cost associated with increased reproductive activity can be gender unbiased, or only one gender bears this cost. Fecundity advantage hypothesis states that egg production in most insects is positively correlated with body weight or size (Leather 1988; Heliovaara et al. 1990; Honěk 1993; Preziosi et al. 1996; Tammaru et al. 1996; Honěk et al. 2008). Hence, big females have a greater fecundity and reproductive activity than small females. Based on the above hypothesis the expectation is that life expectancy will decrease with fecundity, which needs to be addressed. Previous studies indicate that duration of mating affects fecundity and fertility (Obata 1987; Obata and Johki 1991; Omkar and Pervez 2005). Hence, in order to address the problem of body size dependent reproduction, duration of mating also needs to be taken into consideration.

Hippodamia variegata (Goeze) is an aphidophagous ladybird, which occurs in the hilly regions of North India and was used in the present study as an experimental model for addressing the above problem. It is a Palaearctic ladybird, which has successfully established itself worldwide (Natskova 1973; Belikova and Kosaev 1985; Gumovskaya 1985; Gordon 1987; Nicoli et al. 1995; Krafsur et al. 1996; Wheeler and Stoops 1996). There are a few studies on its predation potential (Fan and Zhao 1988; Obrycki and Orr 1990; Kalushkov et al. 1991; Sadeghi and Esmailli 1992; Singh and Singh 1994), but little is known about mating in this species. Female *H. variegata* mated only once can lay large numbers of eggs (Pervez unpublished data). However, its reproductive capacity is greatly enhanced if it mates more than once (Pervez and Maurice 2011). In this paper we determine whether

body size affects the duration of copulation in this species of ladybird and if so which gender is most affected? Additionally, we also tried to determine whether there is a trade-off between life expectancy and reproduction and its evolutionary significance.

Materials and methods

Stock maintenance

Adults of *H. variegata* were collected from fields of *Brassica campestris* L. plants that were heavily infested with the aphid, *Lipaphis erysimi* (Kalt.) at Chamoli, North India, and brought to the laboratory. They were paired in Petri dishes (2 × 9 cm diameter) containing an *ad libitum* supply of *L. erysimi* on twigs of mustard (*B. campestris*) plant and kept in an Environmental Test Chamber (*REMI Instruments*, India) at 25 ± 2 °C, 65 ± 5% R.H and 12L : 12D. The Petri dishes were checked daily for eggs, which were collected and monitored to determine how long it took then to hatch. After hatching, the neonates were transferred to muslin-covered beakers containing an *ad libitum* supply of aphids and reared to the adult stage. The emerging F_1 adults were sexed by carefully examining their genitalia under a stereoscopic trinocular (*Model SZB-46*) connected to a personal computer. These adults were then kept in two groups, *viz.* heavy (males with body sizes ranging from 8 to 11 mg and females ranging from 14 to 16 mg) and light individuals (males with body sizes ranging from 5 to 7 mg and females ranging from 10 to 13 mg).

Reproductive parameters of heavy and light beetles

Ten-day-old virgin pair of ladybirds were kept together in a Petri dish (size and prey as above) and allowed to mate once. After mating, they were isolated and both genders were kept under close observation until they died. The duration of copulation, pre-oviposition period, oviposition period, fecundity, % egg viability, male longevity and female longevity were recorded for each pair. The above data were subjected to a one-way ANOVA and the means compared using a Tukey's Honest Test of significance. Pearson's correlation coefficients of the

relationship between mating duration, fecundity, % egg viability and longevity, and body size in terms of weight were calculated using statistical software, SAS. The relationships between the different parameters and body size were subjected to linear regression analysis using SAS.

Results

The results revealed significant associations between body size and all the reproductive parameters studied (Table 1). The longest duration of copulation was recorded between heavy males and heavy females (504.00 ± 45.93 min). This was significantly longer than that recorded for light males and females (270.00 ± 26.67 min). When light individuals were mated with heavy partners, the duration of copulation of heavy males with light females (483.00 ± 54.73 min) was longer than that of light males and heavy females (378.20 ± 83.03 min). There were significant positive correlations between oviposition period ($r = 0.56$; $p < 0.001$), fecundity ($r = 0.50$; $p < 0.001$) and female longevity ($r = 0.40$; $p < 0.01$), and female body size. There were significant positive correlations between mating duration ($r = 0.48$; $p < 0.01$), percentage egg viability ($r = 0.46$; $p < 0.01$) and fecundity ($r = 0.58$; $p < 0.001$), and male body size. Oviposition period, fecundity and percentage egg viability were significantly greater for females that copulated with heavy than light males.

The reproductive advantage of heavy males resulted in a trade-off in longevity. Heavy males had a significantly shorter life (37.40 ± 1.50 days) than light males (53.10 ± 2.84 days). Male longevity was significantly negatively correlated with their body size ($r = −0.45$; $p < 0.01$). The relationships between mating duration, fecundity and longevity, and body size in terms of weight are presented in Figure (1a–d). This reveals that mating duration was positively correlated with male body weight and fecundity was positively correlated with female body weight (Figure 1a,b). However, male longevity was negatively correlated with duration of copulation (Figure 1c). Similarly, male longevity was negatively correlated with male body weight.

Table 1 The duration of copulation, period of oviposition, fecundity, egg viability and male female longevities recorded for mating pairs of *H. variegata* of different body sizes (HM = Heavy Male, LM = Light Male, HF = Heavy Female, LF = Light Female).

Mating Pair	Duration of copulation (in min)	Oviposition Period (in days)	Fecundity (in eggs)	% Egg Viability	Male longevity (in days)	Female longevity (in days)
HM and HF	504 ± 45.93a	8.00 ± 0.56a	142 ± 15.20a	51.12 ± 2.63a	37.40 ± 1.50b	69.90 ± 2.11a
HM and LF	483 ± 54.73a	5.11 ± 0.58b	94.60 ± 1.19ab	43.73 ± 1.03ab	34.50 ± 1.30b	46.10 ± 2.63c
LM and HF	378.20 ± 26.26ab	4.60 ± 0.72b	85.60 ± 16.69b	40.47 ± 5.33ab	47.10 ± 1.30a	56.20 ± 2.07ab
LM and LF	270.00 ± 26.67b	2.14 ± 0.29c	27.00 ± 2.45c	23.80 ± 6.85b	53.10 ± 8.97a	64.10 ± 1.53ab
F-value	6.96*	18.26*	13.47*	3.55**	8.55*	23.63*

Values are Mean ± SD.
F-values are significant at *P < 0.001 and **P < 0.05; Tukey's test range = 3.80; df = 3, 36.
In each column, means followed by different letters are significantly different.
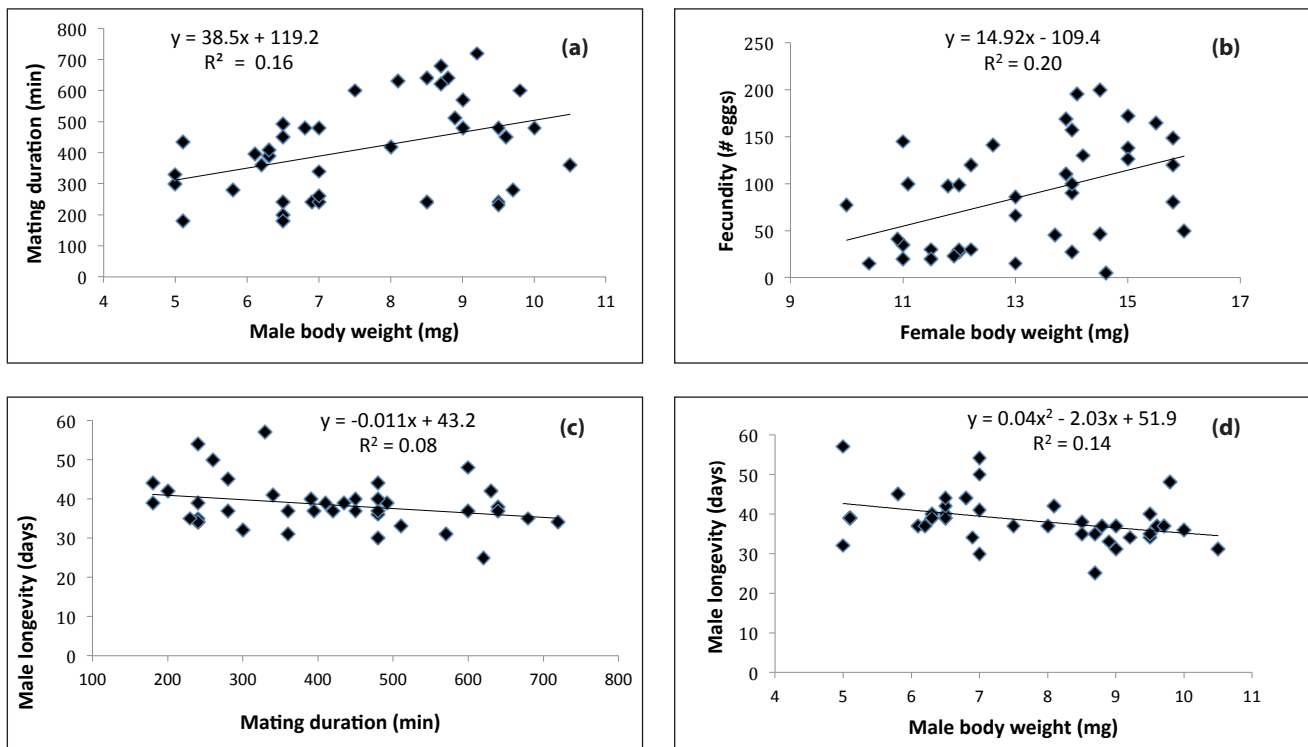
Fig. 1 Relationship between (a) mating duration and male body weight, (b) fecundity and female body weight, (c) male longevity and mating duration, and (d) male longevity and male body weight of *H. variegata* feeding on aphid, *L. erysimi*.

Discussion

It is evident from the results that the body size of adults has a significant role in mating and reproduction. We recorded exceptionally longer durations of mating for *H. variegata* than is recorded for similar sized or even any other ladybirds. Prolonged mating is reported for *P. dissecta*, where it ranges between 176–275 min (Omkar and Pervez 2005). Heavy adults copulated for longer than light adults, which supports our initial hypothesis that bigger is better. Amongst adults, mating with heavy males contributed more in terms of mating duration, which indicates that male body size could be the factor determining the duration of mating. Prolonged mating by heavy males indicates they may ejaculate a greater quantity of sperm (Pervez et al. 2004), which could result in a higher percentage egg viability. Prolonged mating in the ladybird, *Propylea dissecta* (Mulsant) results in a higher percentage of eggs hatching despite the fact that adults mate only once (Pervez 2002).

Mating between heavy males and heavy females resulted in both higher fecundities and egg viabilities compared to those resulting from mating between light males and light females, which again supports the hypothesis that bigger is better. The higher fecundity recorded for light females that copulated with heavy males indicate that the males are providing an excess of non-sperm substances which induce females to lay more eggs. For instance, in many insects, male seminal fluid proteins transferred during a female's first mating stimulate an increase in fecundity and decrease in receptivity to re-mating (Wolfner 2002; Sirat 2011). This provides the first

male to mate with a female with the potential advantage of re-mating with the same partner. This, however, has not yet been recorded in ladybirds.

We recorded a positive relationship between fecundity and body weight. Heavy females were more fecund than light females, which supports the body size-fecundity advantage hypothesis. The fecundity and body size relationship is central to life-history models of age and size at maturity (Kozlowski 1992; Stearns 1992; Roff 2002). The low fecundity recorded in the present study is due to the fact that *L. erysimi* is not a preferred food of *H. variegata* (Omkar and Pervez 2004).

We recorded a significant negative correlation between the longevity of males and their body size, which clearly indicates a trade-off. The longevities of the heavy males were significantly shorter than those of light males. This trade-off, however, was not evident in females indicating that fecundity is not associated with their life expectancy. This is not the case in other insects in which high fecundity is associated with a shorter life expectancy in females (Partridge and Harvey 1985; Hunt et al. 2002). The decrease in male longevity could significantly affect the body size variation in *H. variegata* observed both in the field and stock cultures, as light males outnumber heavy ones (Pervez, unpubl. data). Although the reproductive benefits associated with large body size in males would appear to put them at a selective advantage, other factors such as reduced longevity may constrain further increases in their body size. In addition, large males may experience other costs, e.g. costs of dispersal and physiological maintenance when food is limiting, which are more likely to occur in

wild populations. The cumulative effects of these factors may also constrain the evolution of larger males.

Thus, we conclude that: 1) heavy males mate for longer than light males, 2) females that mate with heavy males have a higher fecundity than those that mate with light males, 3) fecundity is a function of female body size, which supports the body size-fecundity advantage hypothesis, and 4) heavy males have a shorter life span than light males.

Acknowledgements

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DOES *OECEOCLADES MACULATA* (ORCHIDACEAE) REABSORB NECTAR?

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ABSTRACT

Nectar is the most common resource offered by orchid flowers. In some cases, flowers reabsorb nectar as part of a resource-recovery strategy. Nectar is present only in the morning in the widespread orchid *Oececoclades maculata* (Lindl.) Lindl. To determine whether this is due to reabsorption or evaporation of water, the volume of nectar and its concentration in previously bagged flowers were determined throughout the day at two hourly intervals. In addition, the entrance to the nectary of flowers of cultivated plants was obstructed with petroleum jelly in the morning, to prevent the evaporation of water and, in the afternoon, the presence of nectar was recorded. Furthermore, manually self-pollinated flowers, also with the entrance to the nectary obstructed, had their nectary checked 24 hours after pollination to determine whether post-pollination reabsorption occurred. In addition, the period when the pollinators of *O. maculata* foraged for nectar was determined in order to establish whether it was associated with the period when nectar was available. The volume and concentration of nectar in *O. maculata* flowers vary from 0.82 µl (25.10%) between 10–12 h and 0.36 µl (33.73%) between 16–18 h and this difference is caused by evaporation of water. Post-pollination reabsorption does not occur in this species. Pollinators forage most actively between 10–12 h. Thus, *O. maculata* does not reabsorb nectar, but evaporative water loss is a significant factor determining the variation in the volume and concentration of this reward and this is positively correlated with butterfly visitation.

Keywords: Epidendroideae; water evaporation; pollination; *Heliconius*; butterfly; nectar reabsorption

Introduction

Orchid flowers offer different floral resources to pollinators, such as oils, fragrance, pollen, edible trichomes and nectar (van der Pijl and Dodson 1966). Some species do not produce any kind of reward and, in those cases, the pollinators are deceived into visiting the flowers, a very common phenomenon among Orchidaceae (Ackerman 1986; Kindlmann and Roberts 2012). The most common resource offered by orchid flowers is nectar (van der Pijl and Dodson 1966; Arditti 1992; Dressler 1993), which can be produced in many different structures, including spurs (Neiland and Wilcock 1998). The presence of nectar can influence numerous aspects of pollinator behaviour, such as the number of flowers probed per plant and the probing duration, which affects the export and import of pollen (Jersáková and Johnson 2006; Jersáková et al. 2008). In fact, pollinators tend to make more and longer visits to flowers to which additional nectar has been added. However, this can result in higher levels of self-pollination, if it results in more flowers of the same plant being visited (Jersáková and Johnson 2006; Jersáková et al. 2008).

According to Galetto and Bernardello (2004), there is species-specific rhythmicity in nectar secretion and it is important to determine its dynamics throughout the lifespan of a flower in order to understand the plant-animal relationships. The nectar offering strategies of plants in terms of the activity patterns, frequency and diversity of pollinators cannot be understood without considering the dynamics of nectar production (Galetto and Bernardello 2004).

Some of the cases of nectar reabsorption documented for Orchidaceae (Koopowitz and Marchant 1998; Luyt and Johnson 2002) are attributed to saving energy, which the plant could use to invest in fruit development (Koopowitz and Marchant 1998). In these cases the flowers reabsorb nectar after pollination. A decrease in the volume of nectar accompanied by a decrease in sugar concentration indicates nectar reabsorption (Nepi and Stpiczyńska 2008).

Oeceoclades maculata (Lindl.) Lindl. (Orchidaceae: Epidendroideae: Cymbideae: Eulophiinae) is a widespread species that is pollinated by *Heliconius* butterflies, while obtaining the nectar in the spur at the base of the labellum (Aguiar et al. 2012). The nectar is present only in the morning, which indicates that it is probably reabsorbed by the afternoon (Aguiar et al. 2012).

Based on the above evidence, the main objective of this study is to determine whether nectar reabsorption occurs in flowers of a Brazilian population of *Oeceoclades maculata,* as previously suggested by Aguiar et al. (2012). Thus, the volume and concentration of sugar in the nectar of *O. maculata* were recorded throughout the day and a nectar reabsorption experiment was done using petroleum jelly. Furthermore, the timing of nectar production was compared with when butterflies were active during the flowering period of *O. maculata*, to determine if there is positive relation between nectar availability and visits by pollinators.

Materials and methods

Study site

Fieldwork was carried out on the campus of the São Paulo University (FFCLRP-USP), in the municipality of Ribeirão Preto (approximately 21°09′S, 47°51′W), State of São Paulo, southeastern Brazil, during the flowering period of *O. maculata* in 2011 and 2012. The climate there is characterized as 'Cwa' (mesothermic with a dry winter) according to Köppen (1948). The summer is hot and rainy, with mean temperatures above 22 °C and average precipitation greater than 250 mm. The winter is dry, with mean temperatures below 18 °C and average precipitation less than 30 mm in the colder months. Most of this region is covered with mesophytic semideciduous forests. Our study was carried in a natural population located in an anthropogenically-disturbed area, underneath a closed canopy, at approximately 500 m a.s.l. The total area of the campus is approximately 450 ha, but the fieldwork was done in a six ha area in which there were approximately 250 adult individuals of *Oeceoclades maculata*.

Study species

Oeceoclades maculata (Orchidaceae: Epidendroideae: Cymbideae: Eulophiinae) is a widespread terrestrial species, occurring throughout Florida, Panama, West Indies, South America and tropical Africa. Plants are commonly found in disturbed areas of dry, moist and wet forests (Ackerman 1995). In Brazil, the species is widely distributed, occurring in many types of vegetation (E. R. Pansarin, pers. obs. 2010).

According to Pansarin and Pansarin (2010), this species characteristically has oval, laterally compressed and unifoliate pseudobulbs. The leaves are elliptical, green mottled with darker green, coriaceous and erect. The inflorescence is lateral and erect with up to 15 resupinate flowers. The flowers are predominantly pinkish, with sepals connivent with the petals. The labellum is three-lobbed, with a spur at the base, pale-pink with two pink spots internally. Flowers are scentless to humans (Ackerman 1995). A voucher specimen (E. R. Pansarin and F. D. Galli 1280) is deposited in the herbarium of the Universidade de São Paulo (SPFR).

Nectar reabsorption experiments

In order to determine if the reabsorption of nectar in flowers of *Oeceoclades maculata* occurs throughout the day, 21 plants were used. The plants were previously collected in the field and maintained in the Orchidarium LBMBP on the campus of São Paulo University (FFCLRP-USP), in the municipality of Ribeirão Preto, State of São Paulo, southeastern Brazil. On each individual plant, three flowers were sampled. The nectar in the first flower was drained off by 10:00 h in order to confirm the plant was producing nectar. The entrance to the nectary of the second flower was obstructed with petroleum jelly at 10:00 h and the nectar removed by 17:00 h.

The nectar produced by the third flower was measured at 17:00 h and acted as the control.

In addition, 20 plants previously collected and kept in the Orchidarium LBMBP were used to determine whether nectar reabsorption occurred after manual pollination of these plants. On each plant, three flowers were sampled. The nectar of the first flower was collected before 10:00 h to make sure the plant was producing nectar. The second flower was self-pollinated and the entrance to its nectary obstructed with petroleum jelly. The third flower was self-pollinated but the entrance to its nectary was not obstructed. The nectar in the second and third flowers was measured 24 h later.

The volume of nectar collected in all of the experiments was measured using a 10 microliter syringe (Hamilton, NV, USA) (Sazima et al. 2003).

Nectar volume and concentration

In order to determine when *Oeceoclades maculata* produced nectar, 40 inflorescences (40 plants) were previously enclosed in nylon bags in the field. Petroleum jelly was applied to the base of inflorescences to prevent insects from visiting the flowers. Flowers were collected at intervals of two hours: 6–8 h, 8–10 h, 10–12 h, 12–14 h, 14–16 h and 16–18 h. In each interval, 30 flowers were sampled. 30 flowers from 10 plants were used to determine if flowers produced more nectar after it was removed.

Nectar volume and concentration were measured using a 10 microliter syringe (Hamilton, NV, USA) and a hand held refractometer (Eclipse, UK, 0–50%), respectively (Sazima et al. 2003).

Period of activity of pollinators

The observations carried out to determine whether the period when the pollinators of *Oeceoclades maculata* (*Heliconius ethilla narcaea* (Godart 1819) and *H. erato phyllis* (Fabricius 1775; Aguiar et al. 2012)) were active were carried out over total period of 38 h in a natural habitat on sunny days during the flowering period in the 2011. *Oeceoclades maculata* flowers during summer, when on most of the days it rained at the study site in 2011, which made it impossible to sample systematically at selected intervals of the day, as described above. To correct for the effect of differential sampling, the total number of pollinator visits in each interval was divided by the number of times that the respective interval was sampled. Details of the observations are summarized in Table 1.

Data analysis

In order to determine if there is a linear dependence between nectar volume and concentration, a Pearson product-moment correlation coefficient was used. The same analysis was used to verify the existence of linear dependence between nectar volume and visits by pollinators. To determine the effect of time of day on nectar

Table 1 Data on the periods when the visits by pollinators were recorded and the intervals during the course of the day when the volume of nectar volume was measured for the correlation analysis.

Day	Period of observation	Intervals when volume of nectar was measured
March 15, 2011	06:00 h to 15:00 h	06–08h; 08–10h; 10–12h; 12–14h; 14–16h
March 16, 2011	06:00 h to 15:00 h	06–08h; 08–10h; 10–12h; 12–14h; 14–16h
March 19, 2011	09:00 h to 11:00 h	08–10h; 10–12h
March 27, 2011	09:00 h to 18:00 h	08–10h; 10–12h; 12–14h; 14–16h; 16–18h
April 10, 2011	09:00 h to 18:00 h	08–10h; 10–12h; 12–14h; 14–16h; 16–18h

volume and concentration, one-way ANOVA tests were performed. The difference between the volume of nectar in the treated flowers and that in flowers from which the nectar was removed by 10:00 h in the nectar reabsorption experiments was tested for statistical significance using Student's *t*-tests. The data from the same experiments that were not normally distributed were analyzed using the Mann-Whitney *U*-test. IBM® SPSS® version 21 was used to do the statistical analysis.

Results

Reabsorption of nectar

Nectar volume did not differ significantly between flowers that had nectar removed at 10:00 and those whose nectaries were sealed with petroleum jelly (Student's *t*-test; $t_{40} = 0.19$, $p > 0.05$). On the other hand, at 17:00 h there was significantly less nectar in the flowers with the entrance to nectaries not obstructed (Mann-Whitney; $U = 19.00$, $p < 0.001$), with 81.00% of them containing no nectar at this time.

The self-pollinated flowers with obstructed nectaries did not differ significantly in nectar volume 24 h after pollination from those that had the nectar removed at 10:00 h (Student's *t*-test; $t_{38} = 1.85$, $p > 0.05$). In contrast, there was significantly less nectar 24 h after pollination in the self-pollinated flowers with no nectary obstruction (Mann-Whitney; $U = 2.00$, $p < 0.001$), with 80.00% of them containing no nectar.

This indicates that *Oeceoclades maculata* flowers do not reabsorb nectar either during the course of a day or after pollination and the decrease in volume is due to evaporation of water.

Nectar volume and concentration

The volume and concentration of nectar in flowers of *Oeceoclades maculata* varies throughout the day (Fig. 1). Between 10–12 h the mean nectar volume was 0.82 ± 0.08 μl, (mean ± SE) and between 16–18 h it was 0.36 ± 0.5 μl. The concentration of sugar in the nectar was 33.73% ± 0.62% (mean ± SE) between 16–18 h and 25.10% ± 0.84% between 10–12 h. The Pearson correlation test revealed a strong negative correlation ($\rho = -0.8967$) between the volume of nectar and its concentration. The ANOVA revealed that the time of day is a significant factor associated with the variation in volume ($F_{5,174} = 7.25$, $p < 0.001$) and concentration ($F_{5,174} = 37.74$, $p < 0.001$) of nectar. Flowers did not produce more nectar after it was removed.
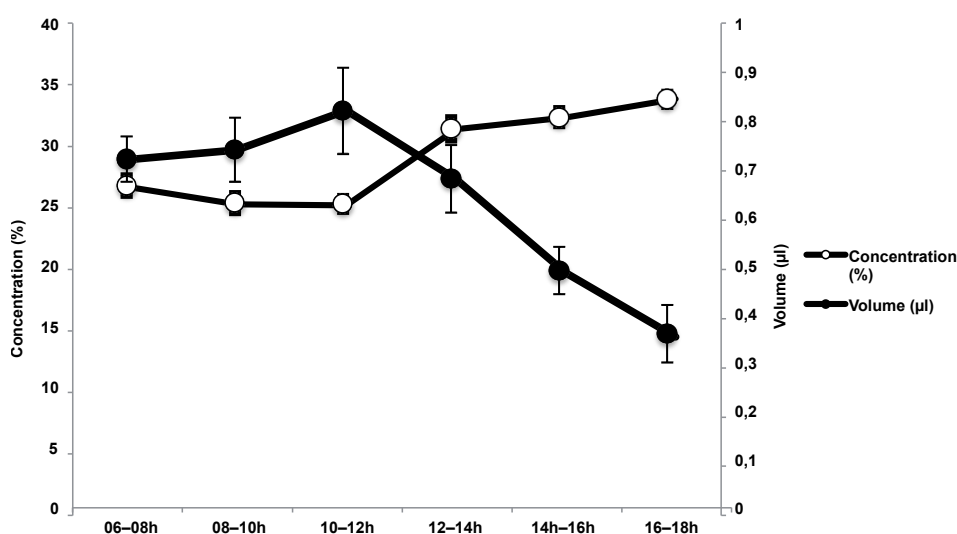
Fig. 1 Variation in volume and concentration (mean ± SE, $n = 30$) of the nectar in flowers of *Oeceoclades maculata*. Note that after the 10–12 h period, the volume of nectar decreases and its concentration increases, indicating evaporation of water.
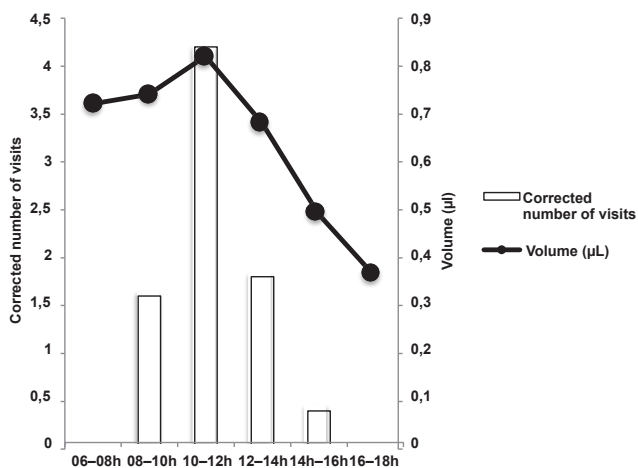
Fig. 2 Number of visits (total number of visits in an interval divided by the number of times that the respective interval was sampled) by both *Heliconius ethilla narcaea* and *H. erato phyllis* to flowers of *Oeceoclades maculata* throughout the course of a day and positive correlation between volume of nectar and the incidence of visits by the butterflies to flowers.

Activity period of pollinators

Pollinators of *Oeceoclades maculata* visit the flowers mainly in the morning, with visits occurring between 8:00 h and approximately 16:00 h (Fig. 2). No visits were recorded between 6–8 h and 16–18 h. The Pearson correlation test revealed that there is a moderate positive correlation ($\rho = 0.69$) between the visits of butterflies to flowers and nectar volume (Fig. 2).

Discussion

Floral nectar is the most commonly resource produced by orchids (van der Pijl and Dodson 1966; Arditti 1992; Dressler 1993) and its production is costly for plants (Koopowitz and Marchant 1998; Nepi and Stpiczyńska 2008). The flowers of some species can reabsorb nectar (Nicolson 1995; Koopowitz and Marchant 1998; Nepi et al. 2001; Luyt and Johnson 2002; Agostini et al. 2011; Stpiczyńska et al. 2012) and this is seen as part of a resource-recovery strategy and a means of maintaining homesostasis in the nectary. This phenomenon is characterized by a simultaneous decrease in volume and increase in concentration (Nepi and Stpiczyńska 2008).

According to Aguiar et al. (2012), in Brazil *Oeceoclades maculata* flowers produce small quantities of nectar, which is used as resource by *Heliconius* butterflies. They infer that by the afternoon the flowers of *O. maculata* reabsorb the nectar.

The results obtained here show that at 6:00 h, when some flowers of *Oeceoclades maculata* are still opening, there is already nectar in the spur of the flower and the volume of nectar increases up to ca. 10:00 h, when each flower contains a mean volume of 0.81 µl. This indicates that the nectar is produced before the flowers open, as is documented for the orchid *Cleistes libonii* (Rchb.f.) Schltr. (as *Cleistes macrantha* (Barb. Rodr.) Schltr.; Pansarin

2003). The concentration of the nectar between 6:00 and 10:00 h remains constant whereas the volume increases, which indicates that nectar is being secreted. After 10:00 h the volume of nectar decreases throughout the day reaching a mean value of 0.36 µl after 16:00 h.

The experiments on reabsorption revealed that the decrease in the volume of nectar is due not to reabsorption but evaporation. There was a significant decrease in the volume of nectar in the nectaries that were not obstructed with petroleum jelly whereas there was no meaningful variation in the volume of nectar in the flowers with the entrance to the nectaries obstructed, which indicates evaporation of water accounts for the decrease in volume. In addition, after 10:00 h, the volume of nectar in the flowers of *O. maculata* decreases, whereas its concentration increases.

Although nectar reabsorption can occur after pollination and is documented for other species of orchids (Koopowitz and Marchant 1998; Luyt and Johnson 2002), this did not occur in *O. maculata*. By obstructing the nectary of self-pollinated flowers we prevented the evaporation of water from the nectar via the nectary entrance and only in the non-treated flowers there was a significant decrease in the volume of nectar. So, it is plausible to affirm that evaporation also accounts for the absence of nectar in many flowers 24 h after pollination.

Most species of plants restrict the visits by pollinators to time-windows of nectar availability, by changing the quantity and quality of this resource (Linnaeus 1751; von Buttel-Reepen 1900). In the squash, *Cucurbita pepo* L., nectar volume and concentration varies significantly during the course of the day and this have influence on when honey bees visit its flowers (Edge et al. 2012). This also seems to be the case for *O. maculata*, in which nectar volume and concentration also varies throughout the day and there is a positive correlation between nectar volume and the frequency of visits by *Heliconius* butterflies. In *Stachytarpheta cayennensis* (Rich) Vahl (Verbenaceae) there is also a positive relation between nectar volume and visits by pollinators (Barp et al. 2011). *Heliconius erato*, a species also recorded as a pollinator of *O. maculata* (Aguiar et al. 2012), prefers to visit the flowers of *S. cayennensis* in the morning when they contain the maximum volume of nectar and the incidence of visits during the course of the day decrease as the volume of nectar in the flowers decreases (Barp et al. 2011). Butterflies were most active between 10:00 h and 12:00 h at our study site, which coincides with the period when the volume of nectar in the flowers of *O. maculata* was at its maximum.

Furthermore, Barp et al. (2011) show that *Heliconius erato* prefers nectar with a concentration of between 20–40%, and this is similar to what we recorded in flowers of *Oeceoclades maculata* between 10:00 h and 12:00 h, when the flowers contained the greatest volume of nectar and were most visited by pollinators (25.10%).

Although many studies indicate that the presence of nectar increases the levels of self-pollination, and thus

inbreeding depression (Johnson et al. 2004; Jersáková and Johnson 2006; Jersáková et al. 2008), in *O. maculata* the presence of nectar is important for cross-pollination. If no resource is available, the number of visits by pollinators would be even lower and the flowers would then have to rely on self-pollination in order to reproduce. Autonomous self-pollination and nectar secretion occurs in the orchid *Epipogium roseum* (D. Don) Lindl. yet its flowers are visited by the Asian honeybee. However, *E. roseum* does not undergo outcrossing mediated by insects, because the bee cannot remove the pollinaria of flowers due to the absence of an adhesive viscidium. Thus, *E. roseum* is obligatorily self-pollinated (Zhou et al. 2012). In *O. maculata,* although the reproductive structures allow the occurrence of biotic cross-pollination, such as the presence of a functional viscidium, development of fruit depends mainly on autonomous self-pollination, but cross-pollination events also occur (Aguiar et al. 2012). Therefore, the nectar in *O. maculata* flowers has an important role in attracting pollinators and thereby maintaining the occurrence of some cross-pollination, unlike what happens in *E. roseum*.

In conclusion, we can affirm that the volume and concentration of nectar in the flowers of *O. maculata* varies throughout the day not as a consequence of nectar reabsorption, as inferred by Aguiar et al. (2012), but due to evaporation of water. This is variation is associated with the attraction of pollinators to flowers of *O. maculata,* which visits the flowers mainly in the morning when the maximum volume of nectar is available. Although *O. maculata* do not depend on pollinators for fruit formation (González-Díaz and Ackerman 1988; Aguiar et al. 2012), the role of *Heliconius* butterflies in providing an opportunity for cross-pollination can, at least presumably, contribute to an increase in genetic variability when compared to strictly autogamous populations (Aguiar et al. 2012).

Acknowledgements

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PRESENCE, DISTRIBUTION AND EFFECT OF WHITE, PINK AND PURPLE MORPHS ON POLLINATION IN THE ORCHID *ORCHIS MASCULA*

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ABSTRACT

How floral polymorphism of flowering plants can be maintained in evolutionary time has long intrigued ecologists and is still debated. In particular, how floral colour polymorphism influences reproductive success is still poorly understood. Here, we investigated the case of *Orchis mascula*, a deceptive orchid species in which the presence of rare white-flowered individuals is known to increase the percentage pollination of co-occurring coloured morphs. In a brief review, we report all the orchid species for which rare colour morphs are recorded and show that colour polymorphism occurs in most orchid genera occurring in France. In this study, more than 20,000 individuals of *O. mascula* were surveyed and some rare clear pink morphs were recorded. The frequencies of white-flowered and clear pink-flowered individuals were 0.59% and 0.28%, respectively. These two rare-colour flowered individuals were not randomly distributed and restricted to a few populations. In addition, the presence of pink-flowered individuals and the use of experimental pink lures resulted in an increase in the percentage pollination of surrounding purple-flowered individuals, as previously shown for white-flowered individuals and white lures. These new observations favour kin selection as the means by which floral colour polymorphism is maintained in this species. We suggest conducting comparative studies of other species in order to evaluate the importance of this mechanism in orchid pollination and that of other plant families.

Keywords: floral colour polymorphism, Mediterranean orchids, pollination, pigment biosynthesis

Introduction

The maintenance of colour polymorphism has long intrigued ecologists. In particular, many flowering plants show substantial intraspecific variation in floral colour (Weiss 1995; Galen 1999; Warren and MacKenzie 2001). The key role of insects through pollinator-mediated selection is the current most often offered explanation for polymorphism in floral signals. Pollinators use diverse floral signals (flower colour, odour, size and shape) to detect flowers, and the various preferences of the different species of insect are a strong selective pressure for the evolution of the flowers they visit in search of rewards (Chittka and Raine 2006; Dormont et al. 2010a). However, recently it was suggested that intraspecific variation in floral traits may also reflect multiple and conflicting selection pressures. Herbivores or local abiotic conditions may also act directly or indirectly, through pleiotropic effects, as selective agents (Warren and MacKenzie 2001; Schemske and Bierzychudek 2007; Coberly and Rausher 2008; Wang et al. 2013). The relative role of these factors on floral polymorphism maintenance is still being debated as their importance as selective agents seems to vary among plant species.

Floral polymorphism is particularly widespread in orchids with a high variation in floral characters, which is generally associated with animal (mostly insect) pollination. Pollination through food deception is a strategy used by about one-third of all orchid species. In such deceptive species, variation in floral traits is expected to be high because pollinators learn to avoid common, unrewarding floral phenotypes (Schiestl 2005; Jersakova et al. 2006a; Dormont et al. 2010a). There is a great diversity of floral colours in orchids and many different kinds of pigments have been identified (Arditti 1992). There are cases of polymorphism in floral colour documented for a few orchids. The European orchid *Dactylorhiza sambucina* (Linné) Soó has yellow- and red-coloured morphs, in frequencies that reflect pollinator preference for the rare colour morph (Gigord et al. 2001). The maintenance of floral polymorphism in this rewardless orchid is explained in terms of negative frequency-dependent selection (Smithson and MacNair 1997; Gigord et al. 2001) and other recent hypotheses (Jersakova et al. 2006b; Smithson et al. 2007). In addition, the pan-tropical species of orchid, *Calanthe sylvatica* (Thou.) Lindl., has three colour morphs with white-, lilac-, and purple-coloured flowers, respectively. These three morphs differ in morphology, floral scent and distribution (altitude and habitat). These differences are hypothesized to reflect a process of on-going speciation (Juillet et al. 2010; Delle-Vedove et al. 2011). Polymorphism in floral colour may thus have different putative ecological explanations (adaptive strategy, on-going speciation), which largely remain to be discovered and elucidated. There are many orchids that exhibit colour polymorphism but there are few studies that explore how this polymorphism is maintained ecologically. For example, the extent to which colour polymorphism influences the reproductive success of such orchids remains poorly understood (Dormont et al. 2010a).

White-coloured flower morphs are reported in various plant families. Insect pollinators have the ability to discriminate among colour morphs and exert a differential selection that may explain (at least partly) the different reproductive success of the colour morphs (Waser and Price 1981; Brown and Clegg 1984; Stanton et al. 1989; Odell et al. 1999; Jones and Reithel 2001; Raguso et al. 2003). Among the wide range of colour variants of orchid flowers, the occurrence of rare hypochromic inflorescences are regularly recorded in natural populations of the common coloured morph (Weiss 1995; Bournérias and Prat 2005; Dormont et al. 2010a). In orchids, the behavioural responses of pollinators to white inflorescences in populations of a coloured morph and the possible consequences for plant reproductive success have been poorly investigated (Koivisto et al. 2002; Ackerman and Carromero 2005). Dormont et al. (2010a) demonstrate for the European species *Orchis mascula* L. that the presence of white-flowered individuals results in a fourfold increase in the percentage pollination of neighbouring purple-flowered individuals. A similar effect is recorded when white lures are placed in natural populations of pure purple morphs. There is no difference in floral scent of purple- and white-flowered morphs. The maintenance of white-flowered mutants in *O. mascula* is hypothesized to be a result of kin selection, in which white morphs act as helpers by increasing the reproductive success of related purple individuals (Dormont et al. 2010b).

The "early purple orchid", *O. mascula,* is a food-deceptive orchid distributed throughout Europe, which typically has red-purple flowers. We recently discovered rare pink-flowered individuals occurring regularly in natural populations of purple-flowered individuals. We investigated this new case of colour polymorphism by addressing the following questions: (1) Do white-flowered individuals currently occur in other species of orchid in France? (2) What are the frequencies and the distributions of white-flowered and pink-flowered individuals in *O. mascula* populations? (3) Do pink-flowered individuals have a similar effect on percentage pollination as that already shown for white-flowered individuals? Based on our results, we offer new arguments to tentatively account for the maintenance of rare colour-flowered plants in orchids.

Material and methods

Study sites and species

The experiments were carried out in a mountainous area in south-central France, located about 70 km north of Montpellier. This study was carried out in area of 308 km^2 (22 × 14 km), which includes both the "Causse de Blandas" and "Causse de Campestre", and part of the "Causse du Larzac", which form a part of extensive limestone plateaux.

Orchis mascula L. is a perennial non-rewarding species of orchid, widely distributed in Europe, western Asia and northern Africa. Inflorescences consist generally of 5–20 purple flowers. In some populations, a few white-flowered (Dormont et al. 2009) and pink-flowered individuals occur mixed with the purple-flowered individuals. *O. mascula* flowers depend on being pollinated by insects (Nilsson 1983; B. Schatz unpublished) and are visited and pollinated by several hymenoptera (bumblebees, cuckoo bumblebees, solitary bees) (Nilsson 1983; Bournerias and Prat 2005; Cozzolino et al. 2005; Dormont et al. 2010a). Flowering occurs early in spring, and *O. mascula* is known to exploit newly emerged insect pollinators, suggesting that pollination in this species is effected mainly by visits of naïve, inexperienced insects (Nilsson 1983; Van der Cingel 1995; Dormont et al. 2010a). Increased abundance of pollinators in the vicinity of nectariferous co-flowering species (van der Cingel 1995; Johnson et al. 2003) is unlikely to be an important factor in the reproductive success of *O. mascula*, as at our study sites there were very few other plants flowering early in the year when *O. mascula* flowers.

Census of white-flowered and "chlorantha" morphs in species of orchid in France

In metropolitan France, we recorded all the species of orchid that have white-flowered morphs (entirely albino flowers) or "chlorantha" morphs (the labellum is yellow-green) (Figs 1 and 2; see discussion for definition of these terms) based on records of personal field observations, naturalist books and photographs found on the web sites of several amateur orchid enthusiasts. We excluded from this census species or genera displaying classically natural white or green flowers in France (*Chamorchis, Coeloglossum, Corallorhiza, Listera, Gennaria, Goodyera, Hammarbya, Herminium, Liparis, Listera, Malaxis, Platanthera, Pseudorchis, Spiranthes*). In other words, this census included 153 species and subspecies of the 174 orchids that are included in the guide book to French orchids (Bournérias and Prat 2005). In certain genera, such as *Dactylorhiza* sp., *Himantoglossum* sp. and *Neotinea* sp., there is a typical dominant colour morph, but some individuals also exhibit many intermediate floral colours. In such genera, white morphs sometimes occurred in natural populations of coloured morphs and these were recorded. We encourage readers to contact us if they have additional data.

Frequency and distribution of colour variants in populations of *O. mascula*

In the area studied, all the individual plants of *O. mascula* were located and surveyed. The different populations, which typically consisted of 20 to 100 individuals, were recorded separately. Populations were patchy distributed and separated from each other by at least 50 m. In the same area were groups of populations, including 17 to 37 populations, separated by at least 500 m (usually 1 km or more), which were also surveyed. The presence of white-flowered and pink-flowered individuals was recorded in each of the above populations.

Fig. 1 White- and pink-flowered individuals in natural populations of purple individuals of *Orchis mascula*.

Fig. 2 "Chlorantha" morphs of *Ophrys scolopax* (left) and *Cypripedium calceolus* (right).

Effect of rare colour-flowered individuals on percentage pollination of *O. mascula*

The role of visual cues in attracting pollinators to flowers of *O. mascula* was estimated by using visual lures during field bioassays. We used white ping-pong balls to mimic white-flowered morphs in a population of natural purple-flowered individuals. This white, spherical standardized object is roughly similar in size to an *O. mascula* inflorescence (Dormont el al. 2010a). Each white ping pong ball was fixed to a wire shaft made of dark green metal, adjusted so that the height of the lure was equal to the mean height of surrounding *O. mascula* purple inflorescences. We placed these lures in populations of purple-flowered individuals at frequencies similar to the mean natural frequency of white inflorescences recorded in mixed populations. The populations of purple plants used in this experiment were selected at random from all the populations of only purple-flowered individuals. The visual lures were placed at random in each population sampled at the very beginning of the flowering period and left there for the whole flowering period.

The reproductive success of plants was assessed by comparing the mean percentage fruit set of seven populations in the area studied: Three populations were natural populations including 1) only purple-flowered individuals (12 populations, 255 individuals, 3736 flowers), 2) purple-flowered individuals with a few white-flowered individuals (12 populations, 255 individuals, 3791 flowers), 3) purple-flowered individuals with a few pink-flowered individuals (10 populations, 202 individuals, 2935 flowers). The four other populations were experimental populations, consisting of only natural purple-flowered individuals to which were added either 4) white lures mimicking white-flowered individuals (4 populations, 74 individuals, 668 flowers), 5) pink lures mimicking pink-flowered individuals (4 populations, 62 individuals, 710 flowers), 6) purple lures, as controls, mimicking pur-ple-flowered individuals (4 populations, 63 individuals, 578 flowers) or 7) green lures, as controls, mimicking the green environment surrounding the *O. mascula* flowers (4 populations, 59 individuals, 687 flowers).

In all populations and for each individual, at the end April (four weeks after placing the visual lures in the experimental populations) we counted the number of mature fruits and total number of flowers in each inflorescence, in order to evaluate percentage fruit set. For each of the seven types of population, individual fruit sets were pooled, because fruit set values were not significantly different among individuals of the same population. In this study, the fruit set was used to estimate plant fitness, but future experiments will also consider seed viability or seed germination. We estimated the influence of the type of population on the number of flowers per inflorescence and fruit set. As the data were not normally distributed (Shapiro-Wilk Test) we performed Permutational ANOVA. All analyses were performed using R 2.14.1 (R Development Core Team 2011).

Results

Census of white-flowered and "chlorantha" morphs of species of orchids in France

We recorded 36 species with white-flowered individuals (Table 1). These individuals are clearly distinct from the classical coloured-flowered individuals and no intermediate colour variant was noted. White-flowered individuals were mainly recorded in the species of three genera, namely *Anacamptis* sp. (11 species) and *Dactylorhiza* sp. (8 species). Most of the species with white morphs typically have purple (19 species) or pink (6 species) flowers and occurred mainly in open-habitats. To our knowledge, white-flowered individuals are not recorded in France in the 10 orchids that have yellow inflorescences, except in *Cephalanthera damasonium*.

Table 1 Literature review: presence of a white morph, together with other coloured variants, in different species of European orchids.

Species in which a white morph is recorded	Main floral colour	Presence of another coloured variant	Reference
Anacamptis pyramidalis	Purple	Yes	B Schatz (pers. obs.); Jouandoudet, 2004; A; B
A. coriophora coriophora	Red-brown	No	B Schatz and R Souche (pers. obs.); Guérin et al. 2007; A; C
A. coriophora fragrans	Red-brown	No	Dormont et al. (in revision.); B
A. lactea	Pink points	No	Souche 2004
A. laxiflora	Purple	No	B
A. longicornu	Purple	Yes	B Schatz (pers. obs.); B; C
A. morio	Purple	Yes	B Schatz (pers. obs.); Jouandoudet 2004; Guérin et al. 2007; A; B; C
A. palustris	Purple	No	B
A. papilionacea expansa	Purple	No	Souche 2004; C
A. papilionacea papilionacea	Purple	No	B
A. picta	Purple	No	Souche 2004; B
Cephalanthera rubra	Pink	No	A
C. damasonium	Yellow	No	Lemoine B and Pessotto L 2007

Species in which a white morph is recorded	Main floral colour	Presence of another coloured variant	Reference
Dactylorhiza elata	Purple	No	Souche 2004
D. fuchsii	Purple	No	B Schatz (pers. obs.); Souche 2004; B; C
D. incarnata	Purple	No	Souche 2004
D. maculata	Purple	No	B
D. majalis	Purple	No	Souche 2004
D. saccifera	Purple	No	B Schatz (pers. obs.)
D. savogiensis	Purple	Yes	B
D. traunsteineri	Purple	No	E
Epipogium aphyllum	Pink-white	No	B
Gymnadenia conopsea	Pink	No	B Schatz (pers. obs.); Jouandoudet 2004; Souche 2004; B
G. corneliana	Red	No	B
Himantoglossum hircinum	Green-brown	No	B Schatz (pers. obs.)
H. robertianum	Purple-green	No	B Schatz (pers. obs.); B
Orchis italica	Pink	No	Souche 2004
O. mascula	Purple	Yes	Dormont et al. 2009; Guérin et al. 2007; B; C
O. purpurea	Purple	No	B Schatz (pers. obs.); Souche 2004; Guérin et al. 2007; B; C
O. simia	Pink	Yes	Dormont et al. (in revision.); Souche 2004; Bournérias and Prat 2005; A; B; C
O. militaris	Pink	No	B Schatz (pers. obs.); A; B; C
Neotinea lactea	Purple-white	Yes	C, D
N. maculata	Purple-white	No	Souche 2004; C
N. tridentata	Purple-white	No	C
N. ustulata	Purple-white	No	B Schatz (pers. obs.)
Traunsteinera globosa	Pink	No	N Juillet (pers. obs.)

Data were also recorded from the following websites:
A) http://www.orchidee-poitou-charentes.org/article2752.html
B) http://perso.numericable.fr/~durbphil/Lusus/LususCouleur.htm
C) http://www.elisajeanluc.fr/orchidées_nature/
D) http://www.guenther-blaich.de/wo_albin.htm
E) http://www.ryenats.org.uk/sandale/sandale.htm

For seven of the orchid species listed in the Table 1 (i.e. coloured-flowered species with the occasional white-flowered morph) no intermediate colour variant is described (Table 1). Such a new colour class would correspond to clear pink individuals, clearly distinct from the white and purple morphs, as in the case of *O. mascula* (Fig. 1).

"Chlorantha" morphs are recorded in 40 different species of orchid, notably in 28 *Ophrys* species (Table 2). The genera in which "chlorantha" morphs are recorded clearly differ from the other orchid genera in Table 1.

In total we recorded 76 species in which hypochromic individuals are recorded. These species are in all the genera with coloured species (see introduction) and are often common. Interestingly, no species of orchid in France is recorded as having both white and "chlorantha" morphs.

Table 2 Literature review: presence of 'chlorantha' morphs in different species of European orchids (see text for explanations, and Figure 2 for examples).

Species in which a "chlorantha" morph is recorded	Main floral colour	Reference
Cypripedium calceolus	Violet and yellow	B Schatz, J Fonderflick and F Nicolé (pers. obs.)
Epipactis atrorubens	Purple-red	Jouandoudet 2004; B
E. helleborine	Green-red	Souche 2004; B
E. palustris	Red-white	B Schatz (pers. obs.)
E. purpurata	Green-purple	B

Species in which a "chlorantha" morph is recorded	Main floral colour	Reference
Limodorum abortivum	Violet	B Schatz (pers. obs.); D
Nigritella rhellicani	Red	Souche 2004
Ophrys apifera	Multicolor	B Schatz (pers. obs.)
O. apifera	Multicolor	A
O. araneola	Multicolor	A; B
O. aranifera	Multicolor	A
O. argentaria	Multicolor	Souche 2004
O. aurelia	Multicolor	C
O. aveyronensis	Multicolor	C
O. aymoninii	Multicolor	B Schatz (pers. obs.); Souche 2004; C
O. bertolinii saratoi	Multicolor	Souche 2004
O. bombyliflora	Multicolor	B Schatz (pers. obs.); Souche 2004
O. classica	Multicolor	Souche 2004
O. exaltata arachnitiformis	Multicolor	Souche 2004
O. exaltata marzuola	Multicolor	Souche 2004
O. forestieri	Multicolor	B; C
O. fuciflora	Multicolor	Souche 2004; A
O. funerea	Multicolor	Souche 2004
O. gresivaudanica	Multicolor	B
O. incubacea	Multicolor	Souche 2004
O. insectifera	Multicolor	B Schatz (pers. obs.); Souche 2004; Guérin et al. 2007; A
O. litigiosa	Multicolor	Souche 2004; Guérin et al. 2007; C
O. lutea	Multicolor	Guérin et al. 2007; A
O. occidentalis	Multicolor	B
O. passionis	Multicolor	C
O. santonica	Multicolor	A
O. scolopax	Multicolor	B Schatz (pers. obs.); Souche 2004; Guérin et al. 2007; B; C
O. sicula	Multicolor	C
O. speculum	Multicolor	C
O. splendida	Multicolor	C
O. sulcata	Multicolor	A
Serapias lingua	Purple	E Sulmont and B Schatz (pers. obs.)
S. neglecta	Purple	Souche 2004
S. parviflora	Purple	Souche 2004; A; B
S. vomeracea	Purple	Souche 2004; A

Data were also recorded from the following websites
A) http://www.orchidee-poitou-charentes.org/article2752.html
B) http://perso.numericable.fr/~durbphil/Lusus/LususCouleur.htm
C) http://www.elisajeanluc.fr/orchidees_nature/
D) http://www.guenther-blaich.de/wo_albin.htm

Frequency and distribution of colour variants in populations of *O. mascula*

A total of 31 groups of *O. mascula* populations were surveyed during this study, involving 774 populations and 20090 individuals. The mean number of populations per group was 25.0 ± 6.1 (mean ± SD). Considering all the 774 populations, the overall percentage of the different colour variants was as follows: 99.13% purple-flowered individuals (n = 19914), 0.59% white-flowered individu-

als (n = 119) and 0.28% clear pink-flowered individuals (n = 57).

The white *O. mascula* morph was recorded in 89 of the 774 populations surveyed and occurred in only 9 of the 31 groups of populations. *O. mascula* populations including a white morph are thus not randomly distributed: a random distribution would have resulted in the presence of the white morph in each of the 31 population groups and in 2.0 to 4.3 *O. mascula* populations in each group

($\chi^2 = 31.07$, df = 1, $p < 0.001$). The pink *O. mascula* morph was recorded in 44 of the 774 populations and in only 7 of the 31 groups of populations. As for the white morph, populations including a pink morph are clearly not randomly distributed: a random distribution would have resulted in the presence of the white morph in 1.0 to 2.1 populations in each population group ($\chi^2 = 35.96$; df = 1, $p < 0.001$). The simultaneous presence, in the same group of populations, of populations with the white morph and other populations with the pink morph, was recorded in only two large population groups. The white and pink morph was recorded occurring sympatrically in only five populations in two different groups of populations.

Effects of the presence of colour variants on the percentage pollination of *O. mascula*

A total of 50 populations, including 973 individual plants of *O. mascula* and 13,105 flowers were surveyed to determine percentage fruit set. In natural populations, the average fruit set of purple-flowered individuals varied significantly depending on the presence or absence of other colour-morphs or lures (Permutational ANOVA: $F_{(6,966)} = 64.47$, $p = 0.001$). The average fruit set of purple-flowered individuals was $5.35 \pm 6.38\%$ (mean $\pm$ SD) in populations with only purple-flowered individuals. The average fruit set reached $26.49 \pm 22.23\%$ in populations with a few white-flowered individuals and $27.8 \pm 14.49\%$ in populations with a few pink-flowered individuals (Fig. 3). The mean number of fruits produced per inflorescence was significantly different among these three

different types of population. Fruit sets of purple-flowered individuals were not significantly different in the populations with white flowers or with pink flowers, but fruit sets in both these populations were more than four times higher than that of purple-flowered individuals in populations where the latter were the sole morph. The mean number of flowers per inflorescence was not significantly different in the three colour morphs (permutational ANOVA: $F_{(2,378)} = 0.059$, $p = 0.94$) and was 14.65 ± 4.50 (mean $\pm$ SD) in the purple-coloured morph (n = 255), 14.49 ± 4.41 in the white-coloured morph (n = 87) and 14.46 ± 4.65 in the pink-coloured morph (n = 39; the relative small sample size is due to the low frequency of this morph in the populations studied).

In experimental populations, the average fruit set of purple-flowered individuals was $29.19\% \pm 26.62\%$ in populations with white lures, $26.18\% \pm 23.26\%$ in populations with pink lures, $6.08\% \pm 8.69\%$ in populations with purple lures and $5.93\% \pm 7.78\%$ in populations with green lures (Fig. 3). Fruit sets of purple-flowered individuals were not significantly different in the populations with white lures or pink lures and not significantly different from the populations with white flowers or with pink flowers. Moreover, fruit sets of purple-flowered individuals were not significantly different in the populations with green lures or with purple lures and not significantly different in the populations with only purple flowers. However, fruit sets of purple-flowered individuals were more than four times higher in the populations with white or pink lures than in the populations with green or purple lures.
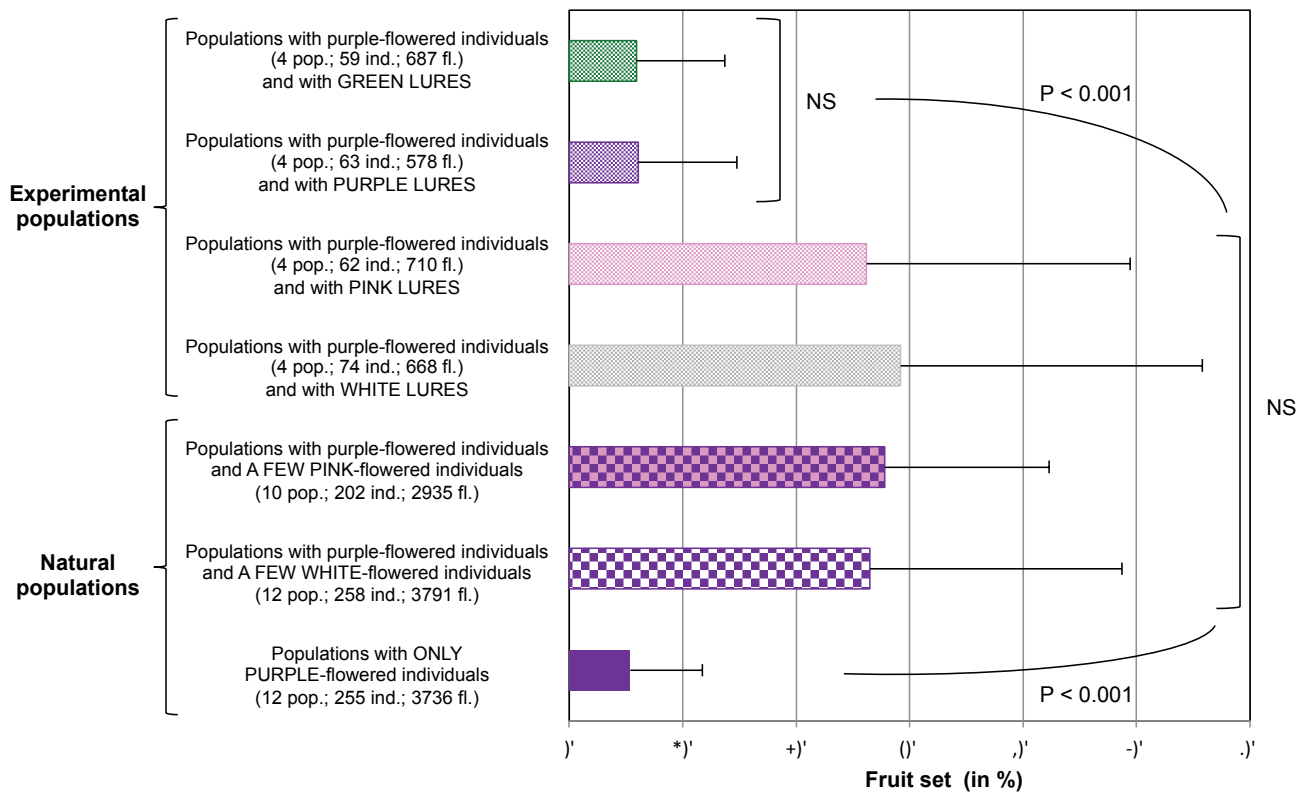
Fig. 3 Percentage fruit set (mean $\pm$ SD) of purple-flowered individuals of *Orchis mascula* recorded in the seven types of populations studied. Results of the Permutational ANOVA are indicated on the figure (n.s.: non-significant).

Discussion

This study provides three new arguments that help to tentatively explain the maintenance of rare colour-flowered plants in orchids: 1) the presence of rare hypochromic individuals is widespread in orchid species in France and this phenomenon both includes hypochromy, such as white and pink morphs, together with "chlorantha"; 2) in addition to the white-flowered morph, pink-flowered individuals also occurred in *O. mascula* populations, and the presence of this clear pink colour morph of *O. mascula* has a similar effect on percentage pollination as that previously recorded for the white morph (Dormont et al. 2010a); and 3) these two rare colour-flowered morphs of *O. mascula* occurred only in certain groups of populations, which is consistent with the predictions of the kin selection hypothesis as suggested for this species by Dormont et al. (2010b).

Presence of rare hypochromic individuals

This brief review of the literature revealed that white-flowered individuals are recorded in 36 species in France and this phenomenon certainly also occurs elsewhere in Europe. They occur in a total of nine genera (*Anacamptis*, *Cephalanthera*, *Dactylorhiza*, *Epipogium*, *Gymnadenia*, *Himantoglossum*, *Orchis*, *Neotinea* and *Traunsteinera*). Interestingly, these nine genera share a common floral trait: the flowers are generally purple or pink and in a few cases red. An intermediate class of colour (between white and the main colour) is recorded in some of these species, e.g. in *O. mascula*. Similar situations are reported for other species of plants, such as the Chalk Milkwort, *Polygala calcarea*, a plant that has violet, pink and white individuals (Schatz B., pers. obs.). The occurrence of such white coloured individuals is generally attributed to spontaneous mutations affecting floral pigmentation, which results in the complete absence of pigments in the flower parts (Waser and Price 1981; Epperson and Clegg 1987; Levin and Brack 1995). The occurrence of an intermediate class of colour, such as pink in *O. mascula*, may be explained by a partial attenuation of pigment biosynthesis. Finally, the presence of rare hypochromic individuals in orchids is different from the situation reported for other polymorphic species of orchid, in which the presence of distinct floral colour morphs (e.g., in *D. sambucina* and *C. sylvatica*) is due to the presence of different pigments.

'Chlorantha' morphs represent a second category of hypochromic individuals, characterized by yellow coloured floral parts. Some naturalists sometimes refer to 'viridism' to describe this phenomenon. To our knowledge, the use of this term is not supported by any scientific study. However, such 'chlorantha' morphs are reported in different groups of orchid taxa: 40 species in six different genera (*Cypripedium*, *Epipactis*, *Limodorum*, *Nigritella*, *Ophrys* and *Serapias*). Taken together, this literature review shows that rare hypochromic individuals are present in all genera with coloured flowers in France and in these species throughout Europe. These rare white-flowered individuals or 'chlorantha' individuals are sometimes described by naturalists as new subspecies (which are called 'albiflora', 'alba', 'flavescens'). This cannot be justified as these rare morphs occur in natural populations of coloured flowers and have similar ecological traits.

Influence of rare floral signals on reproductive success

To our knowledge, the presence of clear pink-flowered individuals in natural populations of coloured individuals, as reported here for *O. mascula*, has not previously been recorded. The presence of these rare coloured-flowered individuals clearly affects the percentage pollination of neighbouring purple-flowered individuals, as already shown for the white *O. mascula* morph (Dormont et al. 2010a). First, the overall average fruit set of *O. mascula* was low (mean fruit set 6%), both for purple-morph individuals in pure purple populations and for the two rare colour individuals (white-morph and pink-morph), as reported in previous studies (Nilsson 1983; Van der Cingel 1995; Jacquemyn et al. 2008; Dormont et al. 2010a). Second, we show here that the presence of either co-occurring clear pink-flowered individuals or co-occurring white-flowered individuals had a similar effect, resulting in a significantly higher reproductive success of nearby purple-flowered individuals (mean fruit set 27%). These results are incompatible with the hypothesis of negative frequency-dependent selection (NFDS) frequently assumed to explain floral colour polymorphism in non-rewarding orchids (Gigord et al. 2001): in this study we found that the common purple morph of *O. mascula* was fitter when the rare morph (pink or white) was present.

In a previous study, we showed that the chemical compositions of the floral volatiles emitted by the two colour morphs of *O. mascula* (white and purple) are not significantly different (Dormont et al. 2010a). Preliminary analyses of the scent emitted by the pink morph of *O. mascula* suggest that floral volatiles of the white, pink and purple morphs do not differ (Schatz et al. unpubl. data). Moreover, pink lures and pink-flowered individuals induced a similar increase in the percentage pollination of neighbouring purple-flowered individuals, which strongly suggest that visual signals alone probably play the key role in colour morph discrimination by insects, as previously shown for the white morph (Dormont et al. 2010a). White flowers are often reported has being less frequently pollinated than coloured flowers, but this is based on observations on nectar rewarding species (Waser and Price 1981; Brown and Clegg 1984; Odell et al. 1999). *O. mascula* is a nectarless species, flowering early in spring when pollinators are attracted through food deception and also because of their inexperience. In other deceptive orchid species (Jersakova et al. 2006a), pollinators deceived by the absence of a reward in one morph tend to visit the other morph, a process globally increasing the total number of visits of the same species of orchid. The presence

of contrasting colours within single plant populations has also been shown to be attractive to bumblebees (Spaethe et al. 2001; Lunau et al. 2006). The increase in percentage pollination, observed both when rare colour-flowered individuals are present and when lures are used, clearly demonstrates the attractiveness of rare white and clear pink colours for naive pollinators. In contrast, the use of purple- or dark green-coloured lures, placed in pure purple populations, had no effect on the reproductive success of neighbouring purple coloured individuals of *O. mascula*. These results confirm that colour contrast is an attractive signal that can increase pollinator visits to flowers. Thus, these two rare flower colours in *O. mascula* (white and pink) may act visual attractants for insects.

Do similar effects on percentage pollination occur in other polymorphic species of orchid with white-flowered individuals? In *O. mascula*, the fourfold increase in fruit set is linked to three factors: 1) the early flowering of this orchid and the naivety of the newly emerged pollinators, 2) the contrast between white and the current purple flower colour is pronounced in *O. mascula*; and 3) the deceptive strategy in *O. mascula*, which results in a very low fruit set (about 6%). The effects on pollination in some other orchids growing in the same study area would be more difficult to evaluate because they differ greatly in their biology. For example, the orchid *Dactylorhiza fuschii* flowers in June, has relatively clear pink flowers and usually a high fruit set (locally about 80%) (Schatz B. pers. obs.). The situation is similar in two other species: *Anacamptis coriophora fragrans* flowers in May, has red flowers and a high fruit set (80%) due to its nectar rewarding strategy and *Orchis simia* flowers in May, has pink flowers and a high fruit set (locally about 80%) (Dormont et al. submitted) in spite of having nectarless strategy (but see Schatz 2006; Schatz et al. 2010). These three factors appear to be important when attempting to detect an effect on the percentage pollination rate of the presence of white-flowered individuals.

Maintenance of the rare white-flowered *O. mascula*

In this study we surveyed more than 20,000 individuals of *O. mascula* and determined the frequency of rare colours in purple populations as 0.28% for clear pink-flowered individuals and 0.59% for white-flowered individuals. Spontaneous mutations affecting floral pigmentation that result in white-flowered variants (and potentially pink variants) are thought to account for the presence of low frequencies of white individuals in natural populations of pigmented flowers (on average 0.1% in these studies) (Waser and Price 1981; Epperson and Clegg 1987; Levin and Brack 1995). The difference between the frequencies reported in the literature (0.1%) and those recorded for *O. mascula* (0.6%) has been tentatively attributed to the possible role of pollinators in plant reproductive success (Dormont et al. 2010a).

Dormont et al. (2010a; 2010b) hypothesized that the maintenance of variant white-flowered individuals in populations of *O. mascula* might be attributed to kin selection (see also Hamilton 1964). The presence of white-flowered individuals might be regarded as an adaptation that benefits the purple-flowered relatives of white-flowered morphs, rather than providing a direct benefit to white-flowered individuals. Moreover, the postulated mechanism of kin selection could work only if the neighbouring individuals that benefit from the proximity of a white-flowered individual are related to it. In this study, white-flowered and pink-flowered individuals were not randomly distributed: only certain groups of populations hosted these rare colour morphs. These groups of populations might be considered as different kins of related individuals; certain kin would consist of only purple individuals, while other kin are characterised by high likelihood of spontaneous mutations affecting floral pigmentation. This hypothesis is consistent with the spatial genetic structure marked by aggregations of related individuals already demonstrated in *O. mascula* (Jacquemyn et al. 2008) and other species of the genus *Orchis* (Chung et al. 2005; Jacquemyn et al. 2006, 2007).

The presence of rare clear coloured-flowered individuals is widespread in European species of orchids. In *O. mascula*, both the presence of white- and pink-flowered individuals resulted in an increase in the fruit set of neighbouring pigmented flowers, which is in accordance with the mechanism proposed recently to explain the maintenance of floral colour polymorphism in this species (Dormont et al. 2010a, 2010b). We also identify important necessary conditions for future similar studies on other species. Some new observations recorded in this study are consistent with kin selection as the possible mechanism. We suggest that other comparative studies should be conducted on other species, in order to evaluate the importance of this mechanism in orchid pollination and that of other plant families.

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INTER-ANNUAL VARIABILITY IN FLOWERING OF ORCHIDS: LESSONS LEARNED FROM 8 YEARS OF MONITORING IN A MEDITERRANEAN REGION OF FRANCE

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ABSTRACT

It is important to evaluate the loss of biodiversity caused by global changes. In the case of orchids, it is still unclear how long the monitoring duration should be chosen in order to achieve a good compromise between the reliability of the orchid dynamics recorded and sampling duration (e.g. years of monitoring). This study aims to propose a method of monitoring orchids. Using a large database, we investigated the inter-annual variability in flowering of orchids in a French Mediterranean region. The database includes an 8-year-long study (2006–2013) of 47 species at 26 locations in three different types of habitats. The number of individual plants that flowered per species varied significantly between years, but not the number of species. Depending on habitat, two to four years were needed to observe the total number of species per location. Therefore, in Mediterranean regions a one-year-study seems to be insufficient to produce reliable results.

Keywords: orchid community, diachronic studies, orchid monitoring, similarity index, conservation

Introduction

In the era of global change that deeply affects organisms on our planet (Parmesan 2006), there is an increasing urgency to evaluate the loss in biodiversity (Barnosky et al. 2011; Thuiller et al. 2011). In order to propose appropriate conservation strategies, it is essential for conservation biologists to precisely evaluate shifts in communities. Limiting our topic in orchids, it has been well acknowledged that orchids are ideal models to evaluate the impact of global change on biodiversity. First of all, they show clear and fast responses to environmental changes, including a current and well documented decline in occurrence or population size (Whigham and Willems 2003; Schatz et al. 2013). Then, many orchids are emblematic species (Cribb et al. 2003) and there is an increasing need to study them with the aim of conservation. Thirdly, they show well-documented patterns of endemism and rarity (Bournérias and Prat 2005; Dusak and Prat 2010).

The availability of long-run data or diachronic studies on the distribution of orchids provides opportunities to document temporal variation in communities (Jacquemyn et al. 2005; Kull and Hutchings 2006). These studies enable us to predict response of organisms to future environmental changes. However, there is a lack of standardized methods of recording and analyzing the data (Kati et al. 2004; Archaux et al. 2009). As a result, it is necessary to document the natural variability in the life-history traits of species (e.g. frequency of flowering) and to develop of sampling methods suitable to monitor orchids.

At the species scale, several studies have shown that the number of flowering individuals varied from year to year in response to climatic fluctuations (Tamm 1991; Sieg and King 1995; Wells et al. 1998; Tali 2002; Oien and Moen 2002; Brzosko 2003; Kindlmann 2003; Hrivnak et al. 2006; Jacquemyn et al. 2007). This inter-annual variability is recorded in several different species of orchids, such as *Anacamptis morio* (Wells et al. 1998), *Dactylorhiza majalis* (Hrivnak et al. 2006), *D. lapponica* (Oien and Moen 2002), *D. sambucina* (Tamm 1991), *D. incarnata* (Tamm 1991), *Gymnadenia conopsea* (Oien and Moen 2002), *Neotinea ustulata* (Tali 2002), *Neottia ovata* (Tamm 1991), *Orchis mascula* (Tamm 1991), *Platanthera praeclara* (Sieg and King 1995), *P. bifolia* (Brzosko 2003) and *Spiranthes spiralis* (Jacquemyn et al. 2007). A common characteristic of these studies is that they are all located in northern Europe. Yet, little is known about such variation in Mediterranean species (but see Sirami et al. 2010; Schatz and Geniez 2011). In Mediterranean regions, a high variability in the incidence of flowering of orchids is expected due to the high intra- and inter- annual variation in climate. In the future, longer and more severe drought periods (IPCC 2007; Giorgi and Lionello 2008) may disturb the phenology of orchids. Moreover, the region hosts different types of habitats, e.g. grassland, shrubland and woodland, which may affect flowering patterns of orchids differently.

We investigated the temporal dynamics of orchid assemblages, at both the species and community level (47 species of orchids) in a Mediterranean region of France (Languedoc-Roussillon). This study aims to address the following questions:

(1) How does the fluctuating Mediterranean climate affect flowering patterns of orchids?

(2) Are there differences in inter-annual flowering of orchids between habitat types?

We documented the inter-annual variations in flowering patterns (e.g. number in flowering individuals and probability of presence) of orchids in order to provide a framework for future monitoring in this region.

Materials and Methods

Study sites

Study sites are situated in the North of the Languedoc-Roussillon region in southern France (43°17´31˝N–44°17´31˝N, 3°05´27˝E–3°50´41˝E). At these sites, the climate is of Mediterranean type with annual precipitation ranging from 950 mm to 1350 mm (Debussche and Escarré 1983). Air temperature varies from 0 °C in winter to 28 °C in summer (Sirami et al. 2010). We sampled at 26 locations, where many species with patchy distributions co-occurred. Three types of habitat were distinguished, corresponding to three stages of succession, hereafter called "grassland" (n = 10), "shrubland" (n = 8) and "woodland" (n = 8). We excluded locations that experienced high levels of disturbance, e.g. intensive tree cutting and grazing. The sampled locations differed in area, ranging from 500 m² to 2000 m². In each location, yearly orchid inventory was always carried out in the same area so that outcomes between inventories can be compared.

The study was conducted for eight consecutive years, from 2006 to 2013. In each year, all the flowering individuals were recorded at each location during the same period (between March and July). In total, 47 species of orchids were included in this study. A full list of the species studied is given in Table S1.

Species traits

Species traits were not measured in the field, but based on the information available in the literature (Bournérias and Prat 2005). The traits included:
1. Number of species of pollinators and mycorrhizal symbionts. They were classified as either a specialist (1 species), an intermediate (2–5 species) or a generalist (more than 5 species).
2. Flower morphology. We considered the number of flowers, the size of the inflorescence (mean in cm) and plant height (mean in cm).
3. Phenology. We considered the duration of flowering (mean in months) and the flowering period (mean in terms of particular months).

Data analysis

We used Wilcoxon or Kruskal-Wallis tests, i.e. two non-parametric tests, to investigate the effect of year sampled on the variability in the presence and number of flowering individuals. For each median, we calculated the confidence interval (IC), as $[1.57 \times (Q_3 - Q_1)] / n^{0.5}$, where, Q_1 and Q_3 are the 25th and 75th percentile of the data, respectively; and n is the number of observations (Chambers et al. 1983).

We averaged the number of species per location and the number of flowering individuals per species and location. Concerning the inter-annual variability between species, we calculated the probability of presence for each species, as the ratio of the number of years when the species was recorded divided by the total number of years monitored, i.e. eight years. We considered that an absence of a record in one or several years did not mean death of an individual, but flowering dormancy. We used Jaccard's similarity index (Jaccard 1901) to determine how similar the species composition was between pairs of consecutive years. The similarity index was calculated as $c/(a + b - c)$, where a is the total number of species in one year; b is the total number of species recorded in the following year; and c is the number of species in common found in both of years (Jaccard 1901). In regard to the number of records, we applied the same similarity index. In this case, the similarity index was equal to the minimum recorded number of flowering individuals divided to the maximum recorded number of flowering individuals between two consecutive years. To compare the similarity index between species, we only considered species that occurred at least at five locations in order to augment the spatial representativeness of the study site.

We used a principal component analysis in order to investigate the relationship between inter-annual variability of flowering (index of similarity and probability of presence) and traits of species. We investigated the species-time relationships using an accumulation curve of species recorded at the site level. We calculated the ratio of the cumulated number of species recorded and the maximum number of species recorded at the same location between 2006 and 2013. This process was repeated for each pair of successive years (e.g. 2007 and 2008, i.e. seven possibilities) and each location.

All analyses were performed using R software (R development core team, version 2.15.0). For the principal component analyses, we used the package FactoMineR (Husson et al. 2007).

Results

Inter-annual variability in flowering at community level

The number of species recorded per location did not differ significantly between years (Kruskal-Wallis test, $K = 3.64$, $p > 0.05$), with the median varying slightly, from 5 ± 1.23 (Median $\pm$ IC, idem for all the following cases) in 2011 to 6.5 ± 1.85 in 2010 (Wilcoxon test, $W = 417$, $p > 0.05$, Fig. 1A). The median of Jaccard-similarity index between consecutive years was 0.94 ± 0.02 for all locations, meaning that the species composition differed

Table 1 Median ± confidence interval of Jaccard-similarity index for pairs of consecutive years for all locations and three habitats. Based on Wilcoxon non-parametric test, different letters indicate a significant difference in the Jaccard similarity index between different pairs of consecutive years for a particular habitat. N is the number of locations.

	All locations			Grassland			Shrubland			Woodland		
N	26			10			8			8		
2006 *vs* 2007	0.77	± 0.11	a	0.77	± 0.10	a	0.92	± 0.27	ab	0.69	± 0.18	a
2007 *vs* 2008	0.97	± 0.05	bd	1.00	± 0.03	bc	0.83	± 0.14	a	0.97	± 0.25	abc
2008 *vs* 2009	1.00	± 0.05	bc	1.00	± 0.00	b	1.00	± 0.02	b	0.8	± 0.12	ab
2009 *vs* 2010	1.00	± 0.00	c	1.00	± 0.00	bc	1.00	± 0.02	b	1.00	± 0.00	c
2010 *vs* 2011	0.77	± 0.07	ad	0.85	± 0.10	a	0.69	± 0.11	a	0.75	± 0.11	ab
2011 *vs* 2012	0.88	± 0.07	b	0.87	± 0.11	ac	0.94	± 0.09	ab	0.87	± 0.12	abc
2012 *vs* 2013	0.97	± 0.05	b	1.00	± 0.00	b	0.81	± 0.12	a	0.92	± 0.08	bc
Total	0.94	± 0.02		1.00	± 0.03		0.87	± 0.06		0.88	± 0.06	

by 6% between consecutive years (Table 1). The Jaccard-similarity index differed significantly between habitats ($p < 0.05^*$) and the medians were $1 ± 0.03$, $0.87 ± 0.06$ and $0.88 ± 0.06$ for grassland, shrubland and woodland, respectively (Table 1).

For the 47 species of orchids studied the average number of flowering individuals per species and location differed between years (Kruskal-Wallis test, $K = 24.75$, $p < 0.001^{***}$), with the median varying significantly from $5.2 ± 3.4$ individuals in 2011 to $28 ± 10.7$ individuals in 2010 (Wilcoxon test, $W = 1619.5$, $p < 0.001^{***}$, Fig. 1B). The similarity index of the number of flowering individuals between two consecutive years did not differ between habitats ($p > 0.05$).
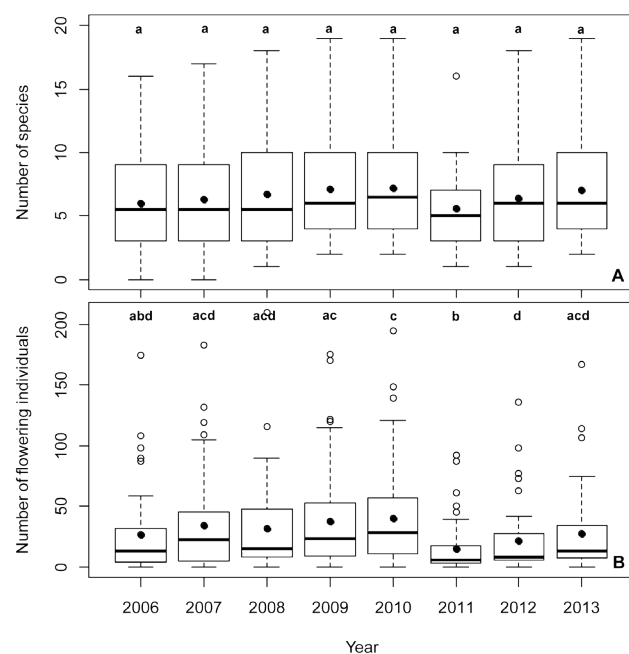
Fig. 1 Boxplots of (A) the average number of species per location and (B) the average number of flowering individuals per species and location recorded per year. Black points correspond to (A) the mean of the number of species per location or (B) the number of flowering individuals per species. Different letters indicate a significant difference based on Wilcoxon non-parametric test. Significant codes: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Inter-annual variability in flowering at species level

In the eight years of inventory, probability of being present differed significantly between species (Kruskal-Wallis test, $K = 69.7$, $p < 0.05^*$, Fig. 2A, Table S2). The median of the average probability of presence was $0.82 ± 0.06$ and $0.87 ± 0.05$ for the 17 species and all the species studied, respectively (Fig. 2B). The probability of presence varied from 0.52 (for *Platanthera chlorantha*) to 0.98 (for *Anacamptis pyramidalis*) (Fig. 2A).
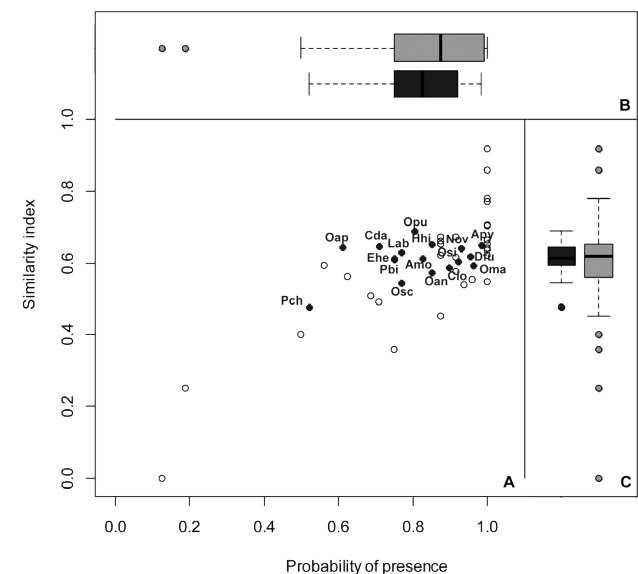
Fig. 2 Plot of the probability of presence and the similarity index of the number of flowering individuals between two consecutive years per year (A). Each point represents the mean probability of presence and similarity index of the orchid species studied that occurred in ≥ 5 locations (dark-grey points) and < 5 locations (hollow points). Boxplots of (B) the probability of presence and (C) inter-annual similarity index are depicted for all the species (in light-grey) and for the 17 species that occurred in ≥ 5 locations (in dark-grey). Abbreviations of species: Amo = *Anacamptis morio*, Apy = *Anacamptis pyramidalis*, Cda = *Cephalanthera damasonium*, Clo = *Cephalanthera longifolia*, Dfu = *Dactylorhiza fuchsii*, Ehe = *Epipactis helleborine*, Hhi = *Himantoglossum hircinum*, Lab = *Limodorum abortivum*, Nov = *Neottia ovata*, Oap = *Ophrys apifera*, Osc = *Ophrys scolopax*, Oan = *Orchis anthropophora*, Oma = *Orchis mascula,* Opu = *Orchis purpurea*, Osi = *Orchis simia*, Pbi = *Platanthera bifolia*, Pch = *Platanthera chlorantha*.

In regard to inter-annual similarity of flowering individuals, there were significant differences between six pairs of species: *P. chlorantha* versus *A. pyramidalis* (Wilcoxon test, $W = 74$; $p < 0.05^*$), *P. chlorantha* versus *H. hircinum* (Wilcoxon test, $W = 35$, $p < 0.05^*$), *P. chlorantha* versus *O. purpurea* (Wilcoxon test, $W = 26$, $p < 0.05^*$), *O. scolopax* versus *H. hircinum* (Wilcoxon test, $W = 49.5$, $p < 0.05^*$), *O. purpurea* versus *O. anthropophora* (Wilcoxon test, $W = 59$, $p < 0.05^*$) and *O. purpurea* versus *O. scolopax* (Wilcoxon test, $W = 37$, $p < 0.05^*$) (Fig. 2, Table S2). Medians of the averaged similarity index were 0.61 ± 0.02 and 0.62 ± 0.02 for the 17 species and all of the species studied, respectively (Fig. 2C). The similarity index varied from 0.48 (for *Platanthera chlorantha*) to 0.70 (for *Orchis purpurea*) (Fig. 2A).

According to the principal component analysis, the first two factorial axes accounted for 34.89% (axis 1) and 20.93% (axis 2) of the total variability, respectively (Fig. S1). The similarity index was negatively related with the duration of flowering. The other traits, e.g. height and number in pollinator species, were neither related to the similarity index, nor to the probability of presence (Fig. S1).

Accumulation in the number of species recorded over time

The increased percentage in the number of the recorded species that accumulated with time differed between habitats (Fig. 3). One year of sampling captured 87.5% of the species in grassland, 88.9% of those in shrubland and 80% of those in woodland. Significant difference was found between shrubland and woodland (Wilcoxon test, $W = 1620.5$, $p < 0.05^*$), but was absent between grassland and shrubland or between grassland and woodland ($p > 0.05$). The total number of species (median-value of 100%) was observed after the 2nd year of sampling for shrubland, the 3rd year for woodland and the 4th year for grassland (Fig. 3).

Discussion

Inter-annual variability in flowering at community level

We showed that the number of orchid flowering individuals varied conspicuously between years in the Mediterranean region (Fig. 1B). These results are in accordance with the previous findings on the variability in the inter-annual records of orchid numbers in northern Europe (Tamm 1991; Kindlmann and Balounova 1999; Oien and Moen 2002; Tali 2002; Brzosko 2003). Inter-annual fluctuations in orchid abundance are mainly induced by variations in climate, in particular temperature and rainfall (Wells et al. 1998; Pfeifer et al. 2006, 2011). 2006 and 2011 were the driest during the sampling period and the lowest number of individuals per species was recorded in 2011 at most of the study sites. This result indicates that orchids may respond to drought by reducing the number of flowering individuals, as observed by Hutch-ings (2010) in the case of *Ophrys sphegodes*. We suppose that
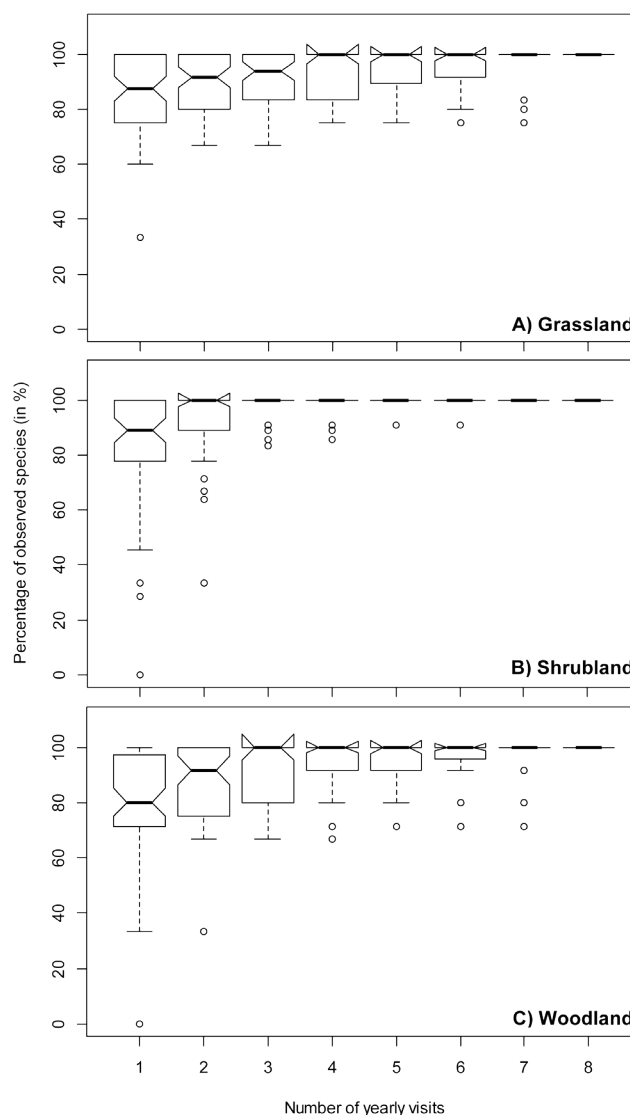
Fig. 3 Boxplot of the cumulative percentage of species recorded in successive yearly inventory, for (A) grassland, (B) shrubland and (C) woodland. A non-overlapping of the notches of two plots denotes a significant difference between the two medians as proposed by Chambers *et al.* (1983).

the high inter-annual variability in flowering of orchids is due to dormancy induced by unfavourable weather conditions. Summer drought can cause premature senescence and death of leaves, which results in insufficient reserves of nutrients for the orchids to flower the subsequent year (Wells et al. 1998).

At community scale, no evidence was detected regarding the significance of the number of species between years (Fig. 1A). In addition, species composition differed only by 6% between consecutive years (Table 1). We suppose that rare species vary less in terms of the incidence of flowering than abundant species, and thus can be maintained between years. As a result, we can distinguish two different strategies, i.e. high numbers of individuals combined with a high incidence of inter-annual fluctuations (abundant species) and few individuals combined with a low incidence of inter-annual fluctuations (rare species). Similarly, Lavergne et al. (2004) evidenced

that narrow endemic plants were locally persistent, despite the fact that they produced fewer flowers than their widespread congeners. The persistence of rare species could be related to a better success in reproduction or establishment (Simon and Hay 2003; Byers and Meagher 1996).

Inter-annual variability in flowering at species level

We evidenced that species responded differently in terms of orchid presence and similarity index (Fig. 2). We attributed this to the disparity of traits between species. The results indicated that the similarity index seems to be negatively related to the duration of flowering. Increasing the flowering duration can lead to an increase in the probability of pollination, and thus to an increase of the reproductive success (fruit production). However, according to Primack and Stacy (1998), a cost of fruit production leads to a reduction in flowering probability in subsequent years (in case of *Cypripedium acaule*). Further analyses need to be carried out, in order to relate reproductive success to traits, as suggested for the nectar presence (Neiland and Wilcock 1998). In particular, our principal component analysis was based on the mean of traits. Traits should be measured locally in order to consider the variability in a trait in individuals and between habitats.

Orchid species can be detected more or less easily. This is probably due to their particular life-state (dormant or flowering state) and species biological traits, e.g. color of the flower and plant height. Our study evidenced a weak probability of presence for *Platanthera chlorantha*, in contrast to *Anacamptis pyramidalis*. This can be due to different levels of difficulty in terms of species detection. *Platanthera chlorantha* has white-greenish flowers, which is more difficult to be detected compared to *Anacamptis pyramidalis* that has purple flowers. In order to take into account the imperfect detection, we suggest using the capture-recapture model proposed by Kery and Gregg (2004) in future orchid inventories. Such model can be used to calculate the extent to which orchid records are underestimated due to imperfect detection of orchids. However, inventories of at least three years are needed in order to estimate and correct for imperfect detection.

Inter-annual variability in flowering in different habitats

Our study revealed that, in Mediterranean regions, a one-year-study is insufficient for monitoring orchids. This may be due to the fact that most of orchid species can stay in dormancy during in which flowering does not occur for one to three years (Shefferson et al. 2001; Brzosko 2003; Kery and Gregg 2004; Coates et al. 2006). Regarding the number of years needed to observe 100% of the species (Fig. 3), we found: shrubland (2 years) < woodland (3 years) < grassland (4 years). We attribute this result to the disparity of microclimate and light availability between habitats. Higher light availability can increase probability of flowering (Diez et al. 2007; Jac-

quemyn et al. 2010). Stable microclimatic conditions, e.g. a warm winter or fresh summer, can favour population performance and avoid dormancy of flowering (Pfeifer et al. 2006). The two factors antagonistically act in the case of grassland and woodland toward probability in flowering. In grassland, there is high light availability, but the microclimatic condition tends to be unstable. On the contrary, in woodland, light availability is low, but the microclimate is more regulated than in open habitat. For example, because of the tree canopy closure, the seasonal variation of air and soil temperature tend to be less contrasted under tree clusters than in open areas (Morecroft et al. 1998; Mao et al. 2013). Therefore, a trade-off effect between microclimate and light availability may exist in both grassland and woodland, thus resulting in higher numbers of years needed to observe 100% of the species (≥ 3 years). Compared to grassland and woodland, an intermediate condition of light availability and microclimate exists in shrubland, resulting in a lower number of years needed to observe all of the species.

Conclusion and perspectives

This study showed that in Mediterranean context, climate affects the inter-annual variability of orchid flowering. We evidenced a species-based response in regard to orchid presence. The number of years needed to observe 100% of species diverged between habitats and last from two to four years. As a result, we suggest that further studies use data collected over periods of a minimum of two years. Species and habitat should be considered equally important when interpreting results.

In the future, relationships between species-specific traits of orchids (e.g. strategies of rare species versus abundant species) and inter-annual variability of flowering need to be better understood in order to enhance the conservation of orchids. Capture-recapture models of orchids, which can take into account imperfect detection, will be a promising tool when characterizing the temporal dynamics of orchid communities.

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SUPPLEMENTARY MATERIALS

Table S1 List of the orchid species studied with the abbreviated names (Abb) and the number of locations where the species was recorded for each habitat (Grassland, Shrubland, Woodland).

Species	Abb	Grassland	Shrubland	Woodland	Total
Anacamptis fragrans	Afr	2	0	1	3
Anacamptis laxiflora	Ala	1	0	0	1
Anacamptis morio	Amo	5	0	0	5
Anacamptis pyramidalis	Apy	10	7	5	22
Cephalanthera damasonium	Cda	1	2	3	6
Cephalanthera longifolia	Clo	1	2	3	6
Cephalanthera rubra	Cru	0	1	1	2
Coeloglossum viride	Cvi	1	0	0	1
Cypripedium calceolus	Cca	0	0	1	1
Dactylorhiza elata	Del	0	0	1	1
Dactylorhiza fuchsii	Dfu	1	3	4	8
Dactylorhiza occitanica	Doc	1	0	0	1
Dactylorhiza sambucina	Dsa	1	0	1	2
Epipactis atrorubens	Eat	0	0	2	2
Epipactis helleborine	Ehe	0	1	4	5
Epipactis microphylla	Emi	0	0	1	1
Goodyera repens	Gre	0	0	1	1
Gymnadenia conopsea	Gco	3	0	1	4
Himantoglossum hircinum	Hhi	4	4	2	10
Himantoglossum robertianum	Hro	1	1	1	3
Limodorum abortivum	Lab	1	3	3	7
Neotinea ustulata	Nus	3	0	1	4
Neottia nidus-avis	Nni	0	0	2	2
Neottia ovata	Nov	2	1	4	7
Ophrys apifera	Oap	4	1	3	8
Ophrys araneola	Oar	1	0	1	2
Ophrys aveyronensis	Oav	1	0	0	1
Ophrys aymonini	Oay	1	0	1	2
Ophrys bilunulata	Obi	0	1	0	1
Ophrys insectifera	Oin	1	1	1	3
Ophrys lupercalis	Olu	0	2	0	2

Species	Abb	Grassland	Shrubland	Woodland	Total
Ophrys lutea	Olut	2	1	0	3
Ophrys massiliensis	Omas	0	1	0	1
Ophrys occidentalis	Ooc	1	1	1	3
Ophrys passionis	Opa	3	0	0	3
Ophrys scolopax	Osc	2	4	1	7
Ophrys sulcata	Osu	1	0	0	1
Ophrys virescens	Ovi	1	0	1	2
Orchis anthropophora	Oan	3	3	4	10
Orchis mascula	Oma	4	3	3	10
Orchis militaris	Omi	2	0	2	4
Orchis provincialis	Opr	0	0	1	1
Orchis purpurea	Opu	4	2	1	7
Orchis simia	Osi	2	4	2	8
Platanthera bifolia	Pbi	4	3	2	9
Platanthera chlorantha	Pch	1	2	3	6
Spiranthes spiralis	Ssp	1	1	1	3
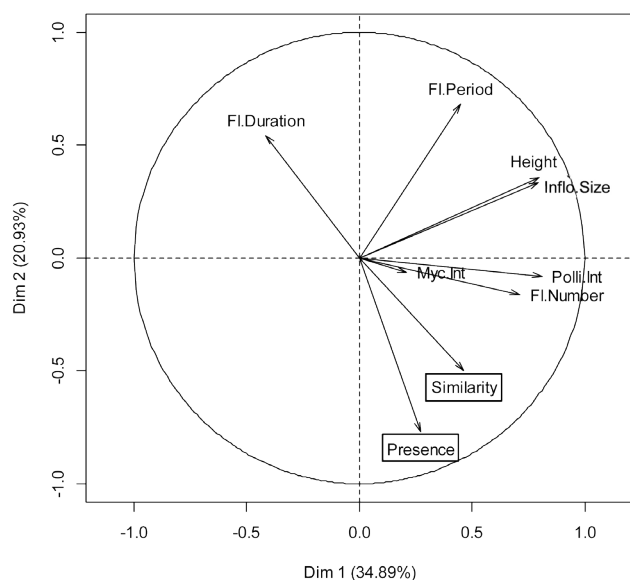
Fig. S1 Principal component analysis of the similarity index, probability of presence and species traits. Similarity = Similarity index, Presence = probability of presence, Polli.Int = number of species of pollinators, Myc.Int = number of mycorrhizal symbionts, Height = plant height, Inflo.Size = inflorescence size, Fl.Number = number of flowers, Fl.Duration = duration of the flowering period, Fl.Period = flowering duration.

Table S2 Results of Wilcoxon test of the comparison of the similarity-index of the recorded numbers (upper right) and probability of presence (bottom left) of species (a $p < 0.05$ is considered as significant). Abbreviations of species: Amo = *Anacamptis morio*, Apy = *Anacamptis pyramidalis*, Cda = *Cephalanthera damasonium*, Clo = *Cephalanthera longifolia*, Dfu = *Dactylorhiza fuchsii*, Ehe = *Epipactis helleborine*, Hhi = *Himantoglossum hircinum*, Lab = *Limodorum abortivum*, Nov = *Neottia ovata*, Oan = *Orchis anthropophora*, Oap = *Ophrys apifera*, Oma = *Orchis mascula*, Opu = *Orchis purpurea*, Osc = *Ophrys scolopax*, Osi = *Orchis simia*, Pbi = *Platanthera bifolia*, Pch = *Platanthera chlorantha*.

	Amo	Apy	Cda	Clo	Dfu	Ehe	Hhi	Lab	Nov	Oan	Oap	Oma	Opu	Osc	Osi	Pbi	Pch
Amo		0.7065	0.7619	0.91	1	0.73	0.84	0.79	0.79	0.63	0.93	0.63	0.31	0.48	0.93	0.93	0.34
Apy	0.24		1	0.26	0.56	0.61	0.80	0.67	0.78	0.11	0.67	0.19	0.47	0.05	0.32	0.53	**0.03***
Cda	0.21	**5.85e-05***		0.40	0.95	0.66	1	0.89	0.94	0.28	0.72	0.49	0.83	0.26	0.44	0.56	0.11
Clo	0.91	0.05	0.13		0.95	0.54	0.43	0.57	0.44	0.74	0.62	0.87	0.07	0.39	0.75	0.75	0.35
Dfu	0.73	0.48	**0.02***	0.42		0.62	0.57	0.96	0.69	0.51	1	0.70	0.15	0.18	0.88	0.64	0.15
Ehe	0.48	**0.002***	0.78	0.37	0.09		0.71	0.57	0.87	0.22	0.93	0.44	0.29	0.33	0.62	1	0.14
Hhi	0.53	**0.003***	0.19	0.63	0.14	0.52		0.81	1	0.17	0.66	0.14	0.20	**0.04***	0.46	0.37	**0.04***
Lab	0.70	**0.01***	0.55	0.57	0.18	0.86	0.79		0.80	0.30	1	0.36	0.08	0.15	0.46	0.64	0.07
Nov	0.70	**0.02***	0.09	0.93	0.33	0.38	0.56	0.57		0.31	0.80	0.42	0.32	0.14	0.61	0.54	0.07
Oan	0.94	**0.04***	0.14	1	0.33	0.33	0.76	0.53	1		0.49	0.85	**0.02***	0.45	0.76	0.72	0.26
Oap	0.07	**1.56e-06***	0.51	**0.02***	**0.003***	0.34	**0.04***	0.24	**0.008***	**0.03***		0.74	0.34	0.39	0.87	0.77	0.16
Oma	0.93	0.21	**0.01***	0.57	0.84	0.11	0.14	0.20	0.40	0.45	**9.15e-05***		0.09	0.31	0.70	0.97	0.19
Opu	0.47	**0.002***	0.46	0.43	0.10	0.80	0.68	1	0.37	0.50	0.14	0.10		**0.02***	0.15	0.28	**0.02***
Osc	0.63	**0.01***	0.46	0.62	0.21	0.86	0.96	1	0.78	0.53	0.14	0.27	0.94		0.23	0.30	0.48
Osi	0.79	**0.03***	0.07	1	0.37	0.30	0.49	0.51	0.95	0.91	**0.007***	0.46	0.31	0.65		0.96	0.11
Pbi	0.42	**0.002***	0.59	0.37	0.10	1	0.70	0.82	0.36	0.39	0.17	0.09	0.96	0.73	0.32		0.21
Pch	0.27	**0.00043***	0.52	0.14	**0.03***	0.40	0.15	0.23	0.11	0.10	0.80	**0.03***	0.27	0.33	0.10	0.33	

CULTURE AND CULTURAL LANDSCAPES AS FUNCTIONAL MATRICES FOR WILDERNESS – AND *VICE VERSA*

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Preparing for the 10th World Wilderness Congress, participants in Germany learn about the cultural dimensions of conservation.

Wilderness received special attention at the 10th World Wilderness Congress (WILD 10), which took place October 4–10 October in Salamanca, Spain. Prior to this congress, a series of events dubbed "The trail to Salamanca" marked preparatory efforts to sharpen the debate about wilderness in Europe. One of the events on this "trail" was a "Wilderness Seminar" held on November 5 to 8, 2012 in the German City of Potsdam. The seminar, which was jointly organized by the Brandenburg Wilderness Foundation and the international WILD Foundation, was attended by 33 participants from 10 nations (Germany, France, Great Britain, the Czech Republic, Kazakhstan, South Africa, Pakistan, Slovakia, the Ukraine and the USA) who considered the "first training component of the WILD 10 process".

Early during this event, a broad perception surfaced that wilderness would always remain a kind of antithesis to culture. Indeed one can think about this in terms of the connotation of the word "culture" in "agriculture", "horticulture" and "silviculture" as standing for "everything manmade", for "improvement" and "refinement".

This is not trivial and often makes for the key dilemma in conservation: in many regions (and "cultures") culture is considered to be something desirable and wilderness therefore undesirable. Thus the advocacy of wilderness and the conservation of free evolving natural processes often face superstition and opposition. Therefore this paper attempts to take the bite out of the perceived wilderness versus culture period after dichotomy. In the course of the of Potsdam seminar it was demonstrated very well that wilderness and culture mutually depend on one another. Due to the high profile of the presenters, such as Vance Martin, President of the WILD Foundation, the event turned out to be an account of the components of the global wilderness movement and quite an eye opener about future strategies for protecting wilderness. The seminar in Potsdam substantiated in many ways that the principle strategies for protecting wilderness are cultural based.

The first example of this is where this seminar was held. The state of Brandenburg (one of 16 of the Federal Republic of Germany) is located in the terrain of former Prussia, which is the political predecessor of the Third Reich and the state famous for its grandiose castles and infamous for its past bellicose culture. Absolutist kings and emperors of the 18th, 19th and early 20th century established Prussia as a global power next to Great Britain, France, Austria and Russia by developing an "unusually well-organized and effective army". In the 20th century, monarchy gave way to the dictatorship of the "Third Reich", then to World War II and finally the Cold War. For this the state of Brandenburg provided vast areas of military training grounds. In 1994, just after the end of the Cold War, the Brandenburg Wilderness Foundation was established in order take responsibility for these former military areas, which currently cover an area of 12,800 hectares.

It was during this period (in 1992) that conservation in Germany gained prominence when Hans Bibelriether, the first director of Germany's first National Park, coined the catchy motto "let nature be" for what later became internationally known as the "non-intervention management" idea of wilderness. Consequently for the Brandenburg Foundation wilderness means "unused landscapes not regulated and managed by humans where nature can develop following its own rules." according to Hans Joachim-Mader, who chairs the Brandenburg Wilderness Foundation.

In their joint foreword to the seminars both Vance Martin and Hans-Joachim Mader acknowledge the cultural value of this conversion: "After several centuries of bellicose, totalitarian regimes (…) large tracts of country are now being allowed to revert back to a wild state and Mother Nature is the new regime (…) This is a major and long-term change, which is occurring not only in the landscape but also, and most importantly, in the minds of people. This is culture, this is progress; this is making history, and yes, lest we forget, this is wilderness at work."

This enthusiastic exclamation about "making history" is not a exaggeration. Considering the close proximity of Potsdam to the capital of this nation, Berlin, the willful transformation of military training grounds into wilderness is a paradigmatic change in this nation's values. This change will receive even more attention as the landholdings near Berlin are to be displayed in a landscape wide "International Nature Exhibition".

The idea of wilderness is pregnant with all kinds of "symbolisms" and "values" and thus a cultural phenomenon. Vance Martin pledges to take the triple value of wilderness seriously: "But what is the value in wilderness protection? If we put this question to conservationists trained in biology, the answer would probably be 'biological intactness' and 'ecosystem function'. If we ask natural resource administrators, they would maintain that socio-economic motives such as 'recreation' and 'subsistence' are valid reasons for wilderness protection. Yet another clientele requests intangible ecosystem services such as 'inspiration', 'beauty', 'mystery' and 'spirit'. So to what means and ends should we protect wilderness? The answer for The WILD Foundation clearly is: all of the above. WILD chooses a three dimensional approach to wilderness protection that includes biological, socio-economic and iconic motives".

An icon of course is a symbol, which stands for certain assumed functions. The icon "wilderness" is often associated with "richness in natural history" and "biodiversity", but also with "chaos", "destruction" and "danger", and is said to "inspire awe" and challenges our para-religious feelings, such as "sublimity." Wilderness travelers swear that exposure to wilderness has a "cleansing effect" and makes us exercise "humility" and "restraint", and questions the spreading of the "culture of convenience". In other words: wilderness is a vector which could help pave the way to certain desirable personal and societal traits. Seen more neutrally, wilderness has cultural functions, such as "shared values and practices", and "aesthetic training" (as described in Princeton University´s "word-net").

These cultural functions of wilderness can be extracted using the cultural skill of communication. The seminar demonstrated that explicitly. Karl Friedrich Sinner, former director of the Bavarian Forest National Park and vice chairman of Europarc-Germany, which is the umbrella organization for all large protected areas in Germany describes the communication skill required: "Wilderness protection is most of all a question of communication, which should address both the mind and the heart (…) It is a story of many plots that need to be related. We have to relate that Urwald (natural forest) is not just about 'big trees', but mostly about different phases of development, which change yearly and often on a daily basis. We have to relate that death in nature is much more than carnivores eating herbivores. The variety of brightly-coloured fungi reveals that the life of a wild forest is largely built by agents of decomposition; the tree mushrooms, the beetles which depend on decaying wood, the many bird species that live on these insects …"

Another presenter, Kevin Hood, Wilderness Manager of the US Forest Service in Alaska, stressed the importance of communication in wilderness conservation and that historically "the shift to a broad wilderness advocacy in American society came with John Muir, a Scottish-born naturalist and author. Muir's articles, books and his special gift of storytelling helped in creating the groundswell that eventually resulted in establishing Yosemite National Park in 1890."

One of the most primeval cultural skills, storytelling around a campfire, was basic to the foundation of WILD in 1974. According to Vance Martin, "Dr. Ian Player, a game ranger of Anglo heritage, and his Zulu mentor, Magqubu Ntombela, pioneered the first walking safaris, or 'trails', in the South African wilderness. They knew this would be the best way to connect people to themselves, one-another and foster a relationship between people and nature."

The accompanying campfire talks between members of indigenous tribes and largely Caucasian "white" scientists and conservationists was in the early days not only in marked contrast to the racial segregation practiced then but later proved to be the key to successful wilderness protection. During later years the friendly camp fire talks would evolve into systematic round table events for stakeholders preparing "wilderness management plans".

In his presentation on this subject Drummond Densham of the Wilderness Action Group of South Africa cautioned: "Extra care must be taken in South Africa to prevent local communities thinking their land is being taken over, thus producing a historical déjà-vu. In reality this does not occur in South Africa. Stakeholders around the planet should feel that they are gaining by having areas in their countries designated as wilderness." Later comments revealed that wilderness management was greatly appreciated, which for some in the audience was quite a new idea. Some even admitted that citizen participation in conservation planning was still quite poor in some European countries.

It is this kind of cultural and cross-cultural communication that made the World Wilderness Congress a success. The first one was held in Johannesburg, South Africa in 1977. Today the World Wilderness Congress is considered to be the longest running public conservation forum and platform. At the most recent WILD 9 venue in 2009 in Mexico, 1800 delegates from more then fifty nations attended.

As the recent Wilderness Congress (WILD 10) took place in Salamanca, many of the presented topics did of course originate from Europe, where the landscape is often even more fragmented than in other continents. The state of Brandenburg is a good example as it largely consists of "cultural landscapes", meaning that most of the features in the landscape are man made. Needless to say the idea of wilderness may be much more challenging to execute here than in many other countries.

In order to meet this challenge the Brandenburg foundation developed the ambitious "Ecological Corridor of South Brandenburg" near Berlin, which facilitates the migration of organisms between Poland and Germany. Wolves have recolonized the state and now count seven family units ("packs"). Systematic monitoring of this corridor revealed an increase in ground-dwelling insects (*Carabidae*), which in turn had positive effects on the

breeding success of birds such as the Red-backed shrike *(Lanius collurio)*.

The "corridor for biodiversity" according to Mader is a "result of a concerto of instruments that work well together: features in the landscape such as edges, linear structures and stepping stones connected by way of underpasses, culverts, green bridges, riparian strips and fish ladders. Great results can often be achieved by working with and enhancing existing structures. This and the continuing support of the people of Brandenburg help to constitute the underlying matrix where wildness can perpetuate itself."

This idea of a large landscape conservation system as a "matrix" to "help wilderness perpetuate itself" works both ways. Wilderness can be beneficial to cultural landscapes as wilderness is more likely to contain the original blend of faunal and floral ingredients of a particular region. In addition, as the scientific report of the Bavarian Forest National Park (2009) concluded, large protected areas provide "threshold values e.g. for forest age, quantities and different dimensions of dead wood, canopy cover and nesting holes. These are the basis for a procedure, by which the conservation relevant areas within a forestry site may be identified (…) on a landscape level as well as on forest stand and individual object level."

Therefore it can be concluded that even concepts of "horticulture" and "silviculture" will benefit from the "gold standard", which is represented in terms of wilderness. The American ecologist Aldo Leopold knew this already in the 1940s as he wrote: "Each biotic providence needs its own wilderness for comparative studies of used and unused land." Consequently wilderness should not be regarded as in opposition to a cultural landscape but a vital ingredient of it.