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Application of Two Way Indicator Species Analysis in Lowland Plant Types Classification

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Abstract: A TWINSPAN classification of 60 sample plots from the Khanikan forest (North of Iran) is presented. Plant types were determined from field observations and sample plot data arranged and analyzed in association tables. The types were defined on the basis of species patterns of presence, absence and coverage values. Vegetation was sampled with randomized-systematic method. Vegetation data including density and cover percentage were estimated quantitatively within each quadrate and using the two-way indicator species analysis. The objectives of the study were to plant type's classification for Khanikan lowland forest in North of Iran, Identification of indicator species in plant types and increase our understanding in regarding to one of Multivariate analysis methods (TWINSPAN). Five plant types were produced for the study area by TWINSPAN, i.e., *Menta aquatica*, *Oplismenus undulatifolius*, *Carex grioletia*, *Viola odarata* and *Rubus caesius*. Therefore, at each step of the process, the program identifies indicator species that show strongly differential distributions between groups and so can severe to distinguish the groups. The final result, incorporating elements of classification can provide a compact and powerful summary of pattern in the data set.

Key words: Classification, TWINSPAN, plant types, Iran

INTRODUCTION

Multivariate analysis methods assume that several random variables have interred relation between each other and that they are important for the researcher (Ajbilou *et al*., 2006). Classification and ordination are two main branches of multivariate analysis and in this study as classification method was used. Due to lack of statistical analysis, understanding the structure of plant species is associated with considerable mistakes, therefore, in the first step of each study, vegetation should be classified (Dollar *et al*., 1992).

TWINSPAN have designed on the basis FORTRAN computer program that use for study of ecological factors and species. Study of plant vegetation in natural sites with compare tables has known Dombois and Ellenberg (1974) handy method (Host and Register, 1991). Hill (1979) has modified Dombois and Ellenberg (1974) handy method to binary clustering analysis. Afterwards with progressive in computer program, this method had displayed with name of TWINSPAN.

Main idea of TWINSPAN is on the basis the first hypothesis of phytosociology that group of quadrates will distinct by group of different species. This species will settle in binary tables. Namely, quadrates have compared each other on the basis presence or absent of species and pseudo species. The quadrates that have more similar to each other are beside each other (White and Hood, 2004).

TWINSPAN simultaneously classify species and samples (Grant and Loneragan, 2001). The program is geared towards ecological data. At its core, TWINSPAN is based on dividing a reciprocal averaging ordination space. The procedure was described in accessible terms by Gauch and Whittaker (1981) and White and Hood (2004). One of the most useful features of TWINSPAN is the final ordered two-way table. Species names are arrayed along the left side of the table, while sample numbers are along the top. The pattern of zeros and ones on the right and bottom sides define the dendrogram of the classification of species and samples, respectively. The interior of the table contains the abundance classes are defined by pseudo species cut levels (White and Hood, 2004).

Currently TWINSPAN is probably the most frequently used procedure for the classification of community data sets (Sebald, 1964; Spies and Barnes, 1985; Kent and Coker, 1996; Mc Cune and Mefford, 1999; Mesdaghi, 2001). Fundamental to TWINSPAN is the concept of pseudo species. Although information on the abundance of species is retained, the pseudo species scaling will reduce is that it allows the occurrence and

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abundance of species to treat as essentially separate variables, recognizing that both can be important characteristics of an association (White and Hood, 2004).

Also, the most popular divisive method (and program of the same name) TWINSPAN (two-way indicator species analysis) was partially inspired be the classificatory methods of the classical phytosociology (use of indicators for definition of vegetation types). The idea of an indicator species is basically a qualitative one. Consequently, the methods work with qualitative data only. In order not to loose the information and the quantity of species, the concept of pseudo species and pseudo species cut levels was introduced. Each species can be present as several pseudo species, according to its quantity in the sample. The pseudo species is present, if the species quantity exceeds the corresponding cut level. Imagine that we selected pseudo species cut levels (Waite, 2000).

The present study is, however, the first comparatively extensive one on the botany of Khamikan lowland forest (North of Iran) which seems to be interesting from the biodiversity viewpoint. The present study, therefore, deals with the species composition and life form structure of this forest. It also aims at identifying the prevailing plant types using technique of multivariate analyses (TWINSPAN). The objectives of the study were to plant type's classification for Khamikan lowland forest in north of Iran, identification of indicator species in plant types and increase our understanding in regarding to one of Multivariate analysis methods (TWINSPAN).

MATERIALS AND METHODS

Study area: Khanikan forests are located in the lowland and midland of Mazandaran province in north of Iran with the area of 2807 ha. (Between 36° 33' 15", 36° 37' 45" latitude and between 51° 23' 45", 51° 27' 45" longitude). The maximum elevation is 1400 m and the minimum elevation is 50 m. Minimum temperature in December (7.5°C) and the highest temperature in June (24.6°C) are recorded, respectively. Mean annual precipitation of the study area were from 698.2 to 102.5 mm at the Noushahr city metrological station, which is 10 km far from the study area. This research was performed in summer of 2006.

Data collection: In lowland region 268.7 ha of this forest was selected. For investigation of tree and shrub covers sixty quadrate (20×20 m AR.) (Hill, 1979; Hedınan and Graceland Ling, 2000; Mesdaghi, 2005) and sub quadrate (1 m^2 AR.) in each quadrate for investigation of herbaceous covers (Monier and Abd, 2000; Mesdaghi, 2005), were taken by randomized-systematic method. Quadrate size was determined for each vegetation type using the minimal area method (Cain, 1959). Considering variation of vegetation, floristic list and canopy cover percentage were determined in each quadrate.

Data analysis method: Data matrix of quadrates number and vegetation type was made. The windows (Ver. 3.0) of PC- ORD (Mc Nab *et al.*, 1999) were used for classification of plant types. Data were analyzed by multivariate techniques as the Two-way indicator species analysis (TWINSPAN). TWINSPAN analysis is a numerical method for classification of vegetation belonging to similar groups. This allows the investigator to recognize the homogenous groups. Two-way indicator species analysis using the computer program TWINSPAN (Waite, 2000), a commonly used program in ecological studies. It is a robust technique, fairly impermeable to sampling errors or noise (Grant and Loneragan, 2001).

Identification of indicator species: Indicator species are those that occur predominantly among the samples at one end of an ordination axis. Their distribution should reflect, i.e., indicate, the environmental characteristic of the samples at either end of the gradient depicted by the ordination axis. Samples along the ordination axis are divided into two groups about the centre of the axis. Those to the right of the centre are placed in one group, called the positive group (+); those to the left are placed in a different group, called the negative group (-). Based on the distribution between these groups, an indicator value is calculated for each species using the formula (White and Hood, 2004):

$$Ij = nj^{+}/N^{+} - nj^{-}/N^{-} \quad (1)$$

where, Ij, the indicator value for species j; nj^{+}, the number of samples in the positive (right-hand) group containing species j; nj^{-}, the number of samples in the negative (left-hand) group containing species j and N^{+}, N^{-}, the numbers of samples in the positive group and the negative group.

RESULTS AND DISCUSSION

In studied area 56 species of 36 families were recognized that the number of woody species and herbaceous were 14 and 42 respectively (Table 1). Life forms were determined by Raunkiaer system and according to the biological spectrum, phanerophytes and cryptophytes (35.71%) and hemicriptophytes (28/57%)

Table 1: Species, life form, endemic and family of recorded species in study area

Vegetation group species	Life form[1]	Cerotype[2]	Endemic	Family	Vegetation group species	Life form[1]	Cerotype[2]	Endemic	Family
Carpinus betulus L.	Ph	H		Betulaceae	*Pteris dentate forssk.*	Cr	H		Pteridaceae
Parrotia persica(dc.)	Ph	H	*	Hamameliadaceae	*Circeae lutetiana* L.	He	H		Onagraceae
Cratagus pentagyna Waldst and kit.	Ph	H,M,IT		Rosaceae	*Oplismenus undulatifolius* p.	Cr	H,M,IT		Graminaceae
Quercus castanifolia c.a.m.	Ph	H,M,IT		Fagaceae	*Calystesia septum* (L.).	He	H		Umbelliferae
Buxus hyrcana pojark.	Ph	H	*	Buxaceae	*Hypericum androsaemus* L.	Ph	H,M		Hypericaceae
Diospyrus lotus L.	Ph	H,IT		Ebenaceae	*Fragaria vesca* L.	Ph	H		Rosaceae
Ilex aquifolium L.	Ph	H	*	Aquifoliaceae	*Prunlla vulgaris* L.	He	H		Labiatae
Ulmus glabra huds.	Ph	H		Ulmaceae	*Euphorbia amygdaloides* L.	He	H		Gramineae
Mespilus germanica L.	Ph	H,M,IT		Rosaceae	*Tamus communis* L.	Cr	M		Dioscoraceae
Alnus glutinosa (L.)	Ph	H	*	Betulaceae	*Sanicula europaea* L.	He	H,M		Umbelliferae
Pterocarya fraxinifolia(lam.)	Ph	H	*	Juglandaceae	*Danae racemosa*(L.)moench	Ph	H	*	Liliaceae
Acer insign boiss.	Ph	H		Acearaceae	*Solarum kieseritzkii* c.a.mey.	Cr	H		Umbelliferae
Ficus carica L.	Ph	POL		Moraceae	*Festuca drymeia mert*, koch	Cr	H		Gramineae
Ruscus hyrcanus L.	Ph	H	*	Liliaceae	*Dryopteris filix-mas*(L.) t	Cr	H		Aspidiaceae
Carex grioletia L.	Cr	H,M,IT		Cyperaceae	*Microstegium vimentium* L.	He	H,M		Gramineae
Smilax exelsa L.	Ph	H,IT		Asparaginaceae	*Ophioglossum vulgatum* L.	Cr	H		Ophioglossaceae
Primula heterocliroma stapf.	He	H	*	primulaceae	*Parieturia officinalis* L.	Cr	H,M		Urticaceae
Brachypodium pinnatum (L.)	He	H,M,IT		Gramineae	*Geum urbanum* L.	He	H,M,IT		Rosaceae
Pteris cretica L.	Cr	POL		Pteridaceae	*Menthe aquatica* L.	He	POL		Labiatae
Scutellaria tournefortii benth.	He	H,IT		Labiatae	*Plantago major* L.	He	POL		Plantaginaceae
Viola odarata L.	He	H,M		Violaceae	*Pimpinella affinis* ledeb.	Cr	H		Umbeliferae
Asplenium adiantum-nigrum	Cr	H		Aspleniaceae	*Oxalis corniculata* L.	He	H		Oxalidaceae
Equisetum ramossisimum desf.	Cr	H		Equisetaceae	*Lamium album* L.	Cr	H		Labiatae
Conyza bonariensis L.	He	POL		Compositae	*Mercurialis prennis* L.	He	H		Euphorbiaceae
Aspelenium trichomanes L.	Cr	H,IT		Aspleniaceae	*Cardamin impatiens* L.	Cr	H		Cruciferae
Phyllitis scdopendrium L.	Cr	H		Aspleniaceae	*Rubus caesius* L.	Ph	H		Rosaceae
Pteridium aquilinum L.	Cr	H,M		Hypolepidaceae	*Urtica dioica* L.var.*dioica.*	Cr	POL		Urticaceae
Hedra pustuchovii woron. Life	Ph	H		Araliaceae	*Carex acutiformis* L.	Cr	H,M		Cyperaceae

form[1]: Ph: Phanerophyte. Cr: Cryptophyte. He: Hemicriptophyte. Chorotype[2]: H: Hyrcanian. M: Mediteranian. It: Irano -Touranian. Pol: Poly zona

were dominant life forms of the studied area. Also, vegetation chorology showed hyrcanian elements with 55.35% was dominant chorotype of khanikan lowland forests. Number of 8 species (14.287%) was endemic of Iran Flores (Table 1).

Five plant types were identified after the application of TWINSPAN programs (Table 2, 3). They are named after the characterizing species as follows: *Menta aquatica* (I), *Oplismenus undulatifolius* (II), *Carex grioletia* (III), *Viola odarata* (IV) and *Rubus caesius* (V) (Table 3). The most number of plant species (36) is relation to III plant type and the least of it (15) is relation to I, IV plant types (Table 4). Also, III plant type and I plant type had the most (23.7) and the least (8.3) mean of cover (%), respectively (Table 4).

The plant types were defined for the Khanikan lowland forests in North of Iran. It was the first attempt to develop such plant types in this area, thus making it impossible to compare this study with other studies. Five plant types are generated, in the present study, after application of two-way indicator species analysis (TWINSPAN) to the cover estimates of 56 species in 60 quadrates (Table 2, 3). Cluster analysis has performed for data of species covering (Table 3).

The first division, 60 quadrates have divided to two clusters that in left direction exists 56 quadrates and exists no indicator species. In right direction exists 4 quadrates with *Menta aquatica* indicator species. The second division, 56 quadrates have divided to two clusters that in left direction exists 23 quadrates with *Brachypodium pinnatum* indicator species and right direction exists 33 quadrates with *Hedra pustuchovii*, *Euphorbia amygdaloides* and *Ruscus hyrcanus* indicator species. The third division, 23 quadrates have divided to two clusters that in left direction exists 4 quadrates with *Rubus caesius* indicator species and right direction exists 19 quadrates with *Viola odarata* indicator species. The fourth division, 33 quadrates have divided to two clusters that in left direction exists 17 quadrates with *Carex grioletia* indicator species and right direction exists 16 quadrates with *Oplismenus undulatifolius* indicator species.

For determination of indicator species in classified types have used the formula (1). Thus, five plant types are named as follows: I plant type includes *Menta aquatica* with *Carpinus betulus* woody indicator species. II plant type includes *Hedra pustuchovii*, *Euphorbia amygdaloides* and *Ruscus hyrcanus* with *Oplismenus undulatifolius*. III plant type includes *Hedra pustuchovii*, *Euphorbia amygdaloides* and *Ruscus hyrcanus* with *Carex grioletia*. IV plant type includes *Viola odarata*, *Brachypodium pinnatum* with *Parottia persica* and *Cratagus* sp. Woody indicator species. V plant type includes *Rubus caesius*, *Brachypodium pinnatum* with *Parottia persica*, *Cratagus* sp. and *Quercus castanifolia*.

Result of this research have showed that III plant type had the most number of plant species (36) and I, V plant types had the least of it (15) (Table 4). Also, have

Table 2: TWINSPAN of the vegetation cover in 60 quadrates and 56 species

	I	II	III	IV	V	Main groups
	4444 3567	14 2312334552345 2298605789467903	23 136 112222345 9421104480236581	34 5 123355511551 3115826610285779095	14 3734	No. of samples Species
000	46-4	----3--4-43-344-	--4-451--133-----	43--64341-3123-5--3	-11--	7 viol oda
000	-3--	-----6-34-4-----	----------	66--8----------	8765	41 rubu cae
000	6667	6755665566661664	4565465554545568	6665664454467654665	4466	43 carpi be
001000	----	----------4-4--	------3-------4--	--333411-----35444-	-2-3	4 brac sp
001000	----	----------	----------	----3----------	----	15 pter den
001000	----	----------	----------	----------4	----	21 prun vul
001000	----	----------	----------	-2----------	----	30 ophi vul
001000	----	----------	----------	----------	---3	34 plan maj
001000	----	----------	----------	----------	---1	35 pimp aff
001000	----	----------	----------	----------1--	----	39 card imp
001000	----	4----------5-	-22-3----13------	4-3-5--43---34-4-52	--2--	45 crata sp
001000	----	----------	----------	------5----------	-4--	46 quer cas
001000	----	----------2-	----------	----------3-	----	51 mesp ger
001000	----	----------	----------	----------	---6	56 care sp
001001	----	-------3-32-34--	44--------1------	-22344--34----------	----	3 prim het
001001	----	45---333-442-35-	3556547-44444465	5746446424564434652	452-	44 parr per
001010	----	----------	--76-4-3---1-----	--13--1-----2-3-----	----	2 smil exc
001010	----	----------	----------1---4--	-1--------1--------	----	23 tamu com
001011	----	346333----3-----	--144453434114 34	431-----1--22-43436	--14	1 care gri
001011	----	----1-----23--4-	4-1----3---------	----------23---2-	-11-	6 scut tor
001011	----	---1-1-------5-	-------31-3------	----------	---5	19 hype and
001011	----	----------54-	---------1-------	----------4	--1-	20 frag ves
001100	----	-----43-43353---	--231413-3--4-52	--334----------	--42	5 pter cre
001100	----	---4---3---2---2	44----1------454	-3---1---------43--	----	12 phyl sco
001100	----	3----354-4343--4	--3---644-43334-	--3----4---1-------	----	40 rusc hyr
001101	----	--------3--------	643-3--3-144-----	----------	----	8 aspl adi
001101	----	----------	--3----------	----------	----	10 cony bon
001101	----	----------	------3----3-----	----------	----	11 aspe tri
001101	----	----------	-----61----------	----------	----	13 pter aqu
001101	----	----------	-------4---------	----------	----	16 circ lut
001101	----	----------	----------3-----	----------	----	24 sani eur
001101	----	----------	----------2--	----------	----	25 dana rac
001101	----	------1---------	----------	----------	----	26 sola kie
001101	----	----------	----------54	----------	----	38 merc pre
001101	----	----------	-2----------	----------	----	49 ilex aqu
001101	----	----------	-1----------	----------	----	50 ulmu gla
001101	----	----------	----------3--	----------	----	55 ficu car
001110	----	---44-4-45---4--	4-------4-4----4	----------	----	22 euph amy
001111	----	--4----3-3---44-	--1---1----------	----------	----	9 equi ram
001111	----	---1-1----------	----------	----------	----	18 caly sep
001111	----	----------45-	----------	----------	----	27 fest dry
001111	----	---------242--34	----------	----------1-----	----	29 micr vim
001111	----	----------3---	----------	----------	----	42 urti dio
001111	----	-4-34--55---6---	----------4-2	----------	----	47 buxu hyr
01	-4--	-333--------321-	64-33331-33-13-1	-1----------	----	14 hedr pus
01	6466	656477444-77-66-	-4--36-------3--1	-2---5------54----1	--36	17 opli und
01	--5-	-4----------4-6	----------	----------	----	28 dryo fil
10	--4-	----------	----------4	----------	----	54 acer ins
110	--6-	----------	-----2----------	----------	----	36 oxal cor
1110	6---	----------	----------	----------	----	31 pari off
1110	53--	----------	----------	----------	----	32 geum urb
1110	7676	----------	----------	----------	----	33 ment aqu
1110	--4-	----------	----------	----------	----	37 lami alb
1110	4-4-	----------	----------	----------	----	52 alnu gl
1110	3---	----------	----------	----------	----	53 pter fra
1111	3---	----------	1----------	----------	----	48 dios lot
	1111	00000000000000 11111111111111 11111111111111 00000000000011 00000111111 0011100000001	00000000000000 11111111111111 00000000000000 00011111111111 0000000000011 00001111111	00000000000000000 00000000000000000 11111111111111111 00000000001111111 00111111111100000 0000111111001111	0000 0000 0000	Groups code

showed that III plant type and I plant type had the most (23.7) and the least (8.3) mean of cover (%), respectively (Table 4).

The use of quantitative values (coverage values) was essential in defining and using plant types. There was a certain degree of overlap among the types; in almost all cases more than one type occurred in a given ecosystem. Such over lapping was observed in Northern Michigan ecosystem studies (Kashian *et al*., 2003) and in West Germany (Dahdouh *et al*., 2002). Nevertheless, some plant types were more characteristic than others of certain types of ecosystems.

Ecological classification and grouping of forest habitats was the main subject of forest management since

Table 3: TWINSPAN indicator species for the first three level of division

Level 1	Level 2	Level 3
Menta aquatica 0	-	-
- 1	*Hedra pustuchovii*0	*Oplismenus undulatifolius*0
	Euphorbia amygdaloides	1 *Carex grioleta*
	Ruscus hyrcanus	
	1 *Brachypodium pinnatum*	0 *Viola odarata*
		*Rubus caesius*1

Table 4: Number and mean cover of species in plant types

Plant types	I	II	III	IV	V
No. of species	15.00	25.00	36.00	26.00	15.00
Mean of cover (%)	23.75	18.23	8.35	10.83	10.76

of 1980 decade (Barbour, 1999). Many methods had used in order to classification of forest habitat (Bairley, 1978; Barnes, 1982) but, they couldn't show, the relation ecosystem components very well. Because, the most of they, have been used of one component similar to soil or plant vegetations, alone (Dahdouh *et al*., 2002).

Using of two-way indicator species analysis in order to plant type's classification have been dealt with in numerous works. Among the main ones are : Host and Register (1991), Dollar *et al*. (1992), Zahedi Amiri (1998), McNab *et al*. (1999), Witte (2002), Picard and France (2003) and Kashian *et al*. (2003).

Now days, multivariate methods have been using in systems of ecological classification, extensively (Witte, 2002; Picard and France, 2003). Traditional systems of classification, had used on the basis dominant species or species similarity that its basis was subjective (Verlinden and Dayton, 2005) but, the new systems of classification is on the basis objective methods that has high precision and researchers reaches to some results with this systems (Ajbilou *et al*., 2006).

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