Pak. J. Bot., 45(2): 593-598, 2013.

MORPHOLOGICAL CHARACTERISTICS FOR CLASSIFYING EVERGREEN AZALEA (*ERICACEAE*) CULTIVARS IN CHINA USING NUMERICAL TAXONOMY

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Abstract

Azaleas are among the 10 famous traditional flowers in China having a long cultivation history since the Tang Dynasty. Today, there are probably over 300 evergreen azalea cultivars preserved in different nurseries, all of which are usually classified as belonging to East, West, Summer and Hairy groups. Numerical taxonomy was carried out to determine the phenetic relationships between cultivars. Sixty six evergreen azaleas cultivars were studied based on 30 morphological characters (13 qualitative and 17 quantitative). Cluster analysis using unweighted pair group method with arithmetic average (UPGMA) and principal coordinate analysis (PCO) was performed by using NTSYS software. Mantel's test showed a good fitness between the cluster analysis and Euclidean distance matrix. The first three axes in PCO explained 49.94% of the total variation, and revealed that 14 characters, most of which are related to flowers, played an important role in numerical taxonomy. Both cluster analysis and PCO suggested that cultivars in the East and summer groups were distinct from other groups in the 4 group classification system based on the morphological characteristics, but the West and Hairy groups were closer when concerned primarily with phenetic relationships.

Introduction

The genus *Rhododendron* (*Ericaceae*) is famous world-wide for the diversity of floral colors and forms of its more than 1000 species. Among them, over 800 species are distributed throughout the Northern Hemisphere, ranging from tropical to polar climates and more than 500 species are native to China. It is divided into 8 subgenera (*Azaleastrum*, *Candidastrum*, *Hymenanthes*, *Mumeazalea*, *Pentanthera*, *Rhododendron*, *Therorhodion* and *Tsutsusi*; Chamberlain *et al.*, 1996). The three subgenera viz. *Azaleastrum* (evergreen) *Tsutsusi Pentanthera* comprise the 'azaleas' (De Keyser *et al*., 2010).

Azaleas are among the most important ornamental shrubs in Europe since the 18th century (Scariot *et al.*, 2007). In the last hundred years, many species brought to Europe from China and Japan. The hybridization activity reached remarkable levels in Europe since then, especially in Belgium and the United Kingdom. There are at least 4 or more well defined groups of evergreen azalea cultivars: Belgian or pot azaleas (*Rhododendron simsii* Planch. putative hybrids) (De Riek *et al.*, 1999), Hirado azaleas (*R. scabrum* G. Don putative hybrids), Kurume azaleas [putative hybrids of *R. kiusianum* Makino var. *kiusianum* and var. *sataense* (Nakai) D.F. Chamb. and *R. kaempferi* Planch.] and Satsuki azaleas [putative mutants or hybrids of *R. indicum* (L.) Sweet and *R. eriocarpum* [(Hayata) Nakai] (Scariot *et al.*, 2007).

In China, azaleas became popular as ornamental flowers in the Tang Dynasty and are now recognized as one of the ten famous traditional flowers. Exotic cultivars arrived from Japan and Europe in the 19th century. It is believed that one of the first arrivals is now reserved in Jiashan county (Zhejiang Province), where azaleas are so popular that could be found in almost every family. National azalea exhibition is organized continuously to encourage and reward the gardeners for new cultivars and well-shaped bonsai since 1987. Cultivars have been gathered, selected by local gardeners and hybridized. There are now over 300 cultivars collected in Dandong (Liaoning Province), Wuxi (Jiangsu Province) and Jiashan. The present classification system is put forward by Huang & Qiang (1984) according to the phenotype and the origin: (1) East azaleas, formed by cultivars with small leaves and hose-in-hose flowers; (2) West azaleas, including plants with large flowers and semi-double to double petals; (3) Hairy azaleas, characterized by large simple flowers and finely pubescent stems and leaves; and (4) Summer azaleas, cultivars blooming in the early summer. The group 'East' should be called this way because it is believed that most cultivars were brought from Japan which lies right to the east of China. The name 'West' came out for the similar reason. Azaleas in this group arrived from western countries decades ago, probably having their origin in Belgium and so called Belgian azaleas sometimes.

From many years, Chinese taxonomists are keep focusing on the discovery of new species (Li, 2001) and classification of wild species of genus *Rhododendron* using molecular techniques as RAPD (Tan *et al*., 2005) and ITS sequences (Gao *et al.*, 2002). Studying on the cultivars just started a few years ago, beginning with the basic survey on the cultivars commonly seen in Dandong (Zhang *et al.*, 2005) and Changsha (Hunan Province) (Hou *et al.*, 2006).let alone the genetic diversity. It is not surprise to see different cultivars sharing the same name or the same plant called differently. On the contrary, a lot of work has been done on evergreen azalea morphology in Europe, including diversity of flower colors (Heursel, 1981), corolla size, number of stamens and percentage of plants with petaloid stamens (Heursel & Garretsen, 1989) and other taxonomic characters (Chamberlain & Rae, 1990).

The main goals of this research were to: (1) set a series of characters using all the traits available in the literature (with minor modifications) to describe cultivars, as there is no detail about most of the cultivars; (2) do a thorough morphological investigation and statistical analysis based on these characters; (3) classify evergreen azaleas in China using morphological traits prior to the genetic diversity investigation.

Materials and Methods

Plant materials□Sixty-six evergreen azalea cultivars were investigated (Table 1). Most of them were chosen from the 4 local groups: East azaleas (36 genotypes), West azaleas (6 genotypes), Hairy azaleas (9 genotypes) and Summer azaleas (13 genotypes). Within the East azaleas and Hairy azaleas, some cultivars obtained from *bud sporting* were included (‘Yuhudie’ with pink flower and ‘Baihudie’ with pure white flower are bud sports of ‘Zihudie’; ‘Fenshanhu’ is a bud sport of ‘Hongshanhu’). Most of the plants were potted and maintained in greenhouse at Jiashan or Wuxi. Two unknown genotypes collected from Dandong were also included in this analysis. The criteria used in the selection of the individual plant were age, size and health condition. Three healthy adult plants with similar crown diameter were selected for each cultivar.

Assessment of morphological traits□Ten fully expanded summer leaves and 5 fully blooming flowers were sampled from the mid-to-upper crown of each plant. They must be insect and disease free. A total of 30 morphological characters were measured for each specimen: 13 qualitative and 17 quantitative. Assessment was carried out by the following variables (Table 2).

Data analysis□All the analyses were performed using NTSYS-pc Version 2.10e (Rohlf, 1994). The initial data were converted to rectangular matrix and standardized using STAND module. Genetic distance matrix were calculated by Euclidean distance and then used to do cluster analysis using unweighted pair group method with arithmetic average (UPGMA). Mantel’s (1967) test was computed to examine how well the cluster analysis fits the distance matrix using COPH and MxComp modules. The standardized matrix was also converted to product-moment correlation matrix, and then a principal coordinate analysis (PCO) was performed essentially as described by Meudt & Bayly (2008) based on the correlation matrix.

Table 1. List of names, group types and collection locations of cultivars (all the cultivars belong to the Subgenus *Tsutsusi*).

ID	Name	Type	Collection location	ID	Name	Type	Collection location
B02	‘Shieryichong’	West	Wuxi	C31	‘Yushuang’	East	Jiashan
B05	‘Jinpao’	West	Wuxi	C34	‘Waiguohong’	East	Jiashan
B08	‘Fuguiji’	West	Wuxi	C35	‘Liuqiuhong’	East	Jiashan
B10	‘Hengdi’	West	Wuxi	C36	‘Zhuangyuanhong’	East	Jiashan
B11	‘Wangguan’	West	Wuxi	C41	‘Caiwubao’	East	Jiashan
B13	‘Fengmingjin’	West	Wuxi	C45	‘Zuihaitang’	East	Jiashan
C02	‘Dazhusha’	East	Jiashan	C48	‘Zijuan’	East	Jiashan
C05	‘Wusedaqiao’	East	Jiashan	C49	‘Hongyue’	East	Jiashan
C08	‘Xiaoqinglian’	East	Jiashan	C50	‘Lanying’	East	Jiashan
C09	‘Daqinglian’	East	Jiashan	C51	‘Lanlian’	East	Jiashan
C13	‘Hongshanhu’	East	Jiashan	C54	‘Zhichun’	East	Jiashan
C14	‘Fenshanhu’	East	Jiashan	C58	‘Manao’	East	Jiashan
C15	‘Baipingfu’	East	Jiashan	C64	‘Dahezhichun’	East	Jiashan
C18	‘Fenzhuanglou’	East	Jiashan	C69	‘Bizhi’	East	Jiashan
C19	‘Xiaotaohong’	East	Jiashan	C70	‘Xiaomeigui’	East	Jiashan
C24	‘Yunshang’	East	Jiashan	C74	‘Zihudie’	Hairy	Wuxi
C75	‘Yuhudie’	Hairy	Wuxi	M02	‘Meiguiliuqiuhong’	Hairy	Wuxi
C76	‘Baihudie’	Hairy	Wuxi	M03	‘Wanlihong’	Hairy	Wuxi
D04	‘Hongfushi’	unknown	Jiashan	M04	‘Guoqihong’	Hairy	Wuxi
D05	‘Xiquedengmei’	unknown	Jiashan	M05	‘Taoyuan’	Hairy	Wuxi
G01	‘Bailin’	Summer	Jiashan	M06	‘Qianchongdazi’	Hairy	Wuxi
G02	‘Cuishan’	Summer	Jiashan	PJ003	‘Turuimeigui’	East	Wuxi
G03	‘Dahong’	Summer	Jiashan	PJ007	‘Zigaoyu’	East	Wuxi
G04	‘Shouguangguan’	Summer	Jiashan	PJ010	‘Yanzhi’	East	Wuxi
G05	‘Shouguangzhiyu’	Summer	Jiashan	PJ013	‘Xiayijin’	East	Wuxi
G06	‘Xiaotian’	Summer	Jiashan	PJ034	‘Fendie’	East	Wuxi
G07	‘Qufangzhijing’	Summer	Jiashan	PJ058	‘Tianguinv’	East	Wuxi
G08	‘Fushiying’	Summer	Jiashan	PJ065	‘Duanyang’	Summer	Wuxi
G09	‘Bikong’	Summer	Jiashan	PJ069	‘Wuzi’	East	Wuxi
G10	‘Riguang’	Summer	Jiashan	PJ076	‘Meihuarong’	East	Wuxi
G11	‘Yingzhizhu’	Summer	Jiashan	PJ111	‘Taobanzhusha’	East	Wuxi
G12	‘Rizhaozhiguang’	Summer	Jiashan	PJ113	‘Guoqihong’	East	Wuxi
M01	‘Yuling’	Hairy	Wuxi	PJ135	‘Dantaobai’	East	Wuxi

Table 2. List of morphological variables evaluated for cluster analysis.

No.	Variable	Type	Introduction
1.	Plant habit (PH)	Observed	Scored 1 for leggy; 2 for dense
2.	Lamina color (LC)	Observed	Scored 1 for solid green; 2 for yellow-green
3.	Pubescence (PU)	Observed	Scored 0 for sparse; 1 for dense
4.	Lamina edge (LD)	Observed	Scored 1 for flat; 2 for contorted; 3 for upcurved; 4 for downcurved
5.	Lamina length (LL)	Dimension	Excluded the petiole part
6.	Lamina width (LW)	Dimension	Measured from the widest place
7.	Petiole length (PL)	Dimension	The length of the petiole part
8.	Length of lamina at largest width (WL)	Dimension	Measured from the apex to the widest point
9.	Lamina shape (LS)	Transformed	LS = 100 × WL/LL (the length proportion between the widest part and the lamina)
10.	Petiole ratio (PR)	Transformed	PR = 100 × PL / (LL+PL) (the length proportion between the petiole and the entire leaf)
11.	Blossom time (BT)	Observed	Scored 1 for bloom in mid-April to mid-May; 2 for after mid-May
12.	Number of floret (NF)	Counted	The number of florets that bloom from one flower bud
13.	Flower form (FF)	Observed	Scored 1 for single; 2 for hose-in-hose[a]; 3 for semi-double[b]; 4 for semi-double hose-in-hose; 5 for double[c]; 6 for double hose-in-hose[d]
14.	Flower shape (FS)	Observed	Scored 1 for funnel-shaped; 2 for flat-faced; 3 for saucer-shaped; 4 for spider
15.	Flower diameter (FD)	Dimension	Measured as the flower width, or the distance between the tips of the two upper wing petals
16.	Flower length (FL)	Dimension	Measured from the base of the tube to the level of the top of the petals (the straight height)
17.	Color pattern (CP)	Observed	Scored 1 for self all one color; 2 for blotched; 3 for striped; 4 for margined; 5 for sectored; 6 for compound (at least two different patterns found in one individual)
18.	Sepal length (SL)	Observed	Scored 0 for no sepal; 1 for ≤ 3 mm; 2 for > 3 mm
19.	Petal edge (PE)	Observed	Scored 1 for flat; 2 for wavy; 3 for ruffled
20.	Petal color (PC)	Observed	Using Royal Horticultural Society Colour Chart (RHSCC), scored 1 for white, 2 for orange-red, 3 for red, 4 for red-purple, 5 for purple, 6 for violet, 7 for compound
21.	Petal length (PT)	Dimension	The straight length of the upper lobe
22.	Petal width (PW)	Dimension	The width of the upper lobe
23.	Length of petal at largest width (WP)	Dimension	Measured from the tip of a petal to the widest point
24.	Petal shape (PS)	Transformed	PS= 100 × WP / PT
25.	Flower opening angle (FA)	Transformed	FA = 2 × 180 ×[arctan FD / (2 × FL)] / π
26.	Pedicel length (PD)	Dimension	
27.	Pedicel ratio (PF)	Transformed	PF = 100 × PD / (PD + FL)
28.	Number of Stamens (SN)	Counted	Scored 0 if less than 5; 1 for between 5 to 7; 2 for more than 7
29.	Stamen length (ST)	Observed	Scored 0 for none; 1 for shorter than pistil; 2 for equal; 3 for longer than pistil
30.	Pistil length (PI)	Observed	Scored 0 for none; 1 for shorter than flower length; 2 for equal; 3 for longer than flower length

[a]A flower type with all sepals appearing as petals, usually rotated from the normal (inner) petals
[b]A flower type with some stamens appearing as petals
[c]A flower type with all stamens appearing as petals
[d]A flower type with all stamens and sepals appearing as petals

Results

The resulting dendrogram has a high goodness of fit in Mantel's test (r = 0.844; approximate Mantel t-test: t = 12.873; probability random Z < observed Z: P = 1.000). Six groups were evident on the phenogram based on Euclidean distance (Fig. 1). Almost all the East azalea cultivars clustered in group A, except for 'Fendie' (PJ034), 'Zhuangyuanhong' (C36) and 'Hongyue' (C49). Group B was sister to group A, and consisted of 9 summer azaleas. Two East azaleas named 'Zhuangyuanhong' and 'Hongyue' composed group C. Five out of the 6 West azaleas grouped together with one East azalea ('Fendie', PJ034), one Hairy azalea ('Yuling', M01) and 2 Summer azaleas ('Dahong', G03 and 'Xiaotian', G06) in group D. Group E was comprised 7 Hairy azaleas and 1 Summer azalea ('Cuishan', G02). Group F included 1 West azalea ('Jinpao', B05) and 1 Hairy azalea ('Qianchongdazi', M06).

Principal coordinate analysis (PCO) of the morphological data was performed using product-moment correlation matrix in NTSYS. All the vectors with an eigenvalue greater than 1 explained 78.39% of the total observed variation. The first 3 axes explained 24.50%, 13.58% and 11.87% of the variation in the data (Table 3), respectively.

Mostly consistent with the dendrogram generated in cluster analysis, the 2 dimensional plot of the first 2 axes (38.08% total variation explained) shows that the 4 groups could be clearly separated from each other (Fig.

2). Along the first axis, East azaleas distinguished from Hairy and West azaleas, while the second axis revealed a split between the Summer group and the other 3 groups. Congruent with the cluster analysis, the Summer cultivar 'Xiaotian' (G06) grouped with West azaleas. With regard to the Hairy group and the West group, the segregation was a little weak. Similar results were observed in East and Hairy azaleas. There were more West azaleas distributed together with Hairy azaleas than in cluster analysis. Besides, the Hairy cultivar 'Wanlihong' seemed to be in the East group.

The PCO indicated that different sets of characters showed high loadings in clustering evergreen azaleas on 3 axes (Table 4). Among the 14 characters with the highest 5 factors on the first 3 axes, there are only 2 characters which are not related to flowers. Plant habit, flower diameter, petal width, length of petal at largest width and stamen length had high loadings on the first axis. Blossom time, flower form, color pattern, petal color and pedicel ratio were highly loaded on the second axis. Length of lamina at largest width, flower form, flower shape, petal shape and number of stamens showed high loadings on the third axis.

Table 3. Eigenvalues of 8 vectors, percent eigenvalue and cumulative percent variation explained by each vector (eigenvalue > 1 were shown).

Vector	Eigenvalue	Percent eigenvalue	Cumulative percent
1	6.505	24.50	24.50
2	3.606	13.58	38.08
3	3.151	11.87	49.94
4	2.318	8.73	58.67
5	1.485	5.59	64.26
6	1.346	5.07	69.33
7	1.256	4.73	74.06
8	1.148	4.32	78.39

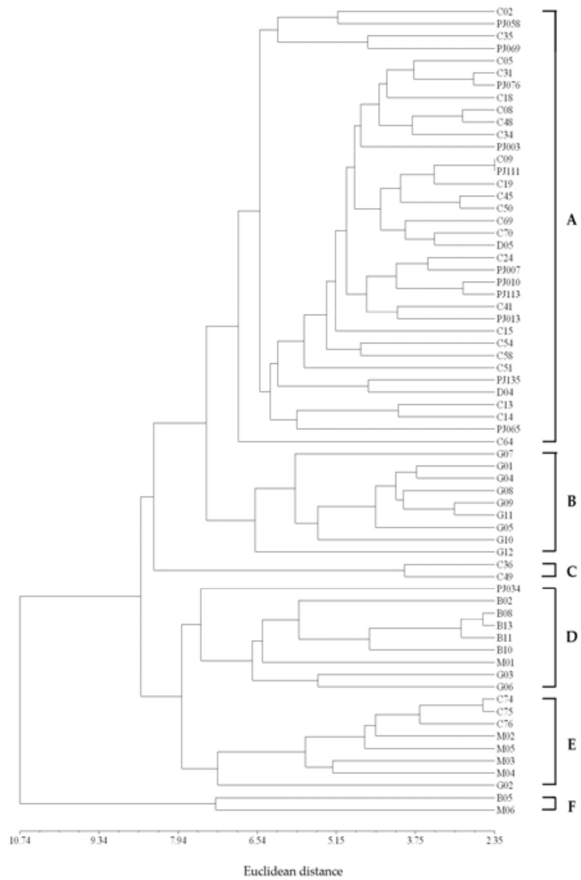

Fig. 1. UPGMA dendrogram based on Euclidean distance showing the relationships of the 66 evergreen azalea cultivars in China using NTSYS software.

Table 4. Summary of morphological characters with the highest 5 factor loadings on the first three axes of principal coordinate analysis.

Characters	Axis 1	Axis 2	Axis 3
Plant habit (PH)	0.775		
Length of lamina at largest width (WL)			-0.487
Blossom time (BT)		0.844	
Flower form (FF)		-0.595	0.571
Flower shape (FS)			0.591
Flower diameter (FD)	-0.656		
Color pattern (CP)		0.675	
Petal color (PC)		0.509	
Petal width (PW)	-0.658		
Length of petal at largest width (WP)	-0.671		
Petal shape (PS)			0.554
Pedicel ratio (PF)		-0.584	
Number of Stamens (SN)			-0.741
Stamen length (ST)	0.786		

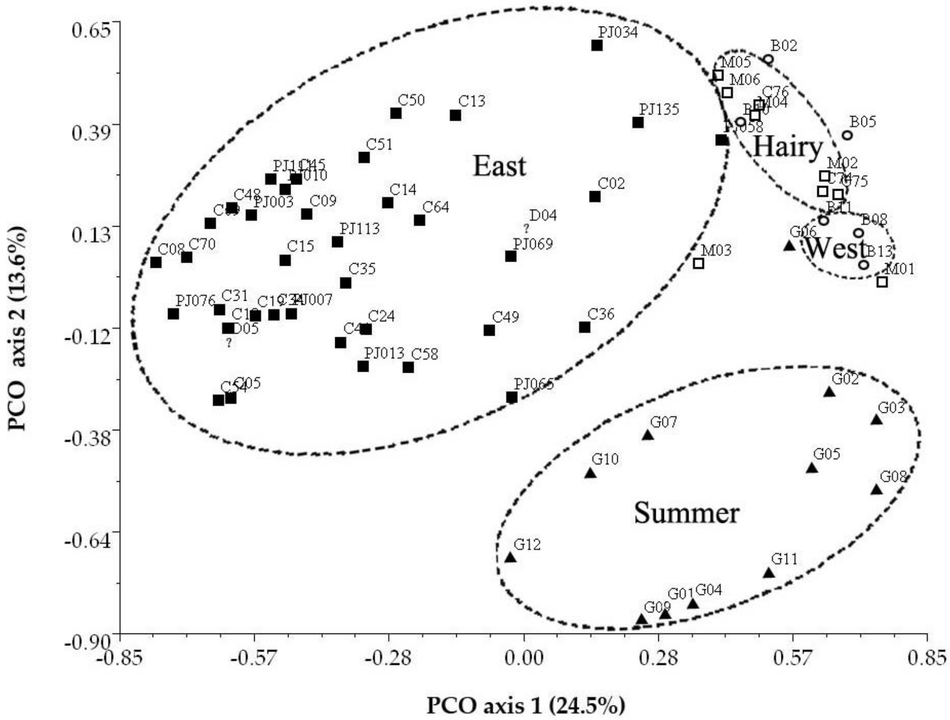

Fig. 2. Principal coordinates analysis of product-moment correlation matrix using NTSYS software. The symbols "■", "□", "▲", "○" and "?" representing East azalea, Hairy azalea, Summer azalea, West azalea and unknown type, respectively.

Discussion

Numerical taxonomy concerned primarily with phenetic relationships developed in the late 1950s and is useful in species classification (Sneath, 2001) based on morphological characters. Our study is a new attempt to classify evergreen azalea cultivars in China using numerical taxonomy. Detailed morphological traits helped to build a profile for each cultivar.

Cluster analysis using UPGMA showed a general agreement with the 4 group classification system in present. The 4 traditional groups making up the main clusters (A, B, D, and E) in the dendrogram revealed that the morphological characters we investigated could be useful in classifying evergreen azalea cultivars. Within group A, 'Dahezhichun' (C64) forming a branch by itself mainly due to its large flowers similar with Summer or West azaleas. Two cultivars called 'Jinpao' and 'Qianchongdazi' comprised group F were separated from others at the beginning because only they had double flowers.

The first 3 axes in PCO explained 49.94% of the total variation, which is nearly the same as Tutel *et al*., (2005), Scariot *et al*., (2007), Sarwar & Qaiser (2012) reported in their studies, and thus proved to be reliable. The cumulative percent of variation explained by vectors with an eigenvalue greater than 1 was less than 80%, which indicated a high variability on cultivars. PCO also revealed that flower characteristics played an important role in phenetic relationships of azaleas, for most of the characters with high loadings on the first three axes were related to flowers, in accordance with Heursel & Heursel (1981) and Garretsen (1989).

Both the UPGMA dendrogram and PCO support the view that cultivars blooming in the early summer should compose a group, in accordance with the present name 'Summer'. The remaining cultivars with the same blossom time in spring were distributed in 2 areas based on different plant habits and flower diameters. Those with dense branches and small flowers composed the East group. But the boundary between West group and Hairy group was a little vague. This problem is likely to be solved by addition of more cultivars belonging to these 2 groups.

Conclusions

Numerical taxonomy using detailed morphological characters was useful in classifying evergreen azalea cultivars. The 4 group classification system presented here are generally fits the phenetic relationships between cultivars only with few exceptions. Our study is the first step to find out the phenetic relationships of azalea cultivars in China, and to contribute towards cultivar conservation and hybridization. Future studies may be carried out by addition of more genotypes and molecular technologies to reveal the genetic conformity between reference accessions and the Chinese gene pool.

Acknowledgements

We wish to thank Wei-dong Zhang from Xi Hui Park in Wuxi, and Hai-Bo Chen from Jiaxing Biyun Garden for their help in cultivar recognition and collection. We are also grateful to Jin Liao, Lu Zhang and Jing-Jue Chen for their assistant on assessment of morphological traits.

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(Received for publication 9 March 2012)