A Morphometric Study of Populations of the *Centaurea jacea* Complex (Asteraceae) in Belgium

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Abstract: **The *Centaurea jacea* aggregate is a polymorphic polyploid complex whose taxonomic treatment is still controversial. A numerical taxonomic approach was applied to 394 individuals of known ploidy level, from 19 populations, based on the main diagnostic characters proposed in earlier revisions. Populations from xeric grasslands were not considered. Principal Component Analysis shows that variation within the complex is continuous. UPGMA Cluster Analysis based on population means supports the recognition of three groups of populations. However, the limits between these groups are blurred to a considerable extent due to extensive within-population polymorphism. It is argued that the Belgian populations of *Centaurea jacea* occurring in mesic grasslands should be treated as a single species, with three subspecies. The two extremes of the morphological gradient can be referred to as *C. jacea* subsp. *jacea* and *C. jacea* subsp. *nigra,* with *C. jacea* subsp. *pratensis* occupying an intermediate position. Most populations from Belgium are tetraploid, a diploid chromosome number being found only in populations of *C. jacea* subsp. *nigra* from the Ardennes massif. On average, diploids grow at higher altitude and on more acidic soils than tetraploids. Finally, a key to the three subspecies is provided.**

Key words: **Knapweeds, numerical taxonomy, polyploidy, flow cytometry, *Centaurea nigra*, *Centaurea jacea*, *Centaurea pratensis*.**

Introduction

In northwestern Europe, knapweeds (*Centaurea* subgenus *Jacea* [Mill.] Hayek, Asteraceae) form an extremely polymorphic polyploid complex in which taxonomic treatments are still controversial. The characters most useful to discriminate taxonomic units are the shape and colour of capitulum phyllaries, the size of the pappus and the presence of ray florets. Briquet (1931[4]) regarded the whole complex as a single polymorphic species. On the contrary, the revisions by Hayek (1918[13]), Arènes (1939[1], 1954[2], 1957[3]) and van Soest (1947[23]) led to a significant inflation of the number of taxa retained for Europe. *Flora europaea* (Dostál, 1976[6]) espoused their views. For Belgium and neighbouring countries, Lambinon et al. (1993[16]) recognize nine taxa (*C. jacea* L., *C. timbalii* Martrin-Donos, *C. debeauxii* Godr. et Gren., *C. nemoralis* Jord., *C. nigra* L., *C. microptilon* (Godr.) Godr. et Gren., *C. thuillieri* (Dostál) J. Duvign. et Lambinon, *C. decipiens* Thuill. and *C. serotina* Boreau). However, these authors question the rank at which these taxa should be treated and stress that intermediate morphotypes, difficult to ascribe to any particular taxon, occur at a high frequency in natural populations.

Significant progress in the understanding of the complex was brought about by the experimental work of Gardou (1972[9]). Based on population samples at a European scale, including Mediterranean populations, she concluded that the complex consisted of morphologically well-defined units at the diploid level (2n = 22), with distinct ecogeographical distributions, linked by intermediate tetraploid morphs (2n = 44). She also demonstrated that reproductive barriers among members of the complex were generally weak, in agreement with previous observations from Marsden-Jones and Turrill (1954[17]) and Saarisalo-Taubert (1966[18]). On these grounds, she argued that the complex could be regarded as a single biological species, but admitted that, for practical reasons, three taxa could be retained, namely *C. nigra* L., *C. jacea* L. and *C. pratensis* Thuill. (now called *C. thuillieri* J. Duvign. et Lambinon). Such a synthetic taxonomic treatment was adopted for France by Guinochet (1982[10]), although Kerguélen (1993[15]) came back to a more analytic treatment. In Germany, the Netherlands and the British Isles, recent works also adopted a very synthetic treatment (Wisskirchen and Haeupler, 1998[25]; Van der Meijden, 1996[22]; Stace, 1997[21], respectively).

An autopolyploid origin of the tetraploids is supported by allozyme markers (Sommer, 1990[20]; Hardy et al., 2000[11]). Although tetraploids are said to be more widespread and ecologically more plastic than diploids (Gardou, 1972[9]; Weeda et al., 1991[24]), the ecogeographical distribution of both cytotypes in Europe deserves further investigation. Hardy et al. (2000[11]) described a parapatric distribution of diploid and tetraploid populations in the Ardennes massif (E Belgium) where the two cytotypes were found to have distinct allozymic gene pools and morphological traits.

An obvious shortcoming of earlier taxonomic treatments of the *C. jacea* complex is the failure to define taxa on statistical grounds and to allow for within-population variation. In the

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Table 1 Morphometric characters studied (see Figs. **1 – 3**). The correlations of all traits with Principal Components 1 and 2 are given

Character		PC1	PC2
1. Bract colour (1, black; 2, intermediate; 3, light brown)	COL	– 0.50	– 0.14
2. Width of central undivided part of bract appendage (mm) (logarithmic transformation)	CWB	– 0.55	– 0.63
3. Length of longest lateral bract tooth (mm)	LLT	0.87	0.01
4. Length of central undivided part of bract appendage (mm)	CLB	0.18	– 0.58
5. Length of apical bract tooth (mm)	LAT	0.59	– 0.26
6. Teeth number (square root transformation)	TNB	– 0.44	– 0.66
7. Bract overlap (1, no overlap; 3, complete overlap; 2: intermediate)	OVL	0.23	– 0.55
8. Appression of bracts (1, bracts tightly adpressed; 2, bracts slightly curved outwards; 3, bracts strongly curved outwards)	APP	– 0.61	– 0.18
9. Involucre diameter (mm)	IVD	– 0.45	– 0.59
10. Involucre height (mm)	IVH	0.12	– 0.22
11. Pappus length (mm) (logarithmic transformation)	PAL	0.58	– 0.25

present study, we try to fill that gap by means of a numerical taxonomic approach, based on a nested sampling design. In order to investigate relationships between cytological and morphometric variation, ploidy level and 11 morphological traits were assessed for 394 plants from 19 populations. In addition, for each population, soil chemical parameters were determined in order to test whether different morphotypes occupy distinct ecological niches. The present paper focuses on populations from mesic grasslands. Populations from xeric grasslands, with narrow leaves and late flowering, corresponding to *C. timbalii, C. decipiens* and *C. serotina* (Lambinon et al., 1993[16]) or *C. angustifolia* (Wisskirchen and Haeupler, 1998[25]) are not considered here.

Materials and Methods

Population sampling

Nineteen populations were sampled in Belgium in 1999. The 19 populations span a wide geographical range from the North Sea coast to the Ardennes massif. Six populations (*Ho, Cha, So, Eu, Ves* and *BM*) (Table **2**) are located in the northeastern part of the Ardennes massif, where diploids and tetraploids are parapatric (Hardy et al., 2000[11]). At the western end of the range, the four populations *Ad, OC, Od* and *Vl* are located at less than 20 km from the sea; they will be referred to as Western Flandrian populations. About 20 individuals were sampled in each of the 19 populations (394 individuals altogether). Herbarium samples and seeds were collected from the 394 individuals for morphological analysis. Herbarium voucher specimens are deposited in BRLU.

Flow cytometry

In each population, fresh leaves were collected from five plants for ploidy level determination. A larger sample was collected in the three populations from the Ardennes district (*Eu, So, Mir*) that comprised two distinct morphotypes (with and without ray florets), because Hardy et al. (2000[11]) showed that such populations were heterogeneous for ploidy level. Ploidy level was assessed by flow cytometry. This technique allows the rapid determination of the relative DNA content of nuclei by measuring the fluorescence of a fluorochrome that specifically binds to DNA (Galbraigth et al., 1983[8]). Small leaf disks were chopped with a razor blade in Petri dishes, after addition of 0.5 ml 0.1 M sodium hydrogen phosphate at pH 7, containing 0.5 % Tween 20. After filtration through a 30 μm nylon filter, 0.5 ml of a solution of 5 g l^{-1} DAPI (fluorochrome) in 0.1 M sodium hydrogen phosphate was added. Flow cytometry measurements were performed with a Partec machine equipped with a UV lamp. The fluorochrome was excited at 340 nm and emitted at 465 nm. A diploid individual and a tetraploid individual, whose chromosome numbers were assessed by chromosome counting (Feulgen method [Jahier et al., 1992[14]]), were used as internal standards for each measurement.

Morphometric analysis

Eleven morphological traits (Table **1**) were assessed on two flower heads of each plant. The characters are those most often used by earlier revisions of the *C. jacea–C. nigra* complex (Arènes, 1954[2]; van Soest, 1947[23]). Three qualitative characters describe the colour of involucral bracts (COL), the overlap of bract rows (OVL) (Fig. **1**) and the degree of bract application (APP) (Fig. **2**). Eight quantitative characters were assessed on bracts, involucres and achenes. Five measurements (CWB, LLT, CLB, LAT and TNB) (Fig. **3**) characterizing involucral bract appendage were performed on the median bract row, i.e., the third or fourth row from the base. Involucre diameter (IVD) was measured without taking into account the outwardly curved bract tips; involucre height (IVH) was measured from the base of the involucre to the top of the uppermost bract row. Pappus length (PAL) was measured to the nearest 0.1 mm on the central achenes of the capitulum. Finally, the occurrence of ray florets was recorded on a population basis (present, absent, or mixed); it was not possible to assess that character for a few individuals collected at the fruiting state. Character ratios were also computed, specifically: IVH/IVD, CLB/CW and LLT/CWB. Quantitative variables were transformed as necessary prior to statistical analysis.

Statistical analysis

A three-level nested analysis of variance was performed on seven quantitative traits to assess the magnitude of variance components attributable to the different factors included in the analysis (cytotypes, populations and individuals). PAL was

Table 2 Location and site characteristics of the populations investigated. The populations are ascribed to their respective UPGMA clusters. nd: not determined. $^{*}p < 0.05$; $^{(*)}$ marginally significant

Population	UPGMA cluster	Location	Phytogeographical district (Lambinon et al., 1993[12])	Cytotype	Habitat	Altitude (m)	pH	P (mg kg^{-1})	Ca ($cmol_c$ kg^{-1})	Mg ($cmol_c$ kg^{-1})	K ($cmol_c$ kg^{-1})
Ad	1	Adinkerke	Maritime	4x	Road verge	3	7.5	5.4	nd	0.17	0.03
BM	3	Baraque Michel	Haute-Ardenne	4x	Pasture	670	5.6	0.1	9.25	0.12	0.08
Cha	1	Champagne	Haute-Ardenne	2x	Road verge	540	5.1	2	3.75	0.61	0.48
Eu	1, 3	Eupen 1	Haute-Ardenne	2x, 4x	Road verge	410	7.0	5	18.50	0.95	0.16
Flo	2	Flobecq	Brabançon	4x	Forest edge	75	6.5	3.2	12.25	0.69	0.54
GG	2	Ramillies	Brabançon	4x	Road verge	130	6.3	12.2	15.75	0.84	0.73
Ho	1	Honsfeld	Haute-Ardenne	2x	Forest edge	620	5.5	3.6	6.05	0.74	0.66
Hoe	2	Hoeilaart	Brabançon	4x	Hay meadow	110	7.5	2.2	nd	0.20	0.03
Mir	1, 2	Mirwart	Ardennais	2x, 4x	Hay meadow	300	4.7	8.2	2.55	0.28	0.12
OC	1	Oost-Cappel	Brabançon	4x	Road verge	16	7.4	14.8	nd	0.12	0.05
Od	1	Oostduinkerke	Maritime	4x	Road verge	3	7.5	11.2	nd	0.22	0.04
Ro	2	Ronquières	Brabançon	4x	Hay meadow	75	5.6	6.8	10.00	1.36	0.19
SM	2	Saint-Médard	Ardennais	4x	Road verge	350	7.5	5.2	nd	0.14	0.04
So	1, 3	Schoenberg	Haute-Ardenne	2x, 4x	Road verge	460	6.7	3.4	nd	0.62	0.12
Te	2	Tellin	Ardennais	4x	Road verge	390	7.5	11.4	nd	0.26	0.05
Ti	2	Tintigny	Lorrain	4x	Road verge	350	7.5	3.2	nd	0.20	0.03
Ves	3	Eupen 2	Haute-Ardenne	4x	Road verge	300	7.0	20.2	25.75	2.02	0.60
Vl	1	Vlissegem	Flandrien	4x	Road verge	4	7.3	14	nd	0.21	0.07
Wi	2	Wiesmes	Mosan	4x	Pasture	200	5.7	2.4	7.75	1.04	0.15
Average cluster 1						61	6.5	7.5		0.44	0.19
Average cluster 2						220	6.5	6.1		0.36	0.21
Average cluster 3						460	6.6	7.2		0.93	0.24
F-value						2.138	0.006	0.169		1.52	0.054
Average 2x						466	5.8	4.44		0.64	0.31
Average 4x						226	6.8	7.58		0.55	0.18
$\mathbf{t_{obs}}$						– 2.56*	$2.07^{(*)}$	1.23		– 0.34	– 1.11

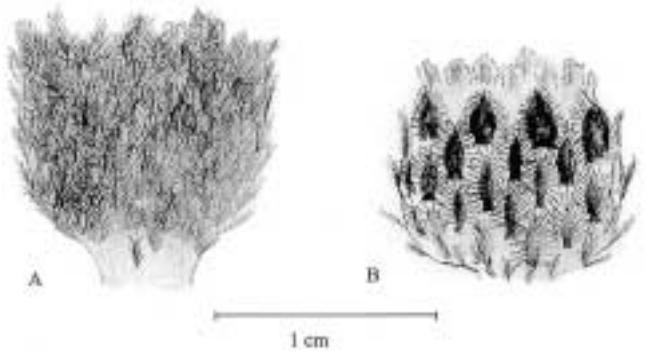

Fig. 1 Overlap of bract rows (OVL). (**A**) Complete overlap; (**B**) no overlap (see Table **1**).

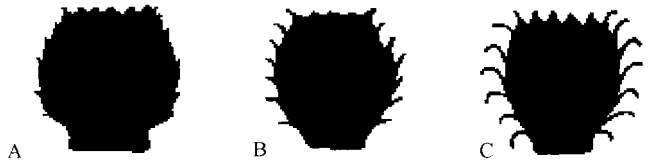

Fig. 2 Silhouettes of capitulum (APP). (**A**) Bracts tightly adpressed; (**B**) bracts slightly curved outwards; (**C**) bracts strongly curved outwards (see Table **1**).

excluded from the analysis because some populations were monomorphic for this character. Satterthwaite approximations were used for significance tests because of unequal sample sizes (Sokal and Rohlf, 1995[18]). Cytotype was considered as a fixed factor whereas population and individual were considered as random factors. In three populations where the two cytotypes existed in sympatry *(Eu, So, Mir),* diploids and tetraploids were treated as distinct populations. A Principal Component Analysis was performed on the whole data matrix (8 quantitative and 3 qualitative traits). Cluster Analysis was performed on population means as follows. The scores of each individual on all 11 Principal Component axes (weighted by the eigenvalue of corresponding axis) were used to compute population mean values for each axis. This new data matrix was subjected to UPGMA (unweighted pair-group method using arithmetic averages) Cluster Analysis based on Euclidean distance. Box plots were constructed to depict variation of each character at the population level. Finally, a Discriminant Analysis was performed using quantitative traits, with the three clusters from the UPGMA dendrogram used as a priori group. Multidimensional scaling based on Euclidean matrix distance was used to provide a two-dimensional representation of the relationships among populations. Statistical analyses were performed with *Statistica* 6 software (StatSoft, Inc., 2001).

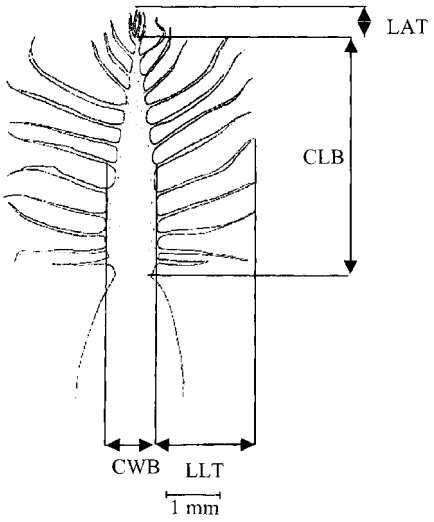

Fig. 3 Bract appendage measurements (see Table **1**).

Soil analysis

Soil samples (bulk samples of five cores at 0 – 12 cm depth) were collected in each site for determination of soil pH (1 : 1 soil/water). Extractable phosphorus and exchangeable cations (Ca^{2+}, Mg^{2+} and K^{+}) were extracted with 0.5 M ammonium acetate-EDTA, pH 4.65. Phosphorus concentration was determined by colorimetry using the Scheel method (Cottenie, 1982[5]) and exchangeable cation concentrations were determined by flame atomic absorption spectrometry. Ca concentration was not assessed for samples containing free $CaCO_3$.

Results

Cytotype distribution (Table **2**)

Fourteen populations, originating from all phytogeographic regions represented in the sample, comprised only tetraploid individuals. Diploids were found in only five populations, all

Table 3 Three-level nested ANOVA on seven quantitative characters. DF and MS are based on Satterthwaite approximations. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns: $p > 0.05$

Trait and source of variation	SS	DF	MS	*F*-ratio	Variance percentage
CWB (logarithmic transformation)					
Cytotype	29.66	1	29.66	2.39 ns	
Population	279.2	20.37	12.42	11.52 ***	35.8
Individual	398.8	348.21	1.21	9.48 ***	52.5
Error	44.7	376	0.12		11.7
LLT					
Cytotype	157.3	1	157.3	9.9 **	
Population	357.6	20.32	15.89	13.89 ***	38.3
Individual	427.6	342	1.29	4.59 ***	40.4
Error	99	376	0.26		21.4
CLB					
Cytotype	0.07	1	0.07	0.07 ns	
Population	20.09	22.12	0.93	2.12 ***	5.2
Individual	157.16	343.34	0.47	5.15 ***	65.1
Error	32.41	376	0.09		29.7
LAT					
Cytotype	8.4	1	8.4	10.32 **	
Population	17.82	21.04	0.81	4.03 ***	10.6
Individual	71.13	321.45	0.21	1.63 ***	22.4
Error	46.26	377	0.12		67
TNB (square root transformation)					
Cytotype	2.07	1	2.07	0.73 ns	
Population	63.32	20.61	2.84	7.38 ***	22.1
Individual	143.75	366	0.43	3.13 ***	41.2
Error	48.67	375	0.13		36.7
IVD					
Cytotype	115.02	1	115.02	3.56 ns	
Population	782.85	20.47	32.3	13.24 ***	38.1
Individual	921.02	312.77	2.95	3.12 ***	33.5
Error	285.155	315	0.91		28.4
IVH					
Cytotype	38.45	1	38.45	4.03 ns	
Population	223.67	22.09	9.54	3.33 ***	10.3
Individual	1045.34	310.28	3.36	2.88 ***	45.8
Error	352.61	316	1.12		43.9

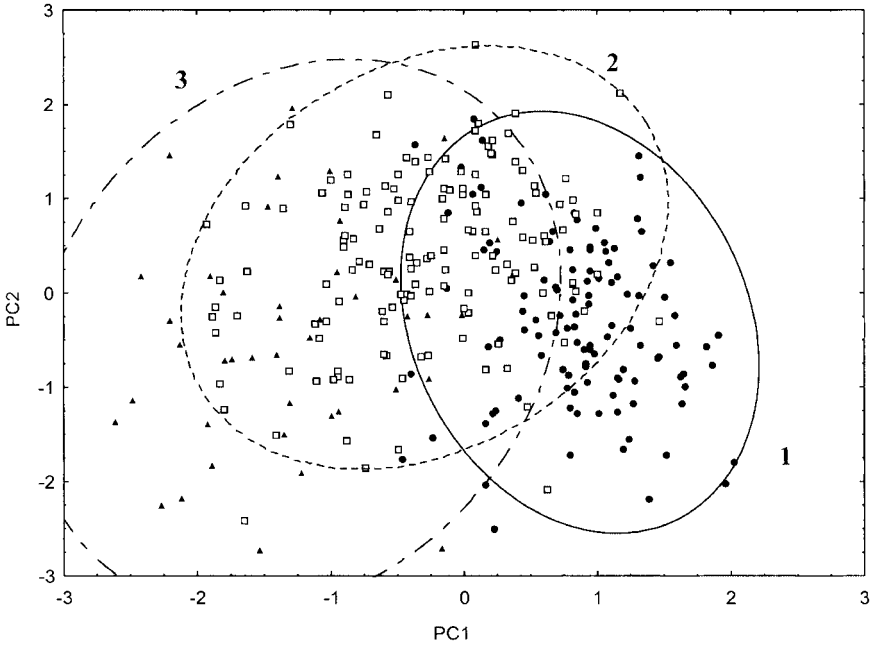

Fig. 4 Scatter plot of individuals on PC1 and PC2. The three UPGMA groups are delineated by 95% confidence ellipses. ●: cluster 1; □: cluster 2; ▲: cluster 3.

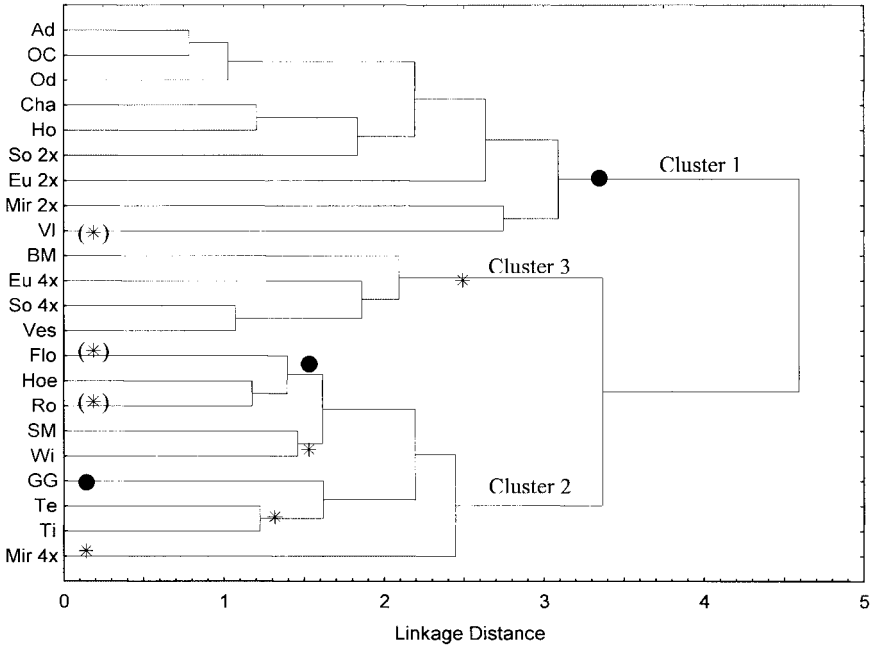

Fig. 5 UPGMA dendrogram based on Euclidean distances. ✳: ray florets always present; ●: ray florets absent; (✳) ray florets in a few individuals. Mixed populations (*Eu, So* and *Mir*) are split according to the ploidy level.

located in the Ardennes massif. In three of them (*Eu, So* and *Mir*), diploids and tetraploids grew intermingled. No triploid plant was found in any population.

Morphometric analysis

For all characters, the three-level nested ANOVA reveals a significant added variance component at the individual and population level (Table **3**). The between individual variance percentage (22.4 – 65.1%) is larger than the between population variance percentage (5.2 – 38.3%) for all traits except IVD. The cytotype effect was significant in only two traits (LLT and LAT).

Principal component 1 (PC1) accounts for 26% of the total variance and is mostly correlated with LLT (r = 0.87), APP (r = – 0.61), LAT (r = 0.59), PAL (r = 0.58), CWB (r = – 0.54), COL (r = – 0.50) (Table **1**). PC2 accounts for 19% of the total variance and is mostly correlated with TNB (r = – 0.66), CWB (r = – 0.62), IVD (r = – 0.59), CLB (r = – 0.58) and OVL (r = – 0.55). PC3 accounts for 11% of total variance and is mostly correlated with CLB (r = – 0.59) and IVH (r = – 0.59). The PCA scatter plot (Fig. **4**) does not reveal any clear-cut group.

The UPGMA dendrogram shows three main population clusters (Fig. **5**). Cluster 1 comprises four tetraploid populations from Western Flanders *(Ad, OC, Od, Vl)* and two diploid popula-

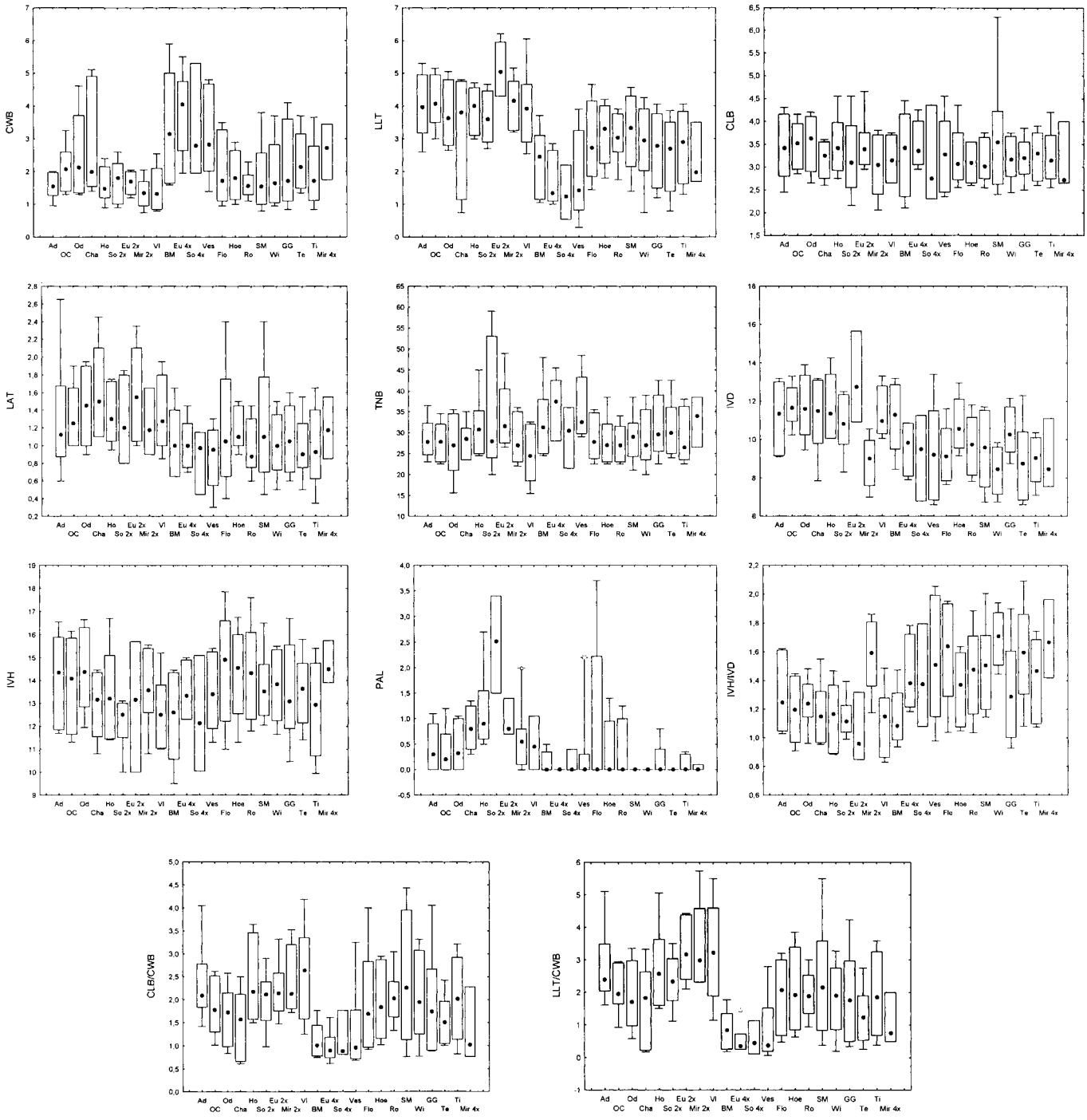

Fig. 6 Box plots for the 8 quantitative traits and ratios. ●: median; ○: outliers; △: extreme values; box: percentile 90, whisker: minimum–maximum interval.

tions from the Ardennes (*Cha* and *Ho*). All individuals in this cluster lack ray florets except population *Vl* in which ray florets occur at a very low frequency (< 5%). Cluster 2 comprises all the tetraploid populations except those from the Western Flanders and those from the eastern part of the Ardennes massif. This cluster is polymorphic for ray florets although most populations within it are fixed for this trait. Cluster 3 comprises four tetraploid populations from eastern Belgium, with all individuals having with ray florets. Most individuals from cluster 1 have positive scores on PC1, while cluster 3 generally has negative scores. The tetraploid populations of cluster 2 occupy an intermediate position (Fig. **4**).

Box plots (Fig. **6**) highlight the extensive within-population polymorphism and the extensively overlapping variation range of the three population clusters defined here above. The individuals included in cluster 1 have black to dark brown bracts with long teeth (LLT mean = 4.0 mm) and a narrow cen-

Table 4 Means and standard deviations of 14 traits and ratios for the three UPGMA clusters

	COL	CWB	LLT	CLB	LAT	TNB	OVL	APP	IVD	IVH	PAL	IVH/IVD	CLB/CWB	LLT/CWB
Means														
Cluster 1	1.29	1.80	3.98	3.34	1.35	28.67	2.42	1.41	11.24	13.35	0.72	1.20	2.06	2.52
Cluster 2	1.82	1.90	2.92	3.23	1.05	28.93	1.88	1.81	9.52	13.84	0.16	1.48	1.93	1.82
Cluster 3	2.07	3.37	1.85	3.33	0.96	33.56	1.76	2.23	9.94	13.08	0.10	1.36	1.09	0.68
All groups	1.64	2.07	3.19	3.29	1.16	29.51	2.07	1.72	10.27	13.54	0.37	1.35	1.86	1.93
Std.dev.														
Cluster 1	0.57	0.73	0.84	0.46	0.34	5.79	0.78	0.59	1.52	1.37	0.67	0.23	0.63	0.96
Cluster 2	0.86	0.73	0.78	0.46	0.32	4.54	0.82	0.78	1.33	1.44	0.48	0.24	0.72	0.90
Cluster 3	0.79	1.10	0.88	0.59	0.26	6.08	0.82	0.84	1.72	1.33	0.34	0.27	0.44	0.53
All groups	0.80	0.96	1.10	0.49	0.36	5.56	0.85	0.78	1.67	1.42	0.61	0.28	0.73	1.07

Table 5 Frequency of qualitative character states for the three UPGMA clusters

Character	COL			OVL			APP		
State	1	2	3	1	2	3	1	2	3
Cluster 1	0.77	0.17	0.06	0.18	0.22	0.60	0.65	0.30	0.05
Cluster 2	0.27	0.38	0.35	0.48	0.28	0.24	0.26	0.24	0.5
Cluster 3	0.47	0.24	0.29	0.42	0.30	0.28	0.34	0.48	0.18

tral part (CWB mean = 1.8 mm), compared to the other clusters (Tables **4,5**). The mean value for teeth number (TNB) is 28. Capitulums are broad (IVD mean = 11.2 mm) and nearly spherical (IVH/IVD mean = 1.2) with recovering and adpressed bracts. Achenes most often have a relatively well-developed pappus (PAL mean = 0.7 mm). The populations in cluster 2 are intermediate between clusters 1 and 3 for many characters (COL, LAT, LLT, TNB, OVL, APP, PAL, CLB/CWB, LLT/CWB). Bracts are clearly pectinate (LLT mean = 2.9 mm) with a narrow central part (CWB mean = 1.9 mm). Teeth number (TNB mean = 29) is similar to that of cluster 1. The capitulum is distinctly longer than wide (IVH/IVD mean = 1.5). Pappus is mostly absent or very short (PAL mean = 0.1 mm). The tetraploid individuals from the mixed population *Mir* uniformly have black bracts, in contrast to the other populations from cluster 2 which are polymorphic for bract colour. Cluster 3 is characterized by short bract teeth (LLT mean = 1.9 mm) and more numerous teeth than in the other clusters (TNB mean = 34). The central part of bract is wide (CWB mean = 3.4 mm) in comparison with teeth length (LLT/CWB mean = 0.7). The capitulum is longer than wide (IVH/IVD mean = 1.3). Pappus is absent or very short (PAL mean = 0.1 mm).

The first dimension of the Multidimensional Scaling (Fig. **7**) best discriminates between the three clusters, with cluster 1 populations presenting negative values, cluster 2 populations being between 0 and 1, and cluster 3 populations reaching values higher than 1. *Mir 2x* and *Vl* differ from the other populations from cluster 1 along the second dimension. *Mir 4x* is separated from the populations from cluster 2, with values of the second dimension identical to those of populations from cluster 3.

79% of the individuals are correctly classified (Table **6**) by Discriminant Analysis. Canonical discriminant roots account for 79.2% and 20.8% of the total variance. The first canonical discriminant root is mainly correlated with LLT (b = – 0.73) and PAL (b = – 0.63). The second canonical root is mainly correlated with CWB (b = 0.81). Incorrectly classified individuals from cluster 2 are equally shared by cluster 1 and 3.

Site characteristics (Table **2**)

Populations of the *C. jacea* complex occurring in mesic grasslands span a wide range of soil conditions. No significant difference between the four UPGMA clusters was found for any site characteristic. However, diploid populations occur, on average, at higher altitudes (mean = 466 m) than tetraploid populations (mean = 266 m). Soil pH is lower for diploids (5.8) compared to tetraploids (6.8), but this was only marginally significant.

Discussion

Populations of the *Centaurea jacea* complex occurring in mesic grasslands in Belgium span a wide morphological gradient. Characters do not vary independently of each other and the extremes of the morphological variation range show opposite combinations of traits, which are usually regarded as diagnostic for *C. nigra* (cluster 1) and *C. jacea* (cluster 3). Plants with more or less intermediate positions in the morphological gradient have usually been treated as a distinct taxon, namely *C. thuillieri* (= *C. pratensis* Thuill. non Salisb.). Cluster analysis, using phenetic distances between populations, tends to support the recognition of three taxonomic units, roughly corresponding to the three aforementioned taxa. However, our results highlight the difficulty to draw objective limits between

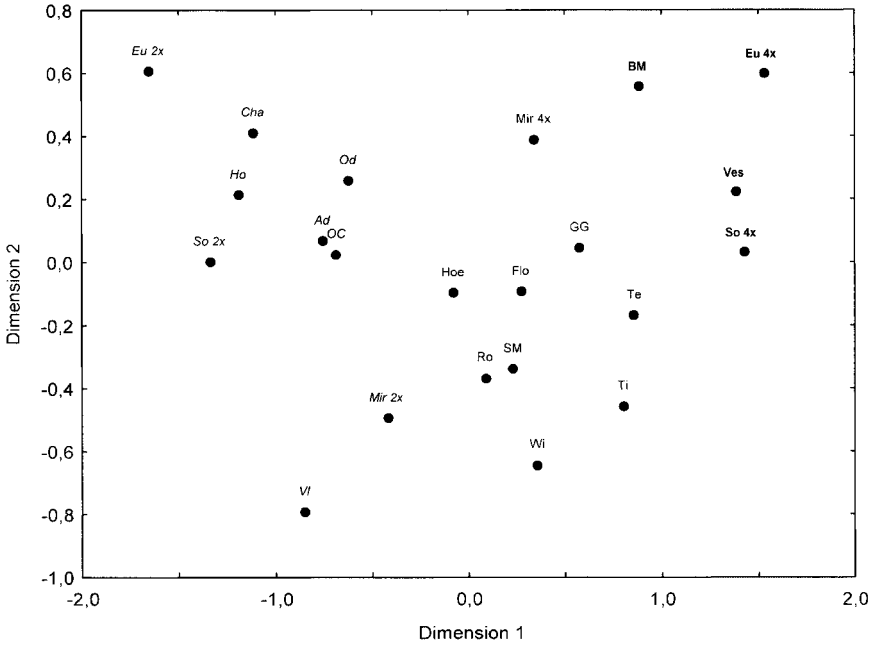

Fig. 7 Multidimensional scaling diagram of the 22 populations. Populations from cluster 1 are in italic and populations from cluster 3 are in bold.

Table 6 Classification matrix of a discriminant analysis performed on the three UPGMA clusters with eight quantitative traits. Rows: observed classification, Columns: predicted classification

	Number of individuals classified into predicted groups			
	Percent correct	Cluster 1, p = 0.37	Cluster 2, p = 0.48	Cluster 3, p = 0.14
Cluster 1	84.2	96	16	2
Cluster 2	78.08	16	114	16
Cluster 3	65.11	1	14	28
Total	78.54	113	144	46

those putative taxonomic units. That difficulty arises from the extensive polymorphism found both within and between populations. Pappus length, a character which is often given a high taxonomic value in the complex, offers a striking example: although mean values do differ among the three clusters, variation ranges overlap widely and most populations comprised at least one plant without any pappus at all.

Gardou (1972[9]) has already reported the occurrence of a whole range of intermediate morphs linking *C. jacea* and *C. nigra.* Gardou (1972[9]), Saarisalo-Taubert (1966[18]), and Marsden-Jones and Turrill (1954[17]) showed that there were no strong genetic barriers between members of the *C. nigra–C. jacea* complex and that hybrids between these morphs were morphologically similar to *C. thuillieri.* Guinochet (1982[10]) rightly insisted on the impossibility to draw objective limits between putative taxa in the *C. jacea* complex. In the British Isles, plants intermediate between *C. jacea* and *C. nigra* are referred to as *C. xmoncktonii* C. E. Britton (Stace, 1997[21]). They are said to derive from introgressions between the native *C. nigra* and *C. jacea,* an introduced species in the British Isles. The relation between *C. xmoncktonii* and *C. thuillieri* still needs to be investigated.

Hardy et al. (2000[12]) demonstrated that the extensive polymorphism existing in many populations of the *C. jacea* complex had a genetic basis and argued that the maintenance of large stores of genetic variation within populations was typical of outcrossing species. Hardy et al. (2000[11]) also showed that, when diploids and tetraploids grow intermingled, they have contrasting morphological traits. Such mixed populations occur close to the cytotype contact zone in the Ardennes region.

Finally, there seems to be good biological reasons not to assign a high taxonomic rank to the segregates of the *C. jacea* complex. This conclusion contrasts strongly with the taxonomic treatments proposed by van Soest (1947[23]), Dostál (1976[6]) and Kerguélen (1993[15]). Gardou (1972[9]) admitted that *C. jacea* sensu lato should be considered as a single biological species, at least at the tetraploid level. However, for practical reasons, considering an eco-geographically structured variation pattern, and the consistent correlations among certain characters, a formal taxonomic recognition can still be useful. We suggest that a subspecies rank might be the most appropriate. Hereafter, we briefly discuss the variation of each subspecies in turn.

C. jacea *subsp.* nigra *(L.) Bonnier et Layens [incl.* C. nemoralis *Jord.]*

In Belgium, the plants most closely agreeing with the description of *C. nigra* are diploid and occur in relatively undisturbed habitats in the Ardennes massif (Duvigneaud and Saintenoy-Simon, 1997[7]). However, even within those populations, many individuals markedly deviate from the *C. nigra* " typical" morphotype for one or several traits (e.g., short pappus, light-coloured bracts). Four tetraploid populations from the lowlands of western Belgium are morphologically very close to two diploid populations from the Ardennes massif (cluster 1). Although minor differences do exist in the mean values of specific traits, no reliable diagnostic feature can be proposed to discriminate these two geographical groups. Van Soest (1947[23]) and Arènes (1954[2]) already reported the existence of *C. nigra* in western Belgium. Gardou (1972[9]) found a tetraploid chromosome number for *C. nigra* from the coastal region of NW France. In her opinion, the diploid cytotype of *C. nigra* is represented by relic populations, mostly in mountain areas. Stace (1997[21]) and Dostál (1976[6]) also reported two chromosome numbers for *C. nigra* (incl. *C. nemoralis*).

Within the *C. nigra* complex, plants having a narrow capitulum, and bract teeth more than twice as long as the width of the central part of the bract, are sometimes treated as a separate taxon, namely *C. nemoralis* Jord. Population *Mir 2x* shows such a combination of characters. However, the shape of the central undivided part of the bracts (expressed as the length/width ratio), and the ratio of teeth length to the width of the central part of the bract show a wide variation range in diploid populations and are therefore of little taxonomic value. British authors also failed to maintain a distinction between *C. nigra* and *C. nemoralis* (Stace, 1997[21]). Thus, our results plead for the recognition of *C. jacea* subsp. *nigra* as a very polymorphic taxon, represented in Belgium by two cytotypes. The diploid cytotype is apparently restricted to the Ardennes massif, where it grows at a higher altitude than the tetraploid *C. jacea* subsp. *jacea.*

C. jacea *subsp.* jacea

Tetraploid individuals from E Belgium (Ardennes) share a number of traits considered as diagnostic for *C. jacea* sensu stricto (pappus lacking or short, apical portion of phyllaries with short teeth, radiate capitula). However, there is considerable variation within these populations for all these traits, suggesting that *C. jacea* subsp. *jacea* and *C. jacea* subsp. *pratensis* gradually merge into each other in that part of the country. The existence of a range of intermediate morphs was implicitly admitted by van Soest (1947[23]) and Arènes (1954[2]), who described several taxa recombining the diagnostic features of *C. jacea* and *C. pratensis* in various ways (e.g., *C. pseudopratensis* van Soest, *C. jacea* subsp. *pectinatisquama* Arènes, *C. subjacea* [Beck] Hayek).

C. jacea *subsp.* pratensis *(W. D. J. Koch) Celak. [=* C. thuillieri *J. Duvign. and Lambinon]*

Most tetraploid populations from Belgium, except those from the extreme east and the coast, are grouped in a single cluster. These populations are more or less intermediate between the former two groups for most quantitative traits and roughly correspond to the description of *C. jacea* subsp. *pratensis.* However, variation within that group of populations is so extensive that many individuals would be impossible to discriminate from either subsp. *jacea* or subsp. *nigra.*

C. jacea subsp. *pratensis* is polymorphic for ray florets, although most populations within it are fixed for that trait. Interestingly, all uniformly radiate populations (*SM, Te, Ti* and *Wi*) had very few individuals with a distinct pappus. On the contrary, populations *Flo, Ro, GG* and *Hoe,* nearly uniformly lacking ray florets, had a noticeably higher proportion of individuals with a distinct pappus. This observation provides circumstantial support to the hypothesis of a hybrid origin of *C. jacea* subsp. *pratensis,* with pappus and ray florets being inherited from *C. jacea* subsp. *nigra* and *C. jacea* subsp. *jacea,* respectively. This hypothesis was put forward by Marsden-Jones and Turrill (1954[17]), Saarisalo-Taubert (1966[18]) and Gardou (1972[9]).

Centaurea microptilon (Gord.) Gord. et Gren. is a segregate taxon in the *C. jacea* subsp. *pratensis* complex, said to differ by its narrowly lanceolate, outwardly curved bracts (Lambinon et al., 1993[16]). A few populations in our sample (e.g., *SM, WI*) comprise individuals with that combination of traits. However, bract shape and position show extensive variation within many populations of *C. jacea* subsp. *pratensis. C. microptilon* therefore appears to be an extreme variant within a continuous variation range, apparently not deserving formal taxonomic recognition.

Conclusions

Extensive population sampling and multivariate statistical treatments demonstrate the low taxonomic value of many characters and the existence of a continuous morphological gradient joining *C. nigra, C. thuillieri* and *C. jacea.* This conclusion is in line with the biosystematic investigations of Gardou (1972[9]). The polymorphism of the three putative taxa, and the high frequency of intermediate morphs, are still not adequately taken into account in most floras of Western Europe. All these observations, in addition to earlier demonstrations of the lack of strong genetic barriers between members of the complex, suggest that the species rank is biologically unjustified for the segregates of the *C. jacea* complex. A subspecies rank might be more appropriate. Further work is needed to clarify the taxonomic status of populations occurring in xeric grasslands, which are not considered in the present work.

Based on the original measurements on Belgian material, we propose the following tentative key for discriminating the three subspecies. Bract measurements refer to a median bract (generally the third row of bracts from the base); identification should be based on mean values from several individuals in a population.

a) Pappus nearly always present, most often exceeding 0.25 mm in length, up to 3.4 mm. Bract appendage most often black to dark brown, regularly and deeply pectinate, with teeth 2.5 – 6 mm long. Width of central undivided part of bracts: 1 – 2.5 (– 4) mm. Ratio teeth length/width of undivided central part of bract: 0.6 – 5.5. Capitulum (5 –) 8 – 16 mm broad, often nearly spherical. Ray florets nearly always absent. *C. jacea* subsp. *nigra*

b) Pappus often absent or, when present, nearly always shorter than 1.5 mm. Bract appendage variable in colour, deeply pectinate, with teeth 1.0 – 4.5 mm long. Width of central undivided part of bract appendage: 1 – 4 mm. Ratio teeth length/width of undivided central part of bract appendage: 0.6 – 3.5 (– 5.5). Capitulum 5 – 12 mm broad, often distinctly higher than wide. Ray florets absent or present.
C. jacea subsp. *pratensis*

c) Pappus always shorter than 0.5 mm, most often absent. Bract appendages variable in colour, irregularly incised, with teeth 0.5 – 2.5 mm long. Width of central undivided part of bracts: 1.8 – 5 mm. Ratio teeth length/width of undivided central part of bract: 0.1 – 1.5. Capitulum 4 – 12 mm broad, often distinctly higher than wide. Ray florets present.
C. jacea subsp. *jacea*

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