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Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum*

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SUMMARY

Populations of diploid and autotetraploid *Anthoxanthum alpinum* A. &. D. Löve formed a narrow hybrid zone in a study area in the Swiss Prealps. Detailed vegetation analyses were performed along transects in several contact zones between the two cytotypes. The vegetation differed according to the position in the hybrid zone. When considering the hybrid zone as a whole, and for one transect that was analysed in detail, there was strong evidence for habitat segregation between the cytotypes. Vegetation transitions, habitat preference and segregation of the two cytotypes differed according to location in the hybrid zone. The origin and dynamics of the hybrid zone are discussed.

Key words: Hybrid zone, polyploidy, habitat segregation, vegetation analysis, *Anthoxanthum alpinum*.

INTRODUCTION

Ecological differentiation of polyploids and their diploid relatives has been emphasized as one of the factors responsible for the widespread occurrence of polyploidy in flowering plants. Polyploidy can induce a change in the ecological requirements of a taxon. Consequently, a shift in the habitats of the cytotypes or a greater ecological amplitude of the polyploid cytotype relative to that of the diploid may occur, thus facilitating their coexistence (Clausen, Keck, & Hiesey, 1945; Lewis, 1967, 1980; deWet, 1971; Jackson, 1976; Thompson & Lumaret, 1992).

Differences in ecology might play a critical role in the establishment of the polyploid and coexistence of the cytotypes. Intercytotype matings often produce offspring of lower fitness than those produced by individuals of the same cytotype. Thus, Levin (1975) showed, that in a mixed population, the minority cytotype will produce less viable progeny than the most abundant one, if hybrid offspring are inviable. The proportion of the minority cytotype in a population would then progressively decline until it disappeared. However, Van Dijk & Bijlsma (1994) demonstrated that reproductive isolation through differences in flowering time alone might be sufficient to allow the coexistence of the two cytotypes. Thus, as Fowler & Levin (1984) demonstrated, niche differentiation may allow, under particular conditions, the coexistence of both cytotypes.

The role of ecological differentiation in the maintenance of mixed populations might be significant when both cytotypes are intermingled (sympatry) or grow as adjacent populations (parapatry). Different survival rates in two environments might allow the co-occurrence of two cytotypes in a mosaic environment (Harrison & Rand, 1989). Moreover, parapatric distributions are largely isolated spatially and reproductively, except at their common boundaries, where ecological differentiation might occur.

Close relationships between taxa distributions and environmental discontinuities do not necessary mean that ecological differentiation is the sole factor of maintenance of hybrid zones. According to Barton & Hewitt (1985), many hybrid zones persist as a result of a balance between random dispersal and selection against hybrids. Those tension zones can be either stable or mobile, depending on the respective densities and dispersal rates on each side of the boundary of the taxa. Moreover, tension zones can be trapped, for example, by environmental discontinuities (Barton, 1979).

In this paper we attempt to determine whether habitat differentiation exists in a hybrid zone be-

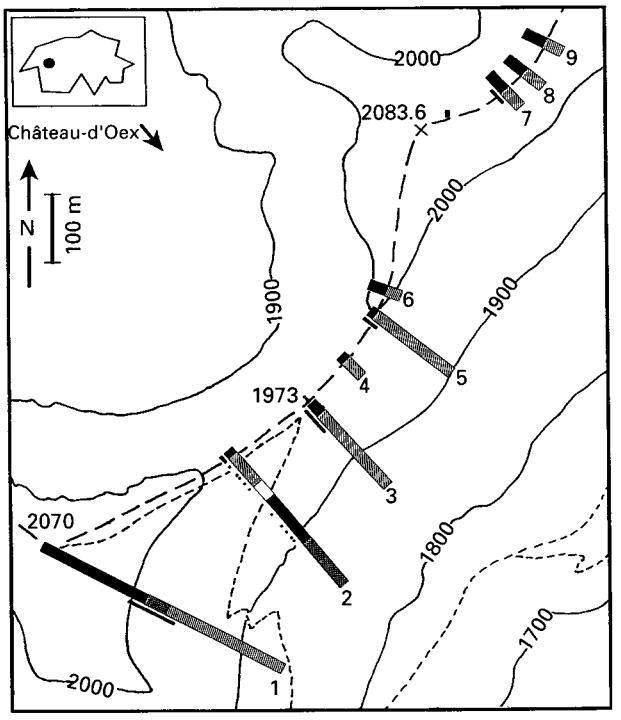

Figure 1. Study area and distribution of the cytotypes of *A. alpinum* (▨, diploid; ★, triploid; ■, tetraploid; ▩, diploid and tetraploid) along transects. Solid lines parallel to the transect indicate the subtransects on which vegetation relevés were undertaken.

tween diploid and tetraploid *Anthoxanthum alpinum* A. &. D. Löve, (*Poaceae*). Both cytotypes have parapatric distributions. The hybrid zone is narrow and consequently the transitions between both cytotypes occur over short distances where triploids have been discovered (Fig. 1).

We test two hypotheses: first, that in the hybrid zone, the two cytotypes have different habitat preferences (in relation to vegetation type), related to ecological differentiation; second, that cytotype segregation occurs in different vegetation transitions found. Our results are discussed in the context of the origin and dynamics of the hybrid zone.

MATERIALS AND METHODS

Study area

The study site is situated in the middle of the calcareous Swiss Prealps, at the limit of the counties of Vaud and Fribourg, northwest of Château-d'Oex. It lies on a SW-NE oriented anticline, limited to the North by the River Jogne and to the South by the River Sarine, and close to the well-known tourist centres of Saanen and Gstaad. The study area (Fig.1) lies approx. between 1850 and 2080 m above sea level.

The region has had a very active tectonic history, hence the marked variation in relief. The steep anticline has undergone intensive erosion through the glaciations and through karst development, which helped to shape the very steep slopes. Bedrock is mainly of limestone and calcareous clays of lower Cretaceous and Jurassic (Malm) age, partly as moraine or scree deposits. The hybrid zone is located entirely on Callovien and Oxfordien outcrops (Spoorenberg, 1952). In general, the climate is Atlantic, characterized by about 2000 mm yr^{-1} rainfall, with maximum in July and August.

The vegetation of the region is strongly influenced by the orientation of the slope. Northern faces can remain covered with snow until June at 2000 m altitude, and these slopes are the habitat for several artico-alpine species. By contrast, on southern slopes vegetation growth begins in March and contains a number of xero-thermophilous relicts associated with the oromediterranean and submediterranean flora (Richard, 1977). Table 1 gives a list of the species most frequently recorded in the vegetation relevés. According to Richard (1977), the vegetation on the northern slopes is classified as *Caricion ferrugineae*, *Seslerion* occurs on the southern slopes. Because of difficulty of access, the study area is protected to a great extent from the impact of tourism.

Cytotype characterization

Diploid and tetraploid *Anthoxanthum alpinum* belong to the *A. odoratum* L. *sensu lato* polyploid complex. Karyological studies have shown that the two species differ in the shape of certain chromosomes that have a secondary constriction (Teppner, 1970). Morphologically, *A. alpinum* differs from *A. odoratum* in having a fertile lemma, which is scabrous in the former and smooth in the latter (Felber, 1987*a*). The two species differ in their sensitivity to a rust fungus (Felber, 1987*b*) and in flowering period (Felber, 1988*a*), but there is no difference between the diploid and the tetraploid of each species. These results support the hypothesis of an autopolyploid origin of both the tetraploid *A. alpinum* and the tetraploid *A. odoratum*. The only characters, apart from chromosome counts, which allow separation of diploid and tetraploid plants of *A. alpinum* are the size of pollen and stomata, both of which are larger in the tetraploid (Humbert-Droz & Felber, 1992). In Europe, diploid *A. alpinum* has an arctic-alpine distribution. In its southern range, it is present in the mountain-ranges of the Balkans, Carpathian and Tatra, Alps and Jura. The tetraploid cytotype has a more restricted range and replaces the diploid in the southern Jura, in the southwest fringe of the Alps and in the Massif-Central (Felber, 1986, 1988*b*).

Sampling and measurements

The distribution of the cytotypes in the hybrid zone was determined by collecting all the plants of

Table 1. *Most frequent species in the overall vegetation and on both south and north facing slopes*

South slope	North slope	Both slopes
Lotus alpinus	*Campanula scheuchzeri*	*Carex sempervirens*
Aposeris foetida	*Primula elatior elatior*	*Festuca rubra rubra*
Trifolium pratense	*Bartsia alpina*	*Leontodon hispidus hispidus*
Helianthemum num. grandiflorum	*Luzula sylvatica*	*Sesleria alb. albicans*
Polygala chamaebuxus	*Salix retusa*	*Knautia dip. dipsacifolia*
Scabiosa lucida lucida	*Vaccinium myrtillus*	*Pulsatilla alpina alpina*
Chaerophyllum hirsutum	*Soldanella alpina*	*Galium anisophyllon*
	Salix reticulata	*Phleum hirsutum*
	Ranunculus alpestris	*Ranunculus nem. nemorosus*
	Arctostaphylos alpinus	*Homogyne alpina*

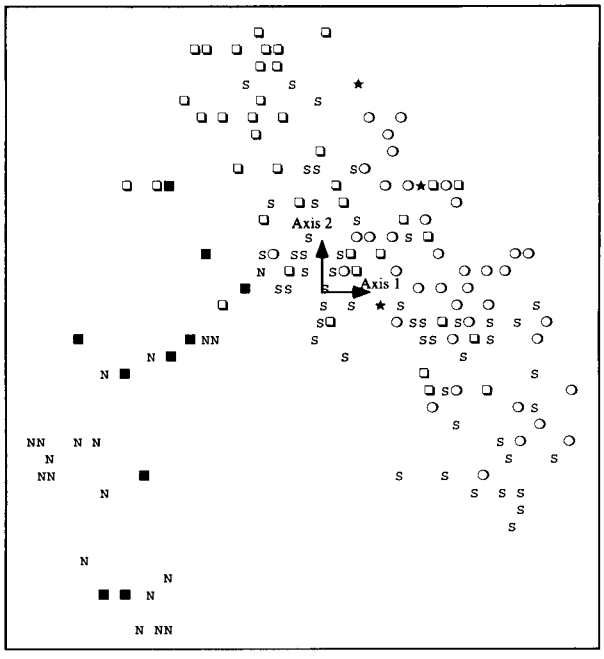

Figure 2. Principal coordinate analysis of all the vegetation relevés from transects 1, 2, 3, 5, and 7; axis 1 and 2 explain respectively 7 and 6 % of the variance. Symbols represent *A. alpinum* cytotypes: ○, diploid on south slope; □, tetraploid on south slope; ★, diploid and tetraploid on south slope; ■, tetraploid on north slope; S, no *A. alpinum* and south slope; N, no *A. alpinum* and north slope.

A. alpinum in flower (a total of 814 plants) on nine linear transects 0·2 m wide and 20–400 m long, during the summer of 1989 (Fig. 1). These plants were grown in the experimental garden of the University of Neuchâtel and the chromosome number of each was determined according to the method described by Felber (1986). Such a large number of plants was collected to allow us to examine the rate of hybridization between cytotypes under natural conditions (Felber, unpublished). In the summer of 1990, vegetation relevés were carried out on five subtransects across the hybrid zone (Fig. 1). The presence/absence of all species growing on contiguous plots 100 cm long and 20 cm wide was recorded. The strategy of sampling along physical axes was carried out under the assumption that: (i) the species in a community being closer together are likely to be influenced by the same generating processes (competition, hybridization, dispersal, etc.); and that (ii) the discontinuities between such patches are important for the structure (species-environment relations) and for the dynamics of species or ecosystems (Legendre, 1987).

The subtransects included 70 relevés on transect 1, 19 on transect 2 (including some isolated plots), 35 on transect 3, 20 on transect 5 and 20 on transect 7. They were selected so as to include the same number of relevés at diploid and tetraploid sites, except in the case of transect 2. Among the 164 plots, 29 lie on the north slope and 135 on the south slope (for simplification, a northwest slope is designated by north and a southeast slope by south). Forty plots had diploids, 51 tetraploids, three both diploids and tetraploids but, in 70, neither cytotype was present. Only seven triploids were recorded in four of the plots, which makes statistical testing irrelevant. Therefore, triploids were excluded from the statistical analyses.

Statistical analysis

Data were analysed in two steps, namely (i) the relation between vegetation and cytotypes, based on the entire set of 164 relevés of the five subtransects, and (ii) a space series analysis on transect 1, comprising 70 relevés.

First, the entire data set of 164 vegetation relevés, based on 122 species, was analysed by principal coordinate analysis (Gower, 1966) using a similarity matrix calculated with the asymmetrical Jaccard's coefficient (Figs 2, 4). This resemblance measure is most appropriate, since the data are qualitative and the absence of many species is considered to represent the absence of information rather than an element of similarity (Legendre & Legendre, 1984: vol. 2, p. 48).

To test the hypothesis of habitat preference and segregation of the cytotypes, Mantel tests (Mantel, 1967) were performed by comparing the same vegetation matrix based on Jaccard's resemblance measure (but calculated without the three *A. alpinum*

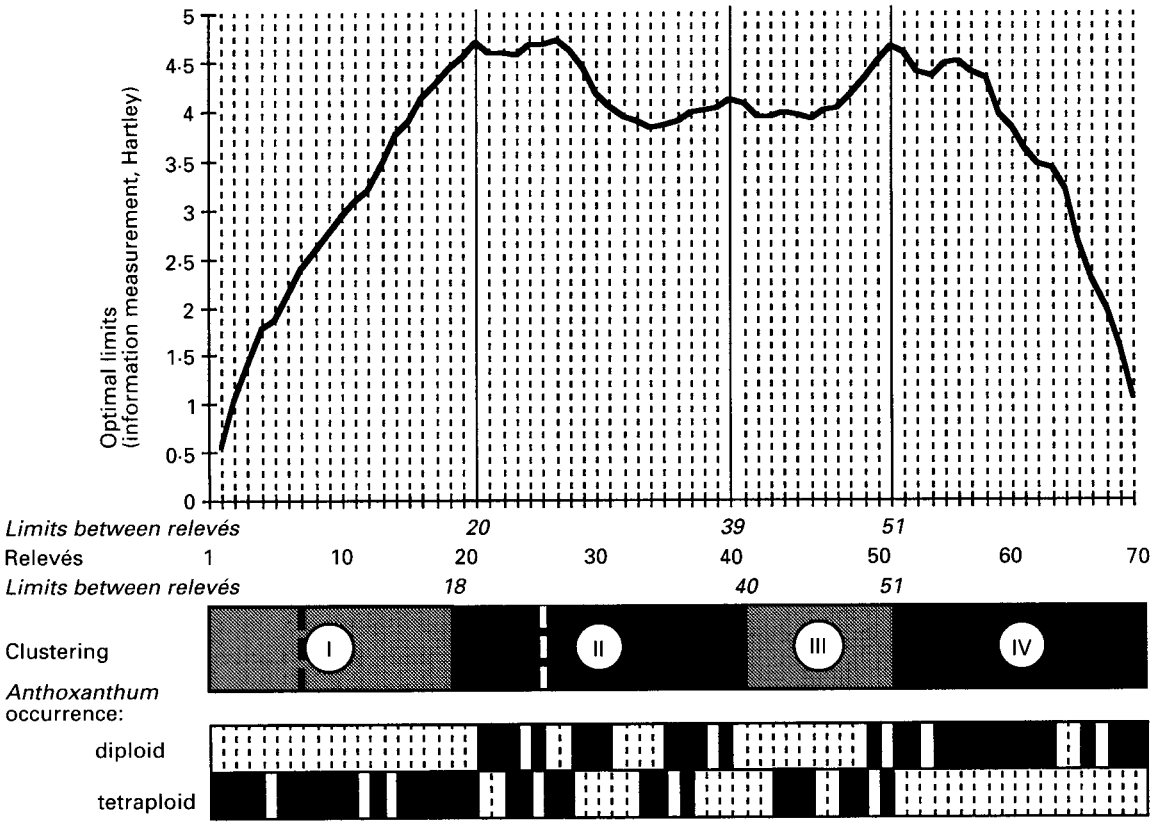

Figure 3. Transect 1 with limits between vegetation communities calculated by means of the optimal limits measurement and clustering with spatial constraint at $P < 0{\cdot}001$ and connexity 0.5 (at $P < 0{\cdot}01$ two additional discontinuities are recognized). Vegetation groups I to IV are significantly different at $P < 0{\cdot}001$. *A. alpinum* occurrences are illustrated for diploid and tetraploid cytotypes.

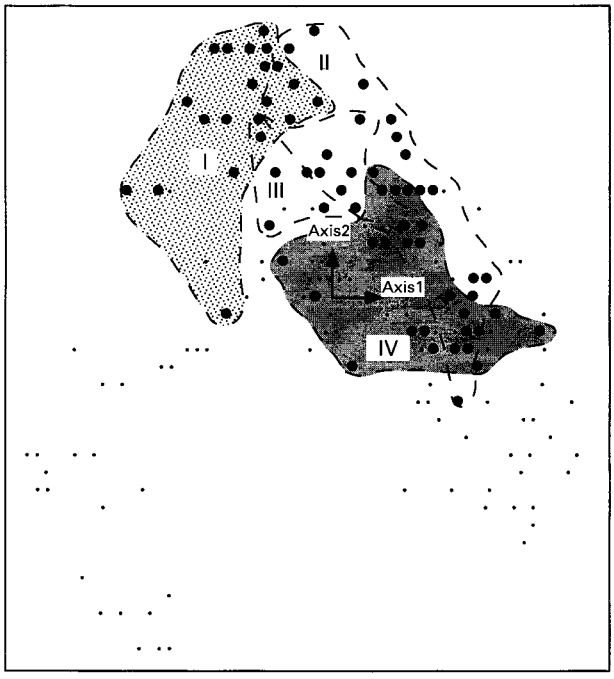

Figure 4. Principal coordinate analysis of all the vegetation relevés from transects 1, 2, 3, 5 and 7 as in Figure 2. Groups of transect 1 are shown by dashed lines.

cytotypes) with different hypothetical similarity matrices (Table 2). These represent the expected values for a perfect preference or segregation of habitats of the diploid and tetraploid cytotypes, following Hudon & Lamarche (1989). These model matrices represent the alternative hypotheses of an existing linear relation between the similarities of the two matrices.

The assumed autocorrelation between relevés (at least along each transect, as a result of the ecological hypothesis that determined the sampling strategy) makes it impossible to use classical inferential statistical tests, since the number of degrees of freedom cannot be calculated accurately (Legendre & Fortin, 1989; Legendre, 1993). Also, both the autocorrelation and the qualitative nature of the data are likely to invalidate the following assumptions: independence of samples, homogeneity of the variance and normal distribution of the errors. With the Mantel statistics, the standardized r-score (standardized form of the z statistics – Smouse *et al.*, 1986), which is equivalent to Pearson's linear correlation, was calculated between corresponding data of both matrices. A reference curve was then generated for each test by randomly permuting rows and columns in one of the matrices and re-computing the statistics. In this way, the sampling distribution of the Mantel statistics can be obtained by repeatedly simulating realizations of the null hypothesis. One thousand permutations were performed for all tests. The observed value was then compared with the

Table 2. *Comparisons of the similarity matrix of vegetation with that of the taxa of* Anthoxanthum alpinum *on all the relevés of transects 1, 2, 3, 5, 7*

Comparison (vegetation vs. taxon)	*r*
Vegetation relevés vs. cytotypes	
Relevés vs. diploid[a]	−0·034 n.s.
Relevés vs. tetraploid[b]	0·017 n.s.
Relevés vs. (diploid + tetraploid)[c]	−0·012 n.s.
Vegetation relevés vs. cytotypes and slopes	
Relevés vs. diploid[d]	0·21**
Relevés vs. tetraploid[e]	0·22**
Relevés vs. (diploid + tetraploid)[f]	0·21**
Vegetation relevés vs. cytotypes, slopes and transects	
Relevés vs. (diploid + tetraploid)[g]	0·36**
Vegetation relevés vs. slopes[h]	0·36**
Vegetation relevés vs. slopes and transects[i]	0·52**

r-statistics were calculated from the Mantel permutation test. Positions on north or south slopes, as well as on the different transects were differentiated for some analyses. Bonferroni's correction was applied to adjust significance levels for multiple comparisons. Differences were then determined as: n.s., non significant; ** $P < 0{\cdot}01$, highly significant.

Codes used for A. alpinum *data and calculation of the model matrices*: The data are binary, with rows representing the releves and columns representing the different descriptors (for example: value 1 when presence of the diploid, 0 when missing). To build the model matrix, data sets a,b,c and h and 8 are used with the simple matching coefficient, and data sets d, e, f, g and i with Jaccard's coefficient.

S, south slope; N, north slope, tr, transect.

[a] Presence of diploid; [b] presence of tetraploid; [c] presence of diploid/presence of tetraploid; [d] S with diploid/S without diploid/N; [e] N with tetraploid/S with tetraploid/N without tetraploid/S without tetraploid; [f] N with tetraploid/S with tetraploid/N without tetraploid /S without tetraploid or diploid/S with diploid; [g] tr1 4X/tr1 2X/tr1 without 2X or 4X/tr2 4X/tr2 2X/tr2 without 4X or 2X/tr3 N 4X/tr3 N without 2X or 4X/tr3 S 4X/tr3 S 2X/tr5 S 2X/tr5 S without 2X or 4X/tr5 N 4X/tr5 N without 2X or 4X/tr7 N 4X/tr7 N without 2X or 4X/tr7 S 2X/tr7 S without 2X or 4X; [h] N; [i] tr1 S/tr2 S/tr3 N/tr3 S/tr5 S/tr5 N/tr7 N/tr7 S.

Table 3. *Main group discriminating species on transect 1 (h, high frequency; l, low frequency)*

Group	I	II	III	IV
Ligusticum mutellina	h			
Agrostis capillaris	h		h	
Hieracium murorum		h		
Helianthemum num. grandiflorum		h		h
Alchemilla conjuncta		h		l
Aster bellidiastrum		h		l
Polygonum viviparum		l		h
Scabiosa lucida lucida		l		h
Trifolium pratense		l		h
Deschampsia cespitosa			l	
Pimpinella major			h	l
Potentilla erecta				h
Campanula rotundifolia				h
Chaerophyllum hirsutum				h
Prunella grandiflora grandiflora		l		h
Viola hirta		l		l
Polygala chamaebuxus	l	l	l	h
Aposeris foetida	h	h	l	h
Homogyne alpina	h	h	l	h

randomly calculated values in order to calculate a probability associated with the null hypothesis, i.e. that there is no relation between the vegetation matrix and the *A. alpinum* model matrix, which means no habitat preference (if the model matrix gives the presence/absence of one cytotype only) or absence of segregation of habitats (if the model matrix uses the presence/absence of both cytotypes simultaneously). If the observed value is greater than 999 calculated random values, then it can be stated, for $P < 0{\cdot}001$, that the null hypothesis can be rejected, and that the idea of habitat preference or segregation must be accepted. Bonferoni's correction (Rice, 1989) was applied in order to calculate significance levels adjusted for multiple comparisons performed on the same data set.

A simple matching coefficient (Legendre & Legendre, 1984: vol. 2, p. 49) was used to calculate

Table 4. *Comparisons of the similarity matrix of vegetation with that of the taxa of* Anthoxanthum alpinum *along transect 1*

Comparison (vegetation vs. taxon)	*r*
Relevés vs. diploid	0·13 **
Relevés vs. tetraploid	0·18 **
Relevés vs. (diploid + tetraploid)	0·20 **
Groups I to IV vs. diploid	0·21 **
Groups I to IV vs. tetraploid	0·21 **
Groups I to IV vs. (diploid + tetraploid)	0·26 **
Groups I and II vs. diploid	0·21 *
Groups I and III vs. diploid	| n.s.
Groups I and IV vs. diploid	| **
Groups II and III vs. diploid	| n.s.
Groups II and IV vs. diploid	| n.s.
Groups III and IV vs. diploid	| **
Groups I and II vs. tetraploid	0·21 n.s.
Groups I and III vs. tetraploid	| n.s.
Groups I and IV vs. tetraploid	| **
Groups II and III vs. tetraploid	| n.s.
Groups II and IV vs. tetraploid	| *
Groups III and IV vs. tetraploid	| *
Groups I and II vs. (diploid + tetraploid)	0·26 *
Groups I and III vs. (diploid + tetraploid)	| n.s.
Groups I and IV vs. (diploid + tetraploid)	| **
Groups II and III vs. (diploid + tetraploid)	| n.s.
Groups II and IV vs. (diploid + tetraploid)	| *
Groups III and IV vs. (diploid + tetraploid)	| **
Relevés of groups II and III vs. diploid	−0·004 n.s.
Relevés of groups II and III vs. tetraploid	0·045 n.s.
Relevés of groups II and III vs. (diploid + tetraploid)	0·027 n.s.

r-statistics were calculated from the Mantel permutation test. Raw data or the groups (I, II, III, and IV) determined by clustering with spatial constraint, described the vegetation. Analyses were performed on the whole matrix or on sub-sets. Bonferroni's correction was applied to adjust significance levels for multiple comparisons. Differences were then determined as: n.s., non significant; * $P < 0{\cdot}05$, significant; ** $P < 0{\cdot}01$, highly significant.

the model matrix (representing the alternative hypothesis) of each of the cytotypes or both together, as well as of the slope (Table 2). In this matrix, a value of 1 was given when the figure of the two compared relevés was totally identical (double presence or absence of one cytotype, or presence or absence of both when comparing the two taxa simultaneously), 0 when totally different (and possibly 0·5 if testing both taxa together, when only one occurrence was identical). When performing the test according to the slope or to the transect, the data were coded (Table 2) in order to allow the use of Jaccard's coefficient. In that case the similarity is 1 when the figure is the same (same cytotype occurrence or absence on the same slope and, possibly, transect), 0 when different.

On transect 1, the 70 contiguous vegetation relevés were used in a space series analysis to identify the discontinuities in the vegetation community and the distribution of diploid and tetraploid *A. alpinum*. Based upon all species present (except *A. alpinum* taxa), those relevés were analysed by means of information theory and the derived optimal limits measurement (Godron, 1971; Godron & Bacou, 1975; Guillerm, Gauthier & Romane, 1976), as well as by means of chronological clustering (Legendre, Dallot & Legendre 1985; Legendre, 1987) which corresponds to a proportional-link linkage clustering (Sneath, 1966) with a constraint of space contiguity. The latter was based on a similarity matrix calculated with Jaccard's coefficient. Figure 3 shows the results of both techniques. The second method was used as a reference in the building of a new group matrix (model matrix).

In order to test the relation between the raw data of each relevé or vegetation group and the distribution of diploid and tetraploid *A. alpinum*, Mantel tests were performed (Table 4). The same matching coefficient as before (see explanation in Table 2) was used to calculate the model matrix for diploid, for tetraploid or for both diploid and tetraploid *A. alpinum*. The vegetation similarity matrix was calculated with Jaccard's coefficient and the vegetation group matrix also, on the same principle as described above (see also explanation in Table 2). A pair of relevés belonging to the same

vegetation group were assigned the value 1, otherwise the value 0. Tests were performed either on all paired matrices or on subsets in order to determine in which pair of vegetation groups the distribution of diploid and tetraploid *A. alpinum* was significantly different.

The location of the relevés along transect 1 and their four groups is specified on the principal coordinate scatter diagram (Fig. 4). The best discriminant species corresponding to those groups were calculated by means of Jancey's ranking of *F*-values (Jancey, 1979; Table 3).

The statistical analyses were performed using statistical packages MULVA-4 (Wildi & Orloci, 1990), 'R' (Legendre & Vaudor, 1985) and ANATRANS (Pagni, 1989).

RESULTS

The relationship between the vegetation and the distribution of diploid and tetraploid *A. alpinum* is shown in Figure 2. The ordination scatter diagram represents the variability of the 164 vegetation relevés, with different symbols representing the different cytotypes and variation in slope. The vegetation differs markedly between the two slopes. Furthermore, the two cytotypes occur in rather different vegetation types on the south slope (only tetraploid *A. alpinum* occurs on the relevés of the north slope).

These relations and the effect of the position of the transects in the hybrid zone are tested in Table 2. On the whole, there is no significant association between vegetation type and diploid distribution, tetraploid distribution or distribution of the two cytotypes when both are considered together. But when the effect of the slope is taken into account, the correlations become significant. When comparing the relevés versus one cytotype the tests are significant, which means that habitat preference occurs. Thus, on each slope, vegetation with one particular cytotype differs from that without it. When comparing the vegetation relevés against both cytotypes, the test is also significant, which indicates that the habitats of the cytotypes exclude each other, and that habitat segregation occurs. Therefore, each cytotype has its specific vegetation.

A significant relation was also detected if the transect effect was included in the test. Thus, habitat segregation occured in various kinds of vegetation, depending upon the location of the transects.

Transect 1 was also studied on its own, in order to test the relation between the two patterns of spatial transition of the cytotypes and the vegetation (Fig. 3). Both methods, the optimal limits information measurement, which allows one to draw a curve where the highest peaks represent the best limits in terms of information, and the constrained clustering, yielded similar results. In general, three major breaks and four groups in the vegetation pattern were observed. The four groups are significantly different, and are characterized by several discriminating species (Table 3). Their position on the ordination scatter diagram is shown in Figure 4. Group I, at one end of the transect, is represented exclusively by tetraploid *A. alpinum*. Group IV, at the opposite end, is characterized by the diploid cytotype. Group III, and particularly group II, include both cytotypes and showed no clear pattern.

Vegetation relevés were significantly correlated with diploid and tetraploid cytotypes when considered separately and together (Table 4). Thus, as previously discovered for the entire hybrid zone, habitat preference and habitat segregation occured within the transect.

If the vegetation group model-matrix is used, a significant relation is again calculated when the four groups are considered together. Tests on subsets illustrate the basis for the diploid and tetraploid habitat preference and segregation. The diploid cytotype had a significant habitat preference in group IV, at the right end of the transect, as compared with groups I and III where this cytotype was missing almost totally (1 presence in group III). Similarly, the tetraploid cytotype was predominant in vegetation group I, at the beginning of the transect, as compared with group IV where it is missing. From the tests with both cytotypes, it can be seen that the two ends of the transect reveal in the clearest way the habitat segregation of diploid and tetraploid *A. alpinum*. No significant relation exists between groups of vegetation and cytotypes when groups II and III are considered. In those groups, cytotype distributions are more random. The relation of groups II and III with the other groups is less significant, except when groups III and IV are considered. The Mantel test was also performed on a subset of relevés' raw data corresponding to groups II and III and it was shown that no significant relation exists between those vegetation relevés and the diploid, the tetraploid or both cytotypes.

DISCUSSION

The distribution of the cytotypes

Close and fine-scale relationships between diploid and tetraploid cytotypes and habitats were found in the hybrid zone. Only these two cytotypes have been considered in the analyses, since triploids were rare and their ecological significance is most probably negligible in the context of our study. Vegetation differed depending on the location in the hybrid zone, according to either the slope or to the lateral position of the transects on the slope. Thus, the ecological determinant of the distribution of the cytotypes was not restricted to two environments, but depended on a range of habitats.

In the hybrid zone as a whole, the two cytotypes both showed a clear habitat preference and, furthermore, habitat segregation occured. Thus, the same vegetation type was not colonized by one cytotype in one location and by the other on another site. This demonstrates that, at least in the hybrid zone, both cytotypes have different ecological requirements in the different transition zones.

These correlations of cytotypes with their habitats were observed not only at the scale of the hybrid zone, but also when the four contiguous groups of transect 1 were considered separately. The four vegetation groups differed in their floristic composition (Table 3), but overlapped partially on the scatter diagram of the principal coordinate analysis (Fig. 4). The absence of significant relationship between vegetation relevés and cytotype when only groups II and III were considered might mean that incomplete habitat segregation occurred. In that case, this zone of mixing might be interpreted as a 'tension zone', and groups I and IV and their surroundings as zones from which individuals of *A. alpinum* disperse in the tension zone. However, the possibility cannot be excluded that the patchiness of the environment was on a smaller scale than the vegetation sampling grid, and therefore remained undetected.

Ecological differentiation in polyploids

The role of ecological differentiation for the coexistence of two cytotypes is generally accepted (Thompson & Lumaret, 1992). However, the ecological requirements of cytotypes belonging to a single polyploid complex might vary, ranging from absence of ecological differentiation, to partial overlapping or net segregation of habitats. The type of the polyploidy might also influence ecological requirements. Allopolyploids might be expected to have intermediate or new ecological requirements, compared to their diploid progenitors, because they contain two different genomes. However, by contrast, autopolyploids might have either different or similar habitats depending upon whether autopolyploidy induces changes in the ecological requirements. These two types of polyploidy will now be considered in turn.

Considering first allopolyploidy, successful colonization of new habitats (intertidal marsh zones) has been recorded for the allopolyploid *Spartina anglica* in Great Britain (Thompson, 1991). A larger ecological amplitude has been noted for allopolyploids in *Draba* compared with their diploid relatives (Brochmann & Elven, 1992). Ecological differentiation has been demonstrated in several hybrid zones between cytotypes of *Ranunculus* sect. *Ranuncella*, which includes several allopolyploids (Vuille, 1986). Habitat segregation has also been reported for the eight sexual species of *Antennaria* and their allopolyploid derivative, *A. rosea* (Bayer, Purdy & Lebedyk, 1991). Moreover, ecological segregation, although incomplete, was found in sympatric populations of diploid *Cardamine rivularis*, diploid *C. amara*, their triploid hybrid (*C. insueta*) and its allohexaploid derivation (*C. Schultzii*) (Urbanska-Worytkiewicz & Landolt, 1978).

With regard to autopolyploidy, similar ecological requirements for diploids and autotetraploids have been found at the level of the range (*Galax*, Baldwin, 1941; *Tripleurospermum inodorum*, Kay, 1969), or when the cytotypes grew in sympatry (*Dactylis glomerata* ssp. *mairei*, Borrill & Lindner, 1971; *Plantago media*, van Dijk, Hartog & van Delden, 1992). However, ecological segregation at the scale of the range has been described for diploids and autotetraploids of *Achlys triphylla* (Fukuda, 1967) and *Deschampsia caespitosa* (Rothera & Davy, 1986). In hybrid zones, habitat differentiation between diploids and tetraploids has been found repeatedly in *Dactylis* (Zohary & Nur, 1959; Borrill & Lindner, 1971; Lumaret *et al.*, 1987) and in *Lotus corniculatus sensu lato* (Jay *et al.*, 1991).

Contrary to what was observed in the hybrid zone that we investigated, diploid and tetraploid *A. alpinum* show no evidence of habitat segregation when they grow in pure and isolated populations on other mountains close to the hybrid zone. Their distribution is independent of aspect and there is no evidence that a particular cytotype 'avoids' the habitat types characteristic of the 'other cytotype' in the hybrid zone we studied (Felber, 1986).

Differences in ecological requirements between diploids and tetraploids may be a direct consequence of polyploidy or may be caused by allopatric differentiation. Thus, the particular preferences of each cytotype might be observed across its whole range (primary habitat differentiation). On the other hand, each cytotype might exist in similar habitats across its range, except in hybrid zones where ecological differentiation occurs. Such secondary habitat differentiation might represent a response to competition between cytotypes and, thus, represent an example of ecological character displacement (Brown & Wilson, 1956; Grant, 1975). We consider that in *A. alpinum*, the ecological differentiation that occurred in the hybrid zone is probably secondary in origin.

Origin of hybrid zones

There has been much debate about the origin of hybrid zones. They might be the consequence of primary intergradation, resulting from the direct response to an environmental gradient (Slatkin, 1973; Endler, 1977; Caisse & Antonovics 1978). They might also result from secondary contact between populations that have differentiated in

allopatry (Mayr, 1942; Chapman, 1892). Many hybrid zones in Europe and North America are probably of secondary origin (Hewitt, 1988), dating from post-glacial warming, when species ranges expanded. Human activities have also probably contributed to the creation of hybrid zones, by extending some habitats and creating new ones. Deforestation occurred early in the history of man, beginning in the Alps as early as the Middle-Ages (Burga, 1988). Forest clearance might have allowed species previously separated by an ecological barrier to meet. Secondary contact between diploid and tetraploid *A. alpinum* is likely. Both cytotypes are allopatric. The diploid has a wide distribution and the tetraploid a narrower marginal range. During the Quaternary glaciations, diploid *A. alpinum* might have been remained in refugia in the Alps. Tetraploid *A. alpinum* may have originated at that time, on the edge of the glaciers (Felber, 1988*b*). The hybrid zone could have originated either during the spread of both taxa during the subsequent warming or, more recently, when deforestation by humans caused an extension of pastures. The present day survival of small trees close to the ridge in our study site suggests that forest might have reached higher altitudes in former times.

A hybrid zone can be only a few metres wide or can occur across several hundreds of kilometres. Very narrow hybrid zones are often described as parapatric distributions (Bull, 1991). Indeed, different definitions of parapatry exist. For example, Grant (1977) excludes interbreeding in defining parapatry. Only in neighbouring sympatry might hybridization occur. However, Key (1981) distinguishes *hybridization parapatry*, where both taxa form a narrow hybrid zone, and *ecological parapatry* where no hybridization occurs. In the hybrid zone between diploid and tetraploid *A. alpinum*, triploid hybrids have been found in the field, as well as in the natural progeny of diploids and tetraploids (Felber, unpublished). Thus, the hybrid zone we studied corresponds to a situation of 'neighbouring sympatry' *sensu* Grant (1977) but to 'hybridization parapatry' *sensu* Key (1981).

Concluding remarks

Niche differentiation is one of the factors that affects the dynamics of the hybrid zone. Hybrid zones might be maintained by the joint action of dispersal of pure species within the hybrid zone and selection against hybrids (tension zone), as well as the occurrence of barriers against gene flow (increased selfing and flowering-time differentiation). The habitat differentiation observed in the hybrid zone between diploid and tetraploid *A. alpinum* is most probably correlated with niche differentiation and this contributes to the maintenance of the hybrid zone. But differential survival in the different environments is probably not the only factor that influences the hybrid zone. Partial flowering-time differentiation has been shown in the field (Felber, 1996) and in the experimental garden (Felber, unpublished). Furthermore, relationships between fitness and heterozygosity could also be important in the tension zone. Thus, for one gene locus there is evidence of hybrid infertility in plants of the hybrid zone, whereas heterosis was found for distinct pure populations (Zeroual-Humbert-Droz, 1995). Consequently, at least three processes may contribute to the maintenance of the hybrid zone: niche differentiation of the cytotypes, flowering-time differences and an equilibrium between random dispersal and selection against hybrids (triploid inviability).

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