# A graphical analysis of the characters of *Calamagrostis stricta* (Timm) Koeler, *C. canescens* (Wigg.) Roth and their hybrid populations in S. E. Yorks., v.c. 61, northern England

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ABSTRACT

Population samples of *Calamagrostis stricta* (Timm) Koeler, *C. canescens* (Wigg.) Roth and the hybrids *C. canescens* × *C. stricta* (Poaceae), 2n = 28 and 2n = 56, as well as a strongly introgressant *C. canescens* population along Leven Canal, S.E. Yorks., v.c. 61 are described using methods of polygraphic analysis: pictorial scatter diagram, hybrid index analysis and polygonal graphs. A polygonal graph is also given for a recombinant represented by a single plant. The overall intermediate nature of the hybrids is demonstrated as well as the loose non-random association of characters. The nature of the hybrids, the effect of polyploidy and the relative merits and limitations of the different methods of polygraphic analysis used are discussed. Evidence for a *C. canescens* population being strongly introgressed is assembled.

KEYWORDS: Poaceae, introgression, multivariate analysis.

## INTRODUCTION

In an earlier paper (Crackles 1994) I described a diverse group of *Calamagrostis* populations along the Leven Canal in S.E. Yorkshire (v.c. 61) and showed that these could be assigned to six 'taxa': *C. canescens* (Wigg.) Roth, *C. stricta* (Timm) Koeler, *C. canescens* × *C. stricta* 2n = 56 ($H_1$ population), *C. canescens* × *C. stricta* 2n = 28 ($H_2$ population), a recombinant individual plant ($H_3$) and an introgressed *C. canescens* ($I_1$ population). These could all be identified in the field.

To explore in more detail the relationship between these various populations and the characters by which they differed, I undertook a comparison of population samples by graphical methods of analysis – using pictorial scatter diagrams, hybrid index analysis (Anderson 1949) and polygonal graphs. The results provide some further insights into the relationships of the Leven *Calamagrostis* populations, as well as illustrating the continuing value of these relatively simple graphical methods and indicating their relative weaknesses and strengths.

## METHOD OF STUDY

25 shoots of *C. stricta*, *C. canescens* and the putative hybrid populations $H_1$ and $H_2$ and 20 shoots of the $I_1$ population (Crackles 1994) were collected at random. Material was collected at the end of July or in early August 1970, when the panicles were in their after-flowering condition so that comparison of the maximum number of floret characters could be made. The range of measurements, the mean and the standard deviation for various characters are given in the previous paper (Crackles 1994).

As axes for each scatter diagram (Figs 1 & 2), the ratio of bract length to bract width was plotted against the ratio of panicle length to length of basal branch of panicle. Ratios were used to minimise the possible effect of varying environmental conditions on lengths of structures. Other information was added to the scatter diagram to produce metroglyphs.

In the case of the ratio of glume length to glume width, and awn length, the intermediate range was calculated by dividing the range between the standard deviations for the two species by four and taking the median two quarters as the intermediate range.

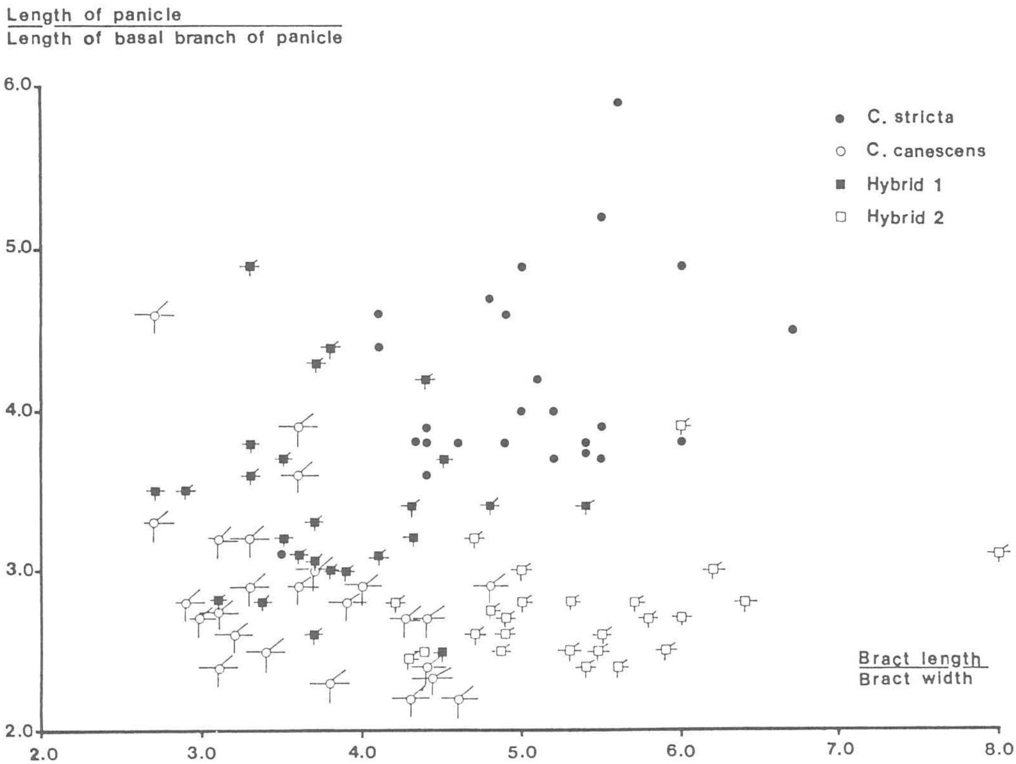


FIGURE 1. Scatter diagram for population samples of *Calamagrostis stricta*, *C. canescens* and *C. canescens* × *C. stricta*, Hybrid 1 and Hybrid 2 ($H_1$ and $H_2$ populations).
Callus hairs: shorter than lemma ●; ± equal to lemma ●; longer than lemma ●.
Awn insertion from: bottom 37·5% of lemma ●; 37·5%–85·0% way up lemma ●; near tip of lemma ●.
Awn length: >1·7 mm ●; 0·9–1·7 mm ●-; <0·9 mm ●–.
Glume length/glume width: <4·5 ●; 4·5–6·0-●; >6·0 –●.

A more arbitrary method had to be used to determine the intermediate range for the position of awn insertion on the lemma. It was known from observation that the awn is inserted just below the middle of the lemma in the hybrid, *C. canescens* × *C. stricta*. In *C. canescens* the awn is almost apical, while in *C. stricta* it is inserted 25%–35% along the length of the lemma from its base. The intermediate range was defined by dividing the range 25%–50% into two and taking the lower half as *C. stricta* and the upper half as intermediate.

The hairs surrounding the floret (callus hairs) are generally at least 0·7 mm longer than the lemma in *C. canescens* and substantially shorter than the lemma in *C. stricta*. Callus hair length between 0.4 mm longer and 0.4 mm shorter than the lemma was taken to be an intermediate character.

The method of hybrid index analysis is described by Anderson (1949) and consists of selecting a number of characters with respect to which the species differ, and recording each character of each plant as identical with that of one of the species or as intermediate between them. Each character is then assigned an arbitrary value. When each of the characters of the plant listed has received a score, the total index score of the plant is obtained by summing the scores of all its characters.

In selecting characters the following principles were borne in mind:

1. in obtaining an index score for an individual plant or a population it is essential that as many characters as possible are taken into account;
2. an index score for each character used must be obtainable for each population analysed;
3. characters which can be observed with the naked eye or with a hand lens and structures which can

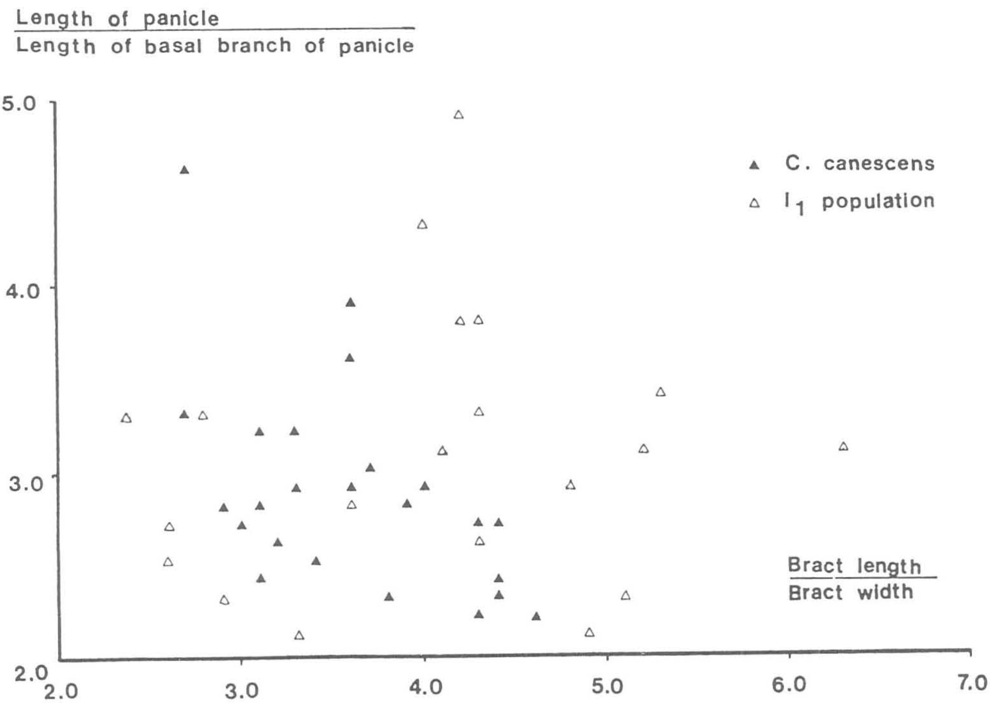


FIGURE 2. Scatter diagram for population samples of *Calamagrostis canescens* and a suspected *C. canescens* introgressant ($I_1$ population).

be measured without the use of a microscope are most useful and were used whenever possible; and

4. floret characters were included in order to achieve coverage of important features although measurements had to be made using a binocular microscope.

TABLE 1. METHOD OF SCORING THE HYBRID INDEX FOR *CALAMAGROSTIS* TAXA

| Character | *C. stricta* Score 0 | Intermediate Score 1 | *C. canescens* Score 2 |
|---|---|---|---|
| Panicle length (cm) | <14·0 | 14·0–15·5 | >15·5 |
| Panicle width (in fruit) (cm) | <1·6 | 1·6–2·5 | >2·5 |
| Length of basal branch of panicle (cm) | <4·0 | 4·0–5·1 | >5·1 |
| Culm rough near panicle | + | | –* |
| Culm width at 2nd node (mm) | <1·6 | 1·6–2·0 | >2·0 |
| Bottom leaf sheath hairy | + | | –* |
| Glume length (mm) | <4·0 | 4·0–4·5 | >4·5 |
| Glume width (mm) | >0·90 | 0·55–0·90 | 0·60–0·70 |
| Glume length/glume width | <4·5 | 4·5–6·0 | >6·0 |
| Callus hairs | <floret | ± = floret | >floret |
| Awn length (mm) | >1·7 | 0·9–1·7 | <0·9 |
| Awn insertion** | <37·5% | 37·5%–85·0% | >85% |
| Ligule length (mm) | <3·0 | 3·0–3·6 | >3·6 |

*scored as 1.
**awn insertion measured from base of floret as % of floret height.

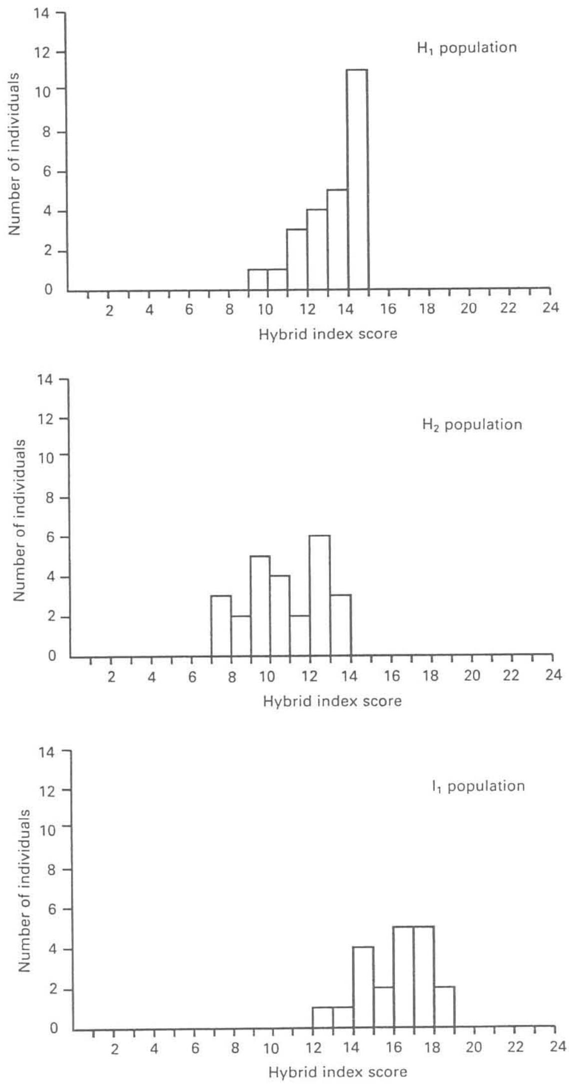


FIGURE 3. Hybrid index scores for Leven *Calamagrostis canescens* × *C. stricta*, $H_1$ and $H_2$, and the *C. canescens* $I_1$ population samples.

A list of characters used and the method of scoring are given in Table 1. The intermediate range for each character was determined in the same way as for the scatter diagrams. The frequency distribution of hybrid index scores for the $H_1$, $H_2$ and $I_1$ populations are shown in Fig. 3.

A polygonal graph was constructed for each of the Leven *Calamagrostis* populations studied and for a single plant, the only one of its kind found, $H_3$ (Fig. 4) The method of scoring is given in Table 2. In using this method, characters in which a hybrid shows a range of measurements which is not intermediate between those of the parents can be included and I have selected lemma length as one of the eight characters. The lemma of individuals of the $H_1$ population is longer than that of both parents (Crackles 1994).

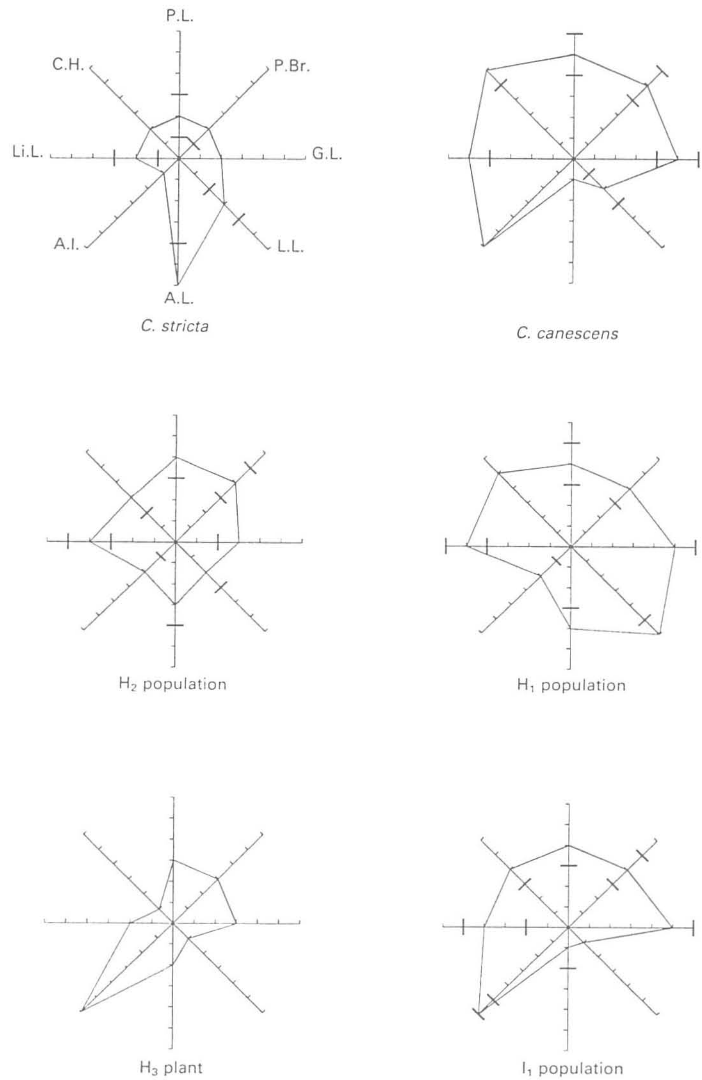


FIGURE 4. Variation in eight characters of the Leven *Calamagrostis* taxa. Each radius has six divisions (see Table 2). In the case of each Leven *Calamagrostis* population sample, except the $H_3$, the polygonal graph was drawn using the mean of the measurements for each character; standard deviations are shown along each axis. The polygonal graph for the $H_3$ was drawn using measurements for one plant only. Abbreviations are given in Table 2.

## RESULTS

The overall intermediate morphological nature of the $H_1$ and $H_2$ populations between that of the two species is demonstrated by the intermediate shape of the polygonal graphs for the two hybrids (Fig. 4) and by the range of hybrid scores for both hybrid populations (Fig. 3).

The tendency of intermediate characters of the $H_1$ and $H_2$ populations to be inherited together, but not invariably so, is shown for four characters by the use of intermediate length arms attached to dots on the scatter diagram (Fig. 1). By examining these four characters, a pair at a time (Table 3), it is seen that such intermediate characters have a strong tendency to remain together, but that the

TABLE 2. METHOD OF SCORING FOR POLYGONAL GRAPHS OF *CALAMAGROSTIS* TAXA

| | Radius division from the centre of a circle* | | | | | |
|---|---|---|---|---|---|---|
| Character | 0 | 1 | 2 | 3 | 4 | 5 |
| Panicle length (P.L.) (cm) | 5·9–8·9 | 9·0–12·0 | 12·1–15·1 | 15·2–18·2 | 18·3–21·3 | 21·4–24·4 |
| Length of basal branch of panicle (P.Br.) (cm) | 1·2–2·4 | 2·5–3·7 | 3·8–5·0 | 5·1–6·3 | 6·4–7·6 | 7·7–8·9 |
| Glume length (G.L.) (mm) | 2·6–3·1 | 3·2–3·7 | 3·8–4·3 | 4·4–4·9 | 5·0–5·5 | 5·6–6·1 |
| Lemma length (L.L.) (mm) | 2·5–2·6 | 2·7–2·8 | 2·9–3·0 | 3·1–3·2 | 3·3–3·4 | 3·5–3·6 |
| Awn length (A.L.) (mm) | 0·3–0·6 | 0·7–1·0 | 1·1–1·4 | 1·5–1·8 | 1·9–2·2 | 2·3–2·6 |
| Awn insertion** (A.I.) | 27%–38% | 39%–50% | 51%–62% | 63%–74% | 75%–86% | 87%–98% |
| Ligule length (Li.L.) (mm) | 1·3–1·8 | 1·9–2·4 | 2·5–3·0 | 3·1–3·6 | 3·7–4·2 | 4·3–4·8 |
| Callus hairs (C.H.) (mm) | 1·9–2·1 | 2·2–2·5 | 2·6–2·9 | 3·0–3·3 | 3·4–3·7 | 3·8–4·1 |

*see Fig. 4.
**measured from the base of the floret as a % of floret height.

TABLE 3. THE FREQUENCY OF ASSOCIATION OF CHARACTERS WITHIN TWO POPULATIONS ($H_1$ AND $H_2$) OF *CALAMAGROSTIS*, EXAMINED TWO AT A TIME

| | Percentage association* | | |
|---|---|---|---|
| Associated character pair | $H_1$ | $H_2$ | $H_1$ and $H_2$ |
| Callus hairs and awn length | 80 | 84 | 82 |
| Awn length and awn insertion | 64 | 84 | 74 |
| Callus hairs and awn insertion | 72 | 72 | 72 |
| Glume ratio and awn insertion | 76 | 60 | 68 |
| Glume ratio and awn length | 80 | 64 | 72 |
| Callus hairs and glume ratio | 96 | 60 | 78 |

*percentages calculated using the number of short 'arms', representing intermediate states, on the scatter diagram shown in Fig. 1.

strength of the affinity between these varies not only from one pair of characters to another, but also usually from one hybrid population to the other.

The $H_1$ and $H_2$ populations were found to be morphologically distinct (Crackles 1994). Differences between these two populations are further demonstrated by:

a. the fact that most individuals of the $H_2$ population appear in a different part of the scatter diagram from those of the $H_1$ population (Fig. 1); and
b. differences between the polygonal graphs for the two populations.

The differences between the polygonal graphs for the hybrid populations $H_1$ and $H_2$ is mainly one of size, there being a general similarity of shape except for the difference caused by the much greater lemma length in the $H_1$ population.

Morphological evidence has already been given to support the view that the *C. canescens* $I_1$ population is introgressed (Crackles 1994). Further evidence of this view is provided by graphical analysis:

a. over half of the individuals of the population occur outside the area occupied by typical *C. canescens* individuals on the scatter diagram (Fig. 2);
b. a hybrid index range of 13–19 was obtained for the $I_1$ population where *C. stricta* = 0 and *C. canescens* = 23; and
c. a polygonal graph for the $I_1$ population (Fig. 4) while of the same shape as that for typical *C. canescens* shows a shift towards the hybrid range for some characters.

The polygonal graph for the Leven $H_3$, represented by a single plant, confirms that this is a recombinant. The position of awn insertion was as in *C. canescens* and the awn length as in some *C. canescens* $I_1$ individuals while the panicle and ligule length were as in *C. stricta*. The basal branch of the panicle and the glume were intermediate in length, being within the range for these characters in the *C. canescens* × *C. stricta* $H_2$ population. The callus hairs tended to be shorter than in *C. stricta*.

## DISCUSSION

The loose non-random association of characters in both the $H_1$ and $H_2$ populations demonstrated by the scatter diagrams (see Table 3) is considered by Anderson (1949) to be critical evidence of hybridization. This evidence together with the intermediate nature of many characters of these taxa (Crackles 1994) and their overall intermediacy demonstrated by the polygonal graphs and their hybrid index range, ($H_1$ 10–15, mode = 15; $H_2$ 8–14, mode = 13; *C. stricta* = 0, *C. canescens* = 23), as well as the fact that they occur in the same locality as both parental species, leave no doubt that both the $H_1$ and $H_2$ populations are the hybrid *C. canescens* × *C. stricta*.

Comparison of the polygonal graphs for the $H_1$ and $H_2$ populations suggest that the main difference between the two taxa is one of size of several characters.

The chromosome number of the Leven *C. stricta*, *C. canescens* and the $H_2$ *C. canescens* × *C. stricta* populations is 2n = 28 (Crackles 1994), a number Nygren (1946) found to be constant for the two species. The chromosome number for the Leven $H_1$ *C. canescens* × *C. stricta* is, however, 2n = 56 so that this taxon is an octoploid (Crackles 1994).

The question of the likely effects of polyploidy on characters of the $H_1$ population thus arises and whether such effects may be the full explanation of differences between the $H_1$ and $H_2$ populations. Stebbins (1971) drew attention to the varying effects of polyploidy in different genotypes and also to the fact that gigas effects are seen most often and most strongly in organs with a determinate type of growth. It would seem that polyploidy is the explanation of increased lemma length which is greater than in either parent. It is also the probable explanation of the longer and wider glumes while an intermediate shape is retained and of greater callus hair length which approaches that of *C. canescens* while remaining more or less the same length as the lemma as in other hybrid individuals. A tendency to greater plant height, greater panicle length and greater awn length would also seem to be due to polyploidy. Features frequently resulting from polyploidy and not occurring in the $H_1$ population are later flowering and reduction of branching (Stebbins 1971). The $H_1$ *C. canescens* × *C. stricta* individuals have a branching culm as does *C. canescens* and the flowering time is intermediate between that of the two species while the $H_2$ population flowers later, only slightly earlier than *C. canescens* or at the same time (Crackles 1994). The $H_1$ population is vegetatively vigorous and this is thought to be a result of polyploidy as the $H_2$ population is not notable in this respect.

There is some overlap of *C. canescens* × *C. stricta* $H_1$ and *C. canescens* individuals on the scatter diagram (Fig. 1) and this might be taken to indicate some backcrossing to *C. canescens*, but the hybrid index scores point to the strictly intermediate nature of this taxon.

The position of most individuals of the $H_2$ population on the scatter diagram mainly away from individuals of other *Calamagrostis* taxa sampled is of considerable interest, although the significance of this is obscure. This positioning of $H_2$ individuals on the scatter diagram results partly from the fact that their bract shape resembles that of *C. stricta* while $H_1$ individuals may resemble *C. canescens* in this respect. The ratio of panicle length to length of basal branch of panicle in the $H_2$ individuals is similar to that for most *C. canescens* individuals while $H_1$ individuals tend to be intermediate in this respect. The low figure for this ratio in $H_2$ individuals is mainly due to a tendency for the basal branch of the panicle to be markedly longer than in $H_1$ individuals and a possible explanation of this is that the *C. canescens* parent had an exceptionally long lowest branch to the panicle. The scatter diagram proved to be a powerful tool in separating individuals of the two species and most of those of the two hybrids.

It is a limitation of hybrid index analysis that individuals with the same hybrid score may have different genetic constitutions and that this is true both for members of the same population and for those of different populations. However the hybrid index range in this study is important in pointing to the overall intermediacy of the two hybrids. The slightly higher hybrid score for $H_1$ compared with $H_2$ individuals is likely to be due to polyploidy.

The polygonal graph describes a population as a characteristic shape which the eye can take in at a glance. By comparing different polygonal graphs differences between taxa with regard to a fixed and limited number of characters can be seen at the same time. Polygonal graphs in this study were particularly important in demonstrating the effect of polyploidy in the $H_1$ population.

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