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Covered and naked barleys from the Himalaya

1. Evidence of multivariate differences between the two types

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Summary. Both reciprocal averaging and discriminant analysis confirm visual impressions that covered and naked barleys from several areas in the Himalaya differ significantly from each other in a multivariate way. Discriminant analysis also suggests similar differences between the 'occidental' and 'oriental' barleys of Takahashi (1955). Either distinction has implications for our understanding of barley phylogeny and evolution.

Key words: Barley – *Hordeum* – Himalaya – Evolution – Phylogeny

Introduction

Cultivated barleys can be classified into those in which the lemma and the palea (the chaff) are tightly fused to the pericarp of the caryopsis (i.e. the single-seeded fruit, commonly referred to as the grain) and those in which the chaff is easily separated from the grain. The former are known as covered, or hulled, and the latter as naked, or hull-less. Where barley forms a major part of the human diet, naked types are preferred. However, pearl barley is produced by grinding covered barley grains to remove the husk. The covered/naked caryopsis character is known to be controlled at a single locus with two common alleles (*N* and *n*), naked caryopsis being completely recessive to covered (Nilan 1964).

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Despite the apparently very simple genetics of the covered/naked caryopsis character, Rao (1974) reported that covered and naked six-rowed barleys from eastern Nepal differ extensively from one another. He grew plants representing 82 accessions in a heated greenhouse and subjected the means of the accessions for several dozen quantitative and qualitative characters to multivariate ordination. Even when the covered/naked caryopsis character itself was excluded from the analysis, the covered and naked barleys separated into two discrete populations. It was decided that the phenomenon merited further study when, in an investigation of 113 six-rowed barley accessions from northern India, the present authors found evidence that these covered and naked types differ from one another in a similar way (Murphy 1982).

In this paper we report an experiment designed to discover whether the phenomenon described above is widespread among Himalayan barleys. The experiment also allowed us to test whether the distinction observed, although apparently into covered and naked barleys, might actually be into 'occidental' and 'oriental' types with respect to the genes controlling brittleness of the rachis (Takahashi 1955).

Materials and methods

Accessions of both covered and naked six-rowed barley (*Hordeum vulgare* L. sensu lato) from Afghanistan, northern Pakistan, northern India and central and eastern Nepal (Table 1 and Fig. 1) were grown in a greenhouse (16 h day; temperature minima: 18 °C day, 15.5 °C night). Each accession was represented (at least initially) by five plants, which were transplanted into John Innes Compost No. 1 in 102 mm plastic pots after a 28 day period of vernalization at 2 °C. The pots were completely randomized on the greenhouse bench and received adequate water throughout the experiment. For each plant, 26 quantitative and 17 qualitative characters were recorded, of which a sub-set of 20 quantitative and 11 qualitative characters were analysed (Tables 2 and 3).

The accessions were randomly selected from those available, except that care was taken (a) to include, where possible,

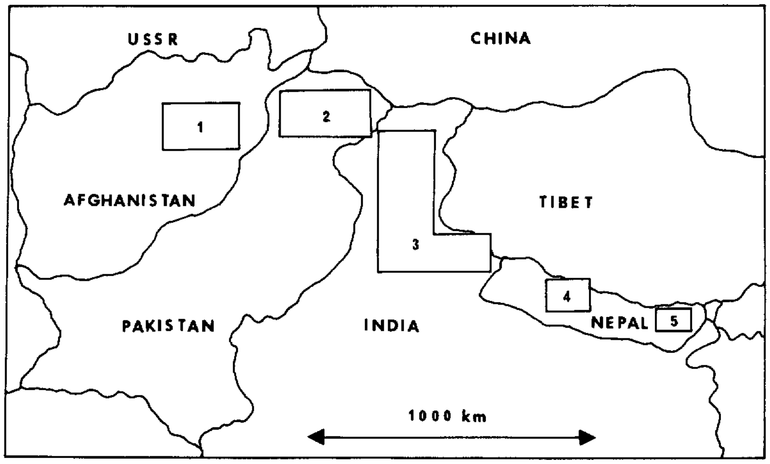

Fig. 1. Schematic diagram of the areas from which the barleys grown in this experiment were collected. *1* Afghanistan *2* Northern Pakistan *3* Northern India *4* Central Nepal *5* Eastern Nepal

Table 1. Number of accessions from each area and their mean altitudes of origin (where known)

Geographical area	Covered barley		Naked barley	
	No. of accessions	Mean altitude (m)	No. of accessions	Mean altitude (m)
Afghanistan	16	–	5	–
Northern Pakistan	21	2,150	21	2,385
Northern India	14	2,255	19	2,743
Central Nepal	11	–	9	–
Eastern Nepal	20	2,199	20	2,427

Table 2. Quantitative characters used in multivariate analyses

1. No. of days to earing
2. No. of productive tillers
3. No. of non-productive tillers
4. No. of leaves
5. Height of top node
6. Length of flag leaf sheath
7. Length of neck
8. Length of top internode
9. Length of flag leaf
10. Breadth of flag leaf
11. Length of penultimate leaf
12. Breadth of penultimate leaf
13. Dry mass of shoot minus ears
14. Length of main ear
15. Mass of main ear
16. Mass of lateral ears
17. No. of grains in main ear
18. No. of grains in lateral ears
19. Mass of grain in main ear
20. Mass of grain in lateral ears

approximately equal numbers of covered and naked types from each of the five areas, and (b) to include, where possible, pairs of naked and covered barley accessions from the same village. Although the vernalization treatment applied was sufficient to induce flowering in all cases, some of the accessions were spring-sown in the Himalaya and others winter-sown. However, both members of each pair of accessions were always either spring- or winter-sown. In areas for which we have data on the altitudes of origin of the accessions, the naked types are generally grown at higher altitudes than the covered (Table 1).

The northern Pakistan, northern India and eastern Nepal accessions had been collected by various genetic conservation expeditions from the University College of North Wales (Witcombe 1974, 1975 a, b, 1978). The Afghanistan accessions were collected by the German Hindu Kush Expedition (DHE) 1935 (Freisleben 1940 a, b). The central Nepal material had been collected at various times by Nakao, Hotta and Pershad (Takahashi et al. 1968) and much of it had been classified by Takahashi into 'occidental' (type W = $btbtBt_2Bt_2$) and 'oriental' (type E = $BtBtbt_2bt_2$) according to its genotypic constitution for non-brittle rachis (Takahashi 1955).

Methods of multivariate analysis

Two methods of multivariate analysis were employed. The first was ordination by means of a reciprocal averaging technique derived from Hill (1973), in which accessions (or individual plants) are described by their distances along a few orthogonal principal axes of ordination. Reciprocal averaging is particularly useful as an exploratory tool, for instance in looking for regional patterns among accessions (Murphy and Witcombe 1981), because the choice of successive axes is not influenced by the researchers' own prejudices. However, reciprocal averaging provides no statistical test of the validity of supposed groups. In this study, therefore, the covered and naked and the 'occidental' and 'oriental' groups were tested by means of discriminant analysis using SPSS version 9 (Nie et al. 1975; Hull and Nie 1981). After preliminary trials, a stepwise procedure of selecting characters for inclusion (or sometimes removal) in order to minimise Wilks' lambda between the groups was chosen. Those characters which failed SPSS's default entrance criteria were excluded from any particular analysis. An F ratio provided a significance test for the Mahalanobis distance between the group centroids and, as an even more rigorous test of the groups' validity, the most probable classification of each accession (or plant) according to the discriminant analysis was compared with its known classification. It should be noted that the covered/naked caryopsis character itself and the classification of a plant into 'occidental' or 'oriental' were both excluded from all analyses. Discriminant analysis was employed very effectively in a study on the morphometric relationships within the *H. vulgare* group as a whole by Baum and Bailey (1983) and Baum (1983).

Table 3. Qualitative characters used in multivariate analyses

21. Hairiness of leaf sheath:
    a. hairy b. hairless
22. Pigmentation of leaf sheath:
    a. green b. red c. purple
23. Nature of collar:
    a. closed b. open c. V-shaped
24. Nature of neck:
    a. straight/curved b. snakey/double snakey
25. Pigmentation of glume streaks:
    a. green b. red c. purple
26. Nature of middle central hood:
    a. unhooded b. hood < = 1 cm & unawned
    c. hood > 1 cm & unawned d. hood < = 1 cm & awned
    e. hood > 1 cm & awned
27. Awn length:
    a. all rows awnless or with short awns
    b. long awns on central rows only
    c. long awns on all six rows
28. Nature of awns:
    a. unawned b. smooth c. semi-smooth d. rough
29. Length of glume:
    a. <0.25 grain length b. 0.25–0.75 grain length
    c. >0.75 grain length
30. Length of glume awns:
    a. < length of glume b. approx. length of glume
    c. > length of glume
31. Colour of caryopsis:
    a. pale b. brown c. blue d. purple e. black

Results

For each of the five areas, clear multivariate differences between the covered and naked barley accessions were revealed by reciprocal averaging and there was no tendency for the covered and naked accessions from the same village to ordinate together. The result for the northern Pakistan accessions is typical (Fig. 2). Discriminant analysis confirmed the statistical validity of the covered and naked groups (Table 4). Furthermore, all of the accessions from each area were correctly classified by the discriminant analysis. The distribution of accessions from northern Indian along the discriminant function, together with the appropriate territorial

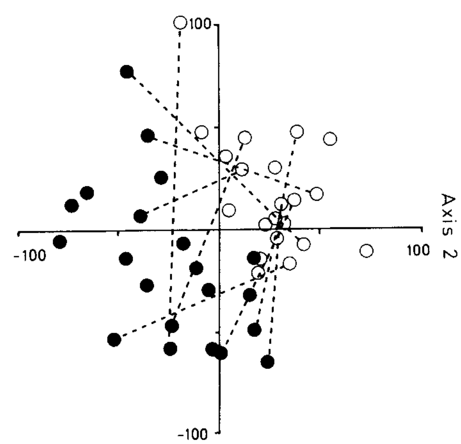

Fig. 2. Scatter diagram of naked (○) and covered (●) barley accessions from northern Pakistan along Axis 1 and Axis 2 of a typical reciprocal averaging ordination. Dashed lines join covered and naked barley accessions from the same village

Table 4. Results of F tests for the Mahalanobis distance between Himalayan covered and naked barley accessions following discriminant analysis (a) using all the characters listed in Tables 2 and 3 and (b) all the characters except 19 and 20

Geographical area	Using all characters		Excluding characters 19 and 20	
	F [df]	Significance	F [df]	Significance
Afghanistan	643.55 [14, 4]	$P<0.001$	228.48 [14, 4]	$P<0.001$
Northern Pakistan	115.50 [18,23]	$P<0.001$	(function not found)	
Northern India	44.60 [14, 6]	$P<0.001$	56.72 [6, 24]	$P<0.001$
Central Nepal	140.76 [12, 7]	$P<0.001$	78.58 [7, 12]	$P<0.001$
Eastern Nepal	68.47 [13, 25]	$P<0.001$	27.76 [12, 26]	$P<0.001$

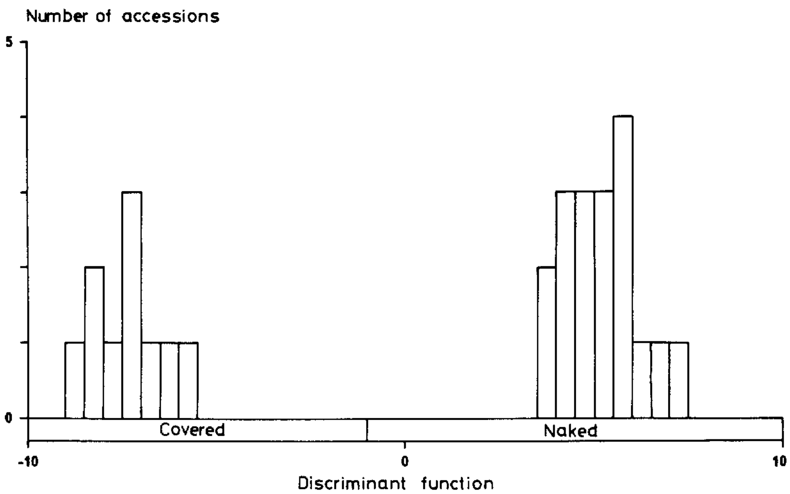

Fig. 3. Histogram showing the distribution of covered and naked barley accessions from northern India along the discriminant function, together with the territorial map of the two types

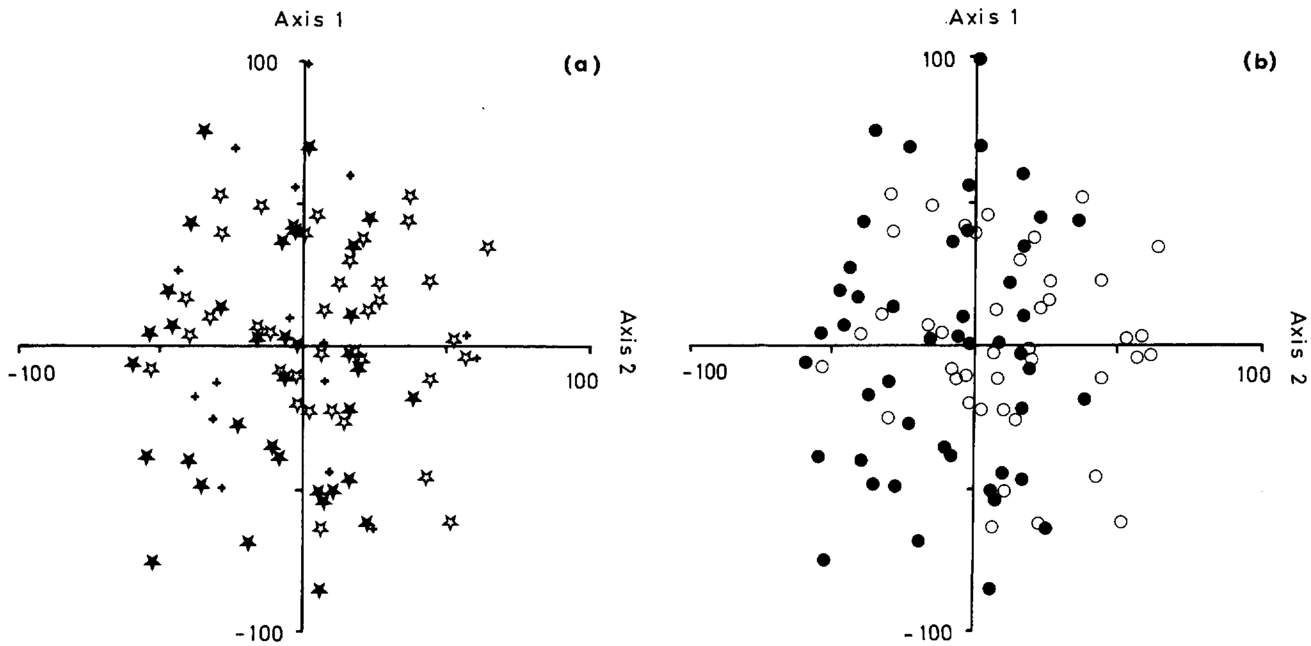

Fig. 4a, b. Scatter diagrams of individual plants from central Nepal along Axis 1 and Axis 2 of a reciprocal averaging ordination showing (**a**) 'oriental' (☆), 'occidental' (★) and unclassified (+) plants and (**b**) naked (○) and covered (●) plants. No other combination of axes resulted in clearer groupings

map, is again typical (Fig. 3). Since the chaff contributes to the mass of grain in both the main and the lateral ears (characters 19 and 20 in Table 2) in covered, but not in naked, barley accessions, this could have accounted for the observed multivariate difference between the two types. However, there were still highly significant differences between the group centroids for the barleys from Afghanistan, India and Nepal when these characters were excluded from the analyses (Table 4). The discriminant function could not be found in the case of the barleys from Pakistan and this analysis had to be abandoned, an unfortunate (but unavoidable) feature of a few discriminant analyses in this study.

Attention must be focused on individual central Nepalese plants in order to consider whether the true distinction might be between 'occidental' and 'oriental', rather than between covered and naked, barleys. Although reciprocal averaging failed to group the plants according to either criterion (Fig. 4), discriminant analysis produced highly significant groups according to both (Fig. 5). The analysis also correctly classified all of the plants into covered or naked type, and all of the plants whose genotype for non-brittle rachis was

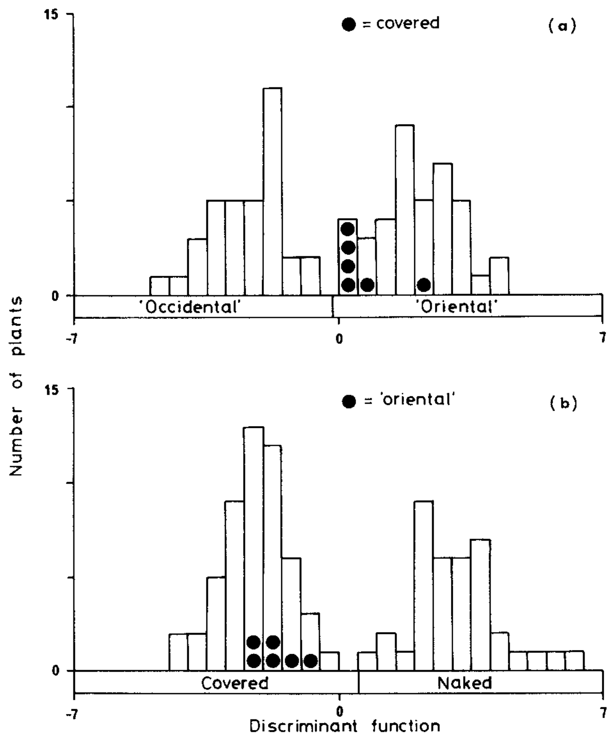

Fig. 5. Histograms showing (**a**) the distribution of barley plants from central Nepal known to be either 'occidental' or 'oriental' along the discriminant function ($F = 18.63$, df=[16, 58], $P < 0.001$) (unclassified plants not shown) and (**b**) the distribution of covered and naked barleys from central Nepal along the discriminant function ($F = 38.21$, df=[14, 76], $P < 0.001$). In each case the territorial map of the two types is also given and the positions of the covered 'oriental' plants indicated

already known into 'occidental' or 'oriental' type. Moreover, the classification of 15 of the 16 plants whose genotype was unknown was in accordance with the known genotypes of other plants from the same village; while the sixteenth plant fell near to the territorial boundary of the two types. Among the central Nepal plants available for this experiment all 'occidental' genotypes were covered and most 'oriental' genotypes were naked. There were only six covered 'oriental' types and these plants grouped with the 'oriental' types according to the 'occidental'/'oriental' function and with the covered types according to the covered/naked function (Fig. 5). Discrimination between covered 'occidental', naked 'oriental' and covered 'oriental' barleys resulted in three non-overlapping groups. It is likely, therefore, that neither classification represents a primary distinction.

From the relative contributions of the different characters to the principal axes of ordination in the case of reciprocal averaging, and to the discriminant functions in the case of discriminant analysis, it is possible to define the general characteristics of the covered and naked barley plants from the Himalaya when grown under the conditions of the experiment. Covered barleys tend to have more tillers than naked barleys. Consequently, the lateral ears of covered barleys generally have both a greater number and a greater mass of grain than those of naked barleys, and the plants also have more leaves. Naked barleys have broader penultimate and flag leaves, but the flag leaves of covered barleys tend to be longer. Covered barleys also usually have shorter glume awns, more pigmentation in the leaf sheaths (but not in the glumes) and paler grains. Naked barleys from central and eastern Nepal are much more likely to be hooded than are covered barleys, but the hooded character is very seldom found in accessions of either type from outside Nepal. However, despite these differences, for not one of the 20 quantitative and 11 qualitative characters considered were there statistically significant univariate differences between the covered and naked barleys for all five of the areas.

In growing the plants, one of the authors (PJM) found it possible to distinguish covered from naked barleys reliably by eye long before ear emergence, naked barleys being generally more robust than covered barleys and also a rather darker green. Thus the differences between the two types extend beyond those formally recorded.

Discussion

Two independent methods of multivariate analysis indicate that for barleys from Afghanistan, northern Pakistan, northern India and central and eastern Nepal there are distinct differences between the covered and naked caryopsis types. These computational methods concur with visual impressions formed while growing the plants. However, univariate analyses failed to produce a consistent picture across all five areas. There is also some evidence that, at least for barleys from central Nepal, the 'occidental' and 'oriental' types of Takahashi (1955) may also differ in a multivariate way. It is not possible to say whether covered/naked or 'occidental'/'oriental' (if either) is the primary distinction.

In our opinion the most likely explanation for covered and naked barleys being so different is that, through being grown as two distinct crops in different areas until quite recent times, and through adaptation to local conditions, the types have come to differ with respect to the alleles fixed at many loci. If this is so, interesting conclusions can be drawn concerning the evolution and phylogeny of barley in the East, and the amount of hybridization that occurs between the covered and naked forms. However, it is also possible that the differences between the covered and naked barleys could be caused by the pleiotropic effect of the covered/naked gene or even a gene very closely linked with it. To distinguish between these two hypotheses we conducted a further experiment, which also provided some more evidence regarding the possible role of the genes determining brittleness of the rachis (Witcombe and Murphy 1986).

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