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Covered and naked barleys from the Himalaya

2. Why do they differ from each other so extensively?

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Summary. Covered and naked barleys from the Himalaya differ significantly from each other in a multivariate way (Murphy and Witcombe 1986). Two hypotheses which could account for this phenomenon are (1) that the caryopsis covering gene (or a gene closely linked with it) exerts a wide-ranging pleiotropic effect, and (2) that the covered and naked types have evolved to differ with respect to the alleles fixed at many loci. Experimental evidence is presented which supports the latter, an explanation which has implications for our understanding of barley phylogeny and evolution.

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

Introduction

Despite the apparently simple Mendelian genetics of the caryopsis covering gene in barley (Nilan 1964), we have demonstrated that covered and naked six-rowed barleys from several areas in the Himalaya differ significantly from each other in a multivariate way (Murphy and Witcombe 1986). Moreover, the two methods of multivariate analysis employed served to confirm the visual impression we gained while growing the plants that the two types are really quite distinct long before ear emergence. Although this difference is suggestive of the classification of barleys into 'occidental' and 'oriental' types with respect to the genes controlling brittleness of the rachis (Takahashi 1955), discriminant analysis separated groups equally well on the basis of 'occidental' versus 'oriental' and covered versus naked, and it is not possible to say which (if either) is the primary distinction.

There appear to be two hypotheses which could reasonably account for the observed phenomenon. The first is that the caryopsis covering gene (or another gene closely linked with it) might exert a wide-ranging pleiotropic effect. The second is that the predominantly homozygous covered and naked barley 'inbred lines' might differ as groups with respect to alleles fixed at many loci.

The naked phenotype can easily be induced in covered barley strains and Scholz (1955) reported that such mutants were identical with the mother strains in all other morphological characters. This is not what would be expected if the gene was having a significant pleiotropic effect. On the other hand, Takahashi et al. (1961) reported that mutation of the covered to the naked caryopsis type was accompanied by diminution of stem length and elongation of the rachis internodes.

Although pleiotropy seems, intuitively, an unlikely explanation of the difference observed, it is easily tested. In crosses between covered and naked forms any pleiotropic effect would manifest itself in succeeding generations; for example, the F1 and the covered F2 plants should resemble their covered parent in a multivariate way and the naked F2 plants should resemble their naked parent (since covered caryopsis is the dominant character, all the F1 plants and three-quarters of the F2 plants would be expected to be covered). If, on the other hand, F1 plants are intermediate between the two parental types, or, at least, the difference between the F1 plants and the naked parental plants is less than the difference between the two parental types, then this supports the hypothesis of quantitative genetic differences.

Under this hypothesis, the F2 plants would not be expected to particularly resemble the parent of like caryopsis character. Furthermore, if the characters under study are quantitatively inherited without pleiotropy then the F2 plants would be more variable than

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* Present address: International Crops Research Institute for the Semi-Arid Tropics, Patancheru P.O., Andhra Pradesh 502 324, India

** Present address: Department of Biology, The Open University, Milton Keynes MK7 6AA, UK either the parental or the F1 plants, and heterosis would be expected in both the F1 and F2 generations. Finally, crossing covered and naked barley plants would test whether they were different with respect to the genes controlling brittleness of the rachis (see "Results"), and thus indicate whether Takahashi's classification has to be taken into account.

Materials and methods

Three accessions of covered, and three of naked, cultivated six-rowed winter barley (*Hordeum vulgare* L. sensu lato), collected in eastern Nepal by the University College Bangor Expedition 1971 (Witcombe 1974, 1975), were used in this experiment. Each accession was represented by three lines or genotypes (i.e. grain derived from three bagged plants grown from original seed in a previous experiment). For convenience, the accession/genotypes were given code letters peculiar to this experiment (i.e. covered barleys A to I, naked barleys N to V).

The experiment had three stages: (1) crossing and selfing of parental plants, (2) selfing of F1 plants and (3) comparison of parental, F2 and control plants. Although reciprocal crosses between many of the covered and naked types were attempted, sufficient grain was obtained only when covered barley was used as the ovule parent. However, there was not enough grain to include F1 plants in the third stage, which consisted of 5 plants from each of the 14 relevant parental genotypes (8 covered and 6 naked), 20 plants (at least initially) from each of the 72 F2 families and 5 plants from each of 32 eastern Nepal accessions (17 covered and 15 naked) to serve as controls. The method of cultivation and the characters recorded in the comparison stage are described in Murphy and Witcombe (1986).

Methods of multivariate analysis

Some important aspects of the two methods of multivariate analysis used in this study, namely reciprocal averaging and discriminant analysis, are described in Murphy and Witcombe (1986).

Results

As predicted by the inheritance of the covered/naked caryopsis character reported by Nilan (1964), all the F1 plants produced covered grain. In the F2 families, 68 from the 72 had at least one member which produced naked grain. The reappearance of this genetically recessive character confirmed that hybridization had been successfully achieved for most (if not all) the families. The 1,061 plants in the 72 F2 families were distributed as 786 covered and 275 naked. This is not significantly different from the 3:1 ratio predicted from the Mendelian expectation ($X^2=0.478$, $P>0.05$).

Although the rachis genotypes of the accessions crossed in this experiment were unknown, most covered barleys would be expected to be 'occidental' (type $W = btbtBt_2Bt_2$) and all naked barleys 'oriental' (type $E = BtBtbt_2bt_2$) if the relative frequencies of the rachis genes in covered and naked barleys from eastern Nepal are similar to those reported for barleys from central Nepal (Takahashi 1955; Takahashi et al. 1968). The F1 plants ($BtbtBt_2bt_2$) would therefore necessarily have brittle, spontaneously shattering rachides (Nilan 1964; Harlan 1968). In fact, none of the F1 or F2 plants had rachides which could possibly be described as brittle or spontaneously shattering. Either the gene frequencies for brittleness in eastern Nepal are quite dissimilar to those in central Nepal, or the resulting brittleness is very different in degree to that found in wild *H. spontaneum* (which was grown at the same time as part of another investigation; Murphy et al. 1982). Since this result was unexpected, no control crosses had been made between $btbtBt_2Bt_2$ and $BtBtbt_2bt_2$ genotypes.

The multivariate difference between covered and naked barley types reported by Murphy and Witcombe (1986) could be clearly detected in the eastern Nepal accessions grown as controls. The two types were emphatically separated by both reciprocal averaging and discriminant analysis (Fig. 1). Furthermore, when the data for even individual parental plants were analysed, both reciprocal averaging (Fig. 2) and discriminant analysis separated the covered and naked genotypes, with no overlap between the two groups in the latter analysis.

When the data for 5 plants of each parent and up to 20 F2 plants from that cross were subjected to reciprocal averaging it was generally found that the parental types formed two distinct clusters according to their caryopsis covering, while the F2 generation displayed considerably greater variability. 17 families (chosen as having relatively high proportions of naked plants) were examined in this way. Sixteen were similar to C×O (1) (Fig. 3), i.e. there was no tendency for the covered and naked F2 plants to form separate clusters, nor for either type to ordinate with the parent of like caryopsis character. It is therefore unlikely that pleiotropy is the explanation of the observed phenomenon. Furthermore, support for the alternative explanation involving multifactorial differences between covered and naked barleys is provided by the fact that parental and F2 plants were generally separated on an axis largely reflecting yield and robustness. In other words, the F2 generation displayed heterosis and this is usually regarded as being due to gene dispersion between the parental lines (Falconer 1981; Mather and Jinks 1971). However, there was one family (I×V (3)) in which the covered parental and covered F2 plants, and the naked parental and naked F2 plants, appeared to form two separate clusters. Since it applied only when Axis 2 was plotted against Axis 4 (Fig. 4), this could well have been due to chance.

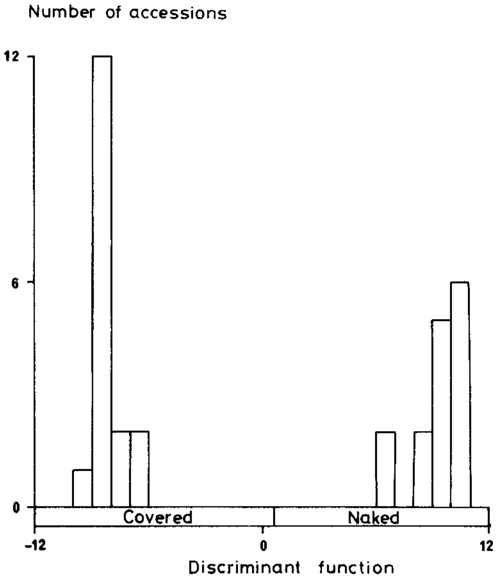

Fig. 1. Histogram showing the distribution of covered and naked barley accessions from eastern Nepal along the discriminant function ($F = 111.07$, df = [13, 18], $P < 0.001$), together with the territorial map of the two types

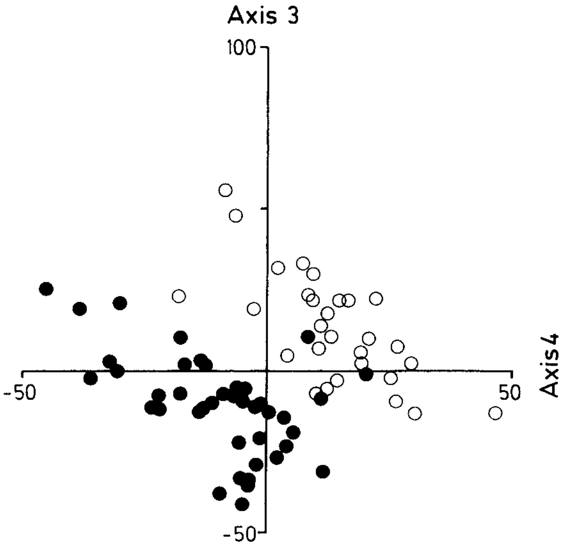

Fig. 2. Scatter diagram of naked (○) and covered (●) parental barley plants from eastern Nepal along Axis 3 and Axis 4 of a reciprocal averaging ordination

Discriminant analysis was applied to the data for the 17 F2 families referred to above and, separately, to that for the relevant parental pairs. For three of the F2 families the discriminant function could not be found, and these analyses had to be abandoned. But for all 8 relevant parental pairs and for all but one of the remaining 14 F2 families the distances between the

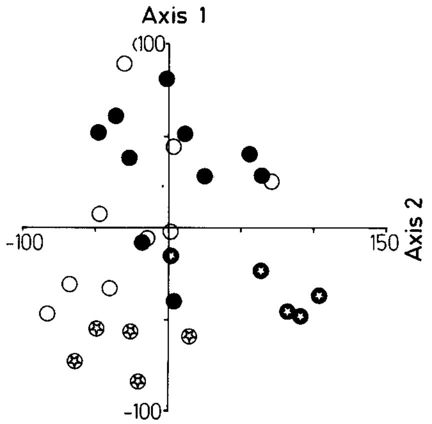

Fig. 3. Scatter diagram of naked (⊛) and covered (✪) parental barley plants and naked (○) and covered (●) F2 barley plants of family C×O (1) along Axis 1 and Axis 2 of a reciprocal averaging ordination

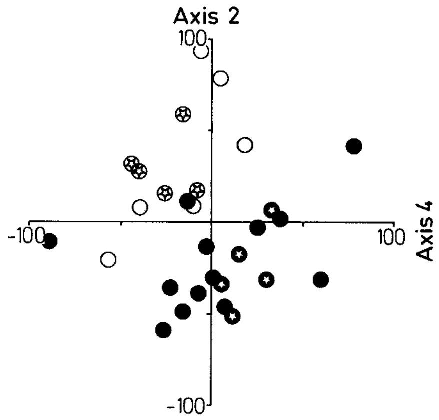

Fig. 4. Scatter diagram of naked (⊛) and covered (✪) parental barley plants and naked (○) and covered (●) F2 barley plants of family I×V (3) along Axis 2 and Axis 4 of a reciprocal averaging ordination

group centroids for the covered and naked barley plants were statistically significant (Table 1). However, in all but two instances Wilks' lambda for the distance between the covered and naked barley plants was greater in the F2 than in the parental generation (Table 2), and generally much greater. The larger lambda is, the lower is the discriminating power between the covered and the naked types (Nie et al. 1975). This again is entirely consistent with there being a multifactorial difference between the two types, which had declined considerably by the F2 generation. It is certainly not what one would expect if the multivariate difference was a result of pleiotropy. The significance of the differences between the group centroids was greater in the F2 than in the parental generation in the majority of families (i.e. the probability levels were

Table 1. Results of *F* tests for the Mahalanobis distance between covered and naked barley plants following separate discriminant analyses on data for (a) 5 parental plants of each type and (b) up to 20 F2 plants derived from 14 covered × naked barley crosses

Cross	Parents		F2	
	F [df]	Significance	*F* [df]	Significance
C × O (1)	694.23 [7, 2]	$P<0.01$	41.60 [5, 14]	$P<0.001$
C × T (1)	447.41 [7, 2]	$P<0.01$	166.58 [3, 5]	$P<0.001$
F × N (2)	733.50 [7, 2]	$P<0.01$	73.81 [13, 6]	$P<0.001$
G × T (2)	2,717.80 [7, 2]	$P<0.001$	67.89 [13, 6]	$P<0.001$
H × Q (1)	1,250.50 [6, 3]	$P<0.001$	1,470.20 [9, 5]	$P<0.001$
H × Q (6)	1,250.50 [6, 3]	$P<0.001$	422.74 [9, 3]	$P<0.001$
H × U (1)	1,030.20 [7, 2]	$P<0.001$	777.58 [10, 2]	$P<0.01$
H × U (3)	1,030.20 [7, 2]	$P<0.001$	153.51 [11,7]	$P<0.001$
H × U (8)	1,030.20 [7, 2]	$P<0.001$	194.04 [5, 1]	ns
I × U (3)	805.55 [8, 1]	$P<0.05$	305.68 [15, 3]	$P<0.001$
I × U (4)	805.55 [8, 1]	$P<0.05$	118.64 [8, 9]	$P<0.001$
I × U (6)	805.55 [8, 1]	$P<0.05$	34.85 [10, 8]	$P<0.001$
I × U (12)	805.55 [8, 1]	$P<0.05$	37.66 [11, 8]	$P<0.001$
I × V (3)	4,009.60 [7, 2]	$P<0.001$	256.92 [13, 4]	$P<0.001$

Table 2. Wilks' lambda for the distance between covered and naked barley plants following separate discriminant analyses on data for (a) 5 parental plants of each type and (b) up to 20 F2 plants derived from 14 covered × naked barley crosses

Cross	Wilks' lambda ($\times 10^7$)	
	Parents	F2
C × O (1)	4,114	630,660
C × T (1)	6,382	99,061
F × N (2)	3,894	62,146
G × T (2)	1,051	67,521
H × Q (1)	3,997	3,777
H × Q (6)	3,997	7,879
H × U (1)	2,773	2,571
H × U (3)	2,773	41,282
H × U (8)	2,773	10,297
I × U (3)	1,551	6,539
I × U (4)	1,551	93,931
I × U (6)	1,551	224,403
I × U (12)	1,551	189,441
I × V (3)	713	11,962

lower) because, although the *F* values were lower in most instances, the numbers of degrees of freedom were generally higher.

Discussion

Several authors (e.g. Freisleben 1940; Takahashi 1955) have argued that barley has a diphyletic origin, with covered barley having originated in the west and naked barley in the east. The distinct differences which appear to exist between western and eastern forms are cited in support of this. For example, Takahashi (1955) demonstrated a marked difference in the genotypes of 'occidental' and 'oriental' barleys with respect to the genes controlling brittleness of the rachis. 'Occidental' and 'oriental' barleys also differ in their resistance to certain races of mildew, in their degree of winter habit (although most are autumn sown), in their degree of post-harvest dormancy and in the relative frequencies of the blue aleurone and purple/black caryopsis characters (Takahashi et al. 1968). In examining barleys from central Nepal and from India, Takahashi et al. (1968) reported that naked barleys are wholly 'oriental' and that 80 per cent of the covered forms are of the 'occidental' type.

Our investigation of covered and naked barleys from central Nepal, whose genotypes with respect to the genes controlling brittleness of the rachis were known, failed to establish whether the covered/naked or the 'occidental'/'oriental' distinction (if either) takes precedence (Murphy and Witcombe 1986). However, since covered and naked barleys from eastern Nepal are certainly distinct, yet artificial hybrids between them do not have spontaneously shattering rachides, we must conclude that the two systems are not completely coincident. Perhaps both the covered and the naked barleys from eastern Nepal are of the 'oriental' type, since this area is east of the line apparently dividing the types (Takahashi 1955; Takahashi et al. 1968). This possibility can be tested only by formal genetic analysis.

The difference between the naked and covered barleys demonstrated by multivariate analysis could be used in support of a diphyletic origin of barley, leading to covered 'occidental' and to naked 'oriental' types. But a convincing diphyletic origin does require evidence of an ancestral form in the east. In the past, much discussion has centered around the existence of a six-rowed wild barley, *Hordeum agriocrithon* Åberg. However, Murphy et al. (1982), using both traditional genetic and electrophoretic techniques, demonstrated that six-rowed 'wild' barley from northern India was derived from natural hybridization between two-rowed wild and six-rowed cultivated barleys. It is clear from Witcombe (1978) and Shao (1981) that wild two-rowed barley occurs as a weed far to the east of its endemic area, the fertile crescent. It is, therefore, probable that all six-rowed brittle rachis forms are of hybrid origin. Although Shao (1981) argues that *H. agriocrithon* is a true species, he has found it only as a weed in cultivated barley. Hammer (1984), on the basis of pollination ecology, found that some six-rowed brittle rachis forms (such as the so-called *H. lagunculiforme*) are the result of introgression of cultivated into wild barleys. Nevertheless, he does maintain that some races of *H. agriocrithon* may not be the result of such introgression and may be implicated in the phylogeny of cultivated barley. Although it would be attractive to explain the difference between covered and naked barley as being the result of them having a separate origin, on balance the evidence is against this. A monophyletic origin for all cultivated barleys somewhere in southwestern Asia is now generally accepted (Harlan 1976). We would also follow the proposal of Harlan and de Wet (1971) and place both the covered and the naked forms of barley, as a single species, in the primary gene pool of the crop. To explain the differences between covered and naked barleys we clearly have to look for some reason other than barley having a diphyletic origin.

Since two methods of multivariate analysis produce essentially the same results, and these objectively confirm our subjective observations of the plants, the phenomenon reported cannot be dismissed as an artifact of the methods of analysis. The (intuitively rather unlikely) possibility that the caryopsis covering gene might be exerting a wide-ranging pleiotropic influence is not supported by the data presented here. We must conclude that the covered and naked barley accessions differ from each other with respect to the alleles fixed at many different loci. Evidence for this hypothesis is provided by the reduced discriminating power between the covered and naked types in the F2 compared to the parental generation, by the increased variability in the F2 and by the heterosis manifest in that generation.

Since even 'sympatric' populations of covered and naked barley, collected in the same village, are clearly separated, there must be a very low rate of natural crossing between the two forms. The absence of natural hybridization between covered and naked barleys is also the only explanation of the hooded and purple/black caryopsis characters being restricted to naked barleys (Takahashi et al. 1968). However, Murphy et al. (1982) have shown that hybridization does take place between weedy, wild two-rowed barley and cultivated six-rowed barley in Ladakh, in the western Himalaya. Shao's *H. agriocrithon* is also almost certainly of hybrid origin. Wild barley must invariably be the ovule parent in these cases or else we would expect to find hybrids between the cultivated forms. Thus, the evidence is that the cultivated forms are overwhelmingly inbred.

The only reasonable explanation of the differences between the covered and naked forms is that naked barley arose by a single mutation from covered barley a long time ago in the crop's history and that, thereafter, cultural and adaptive selection pressures have caused the forms to diverge. Since naked barley is far more variable for qualitative characters in Nepal than elsewhere in the Himalaya (Murphy and Witcombe 1981), Nepal is the most likely place for the origin of the naked form. The other genetic differences could then have arisen as the two types adapted to somewhat different conditions and uses under the influence of natural and conscious selection pressures. South and west of the Himalaya, wheat might long ago have replaced naked barley as a cereal for direct human consumption. In such areas, the (predominantly covered) barleys would be used mainly for animal feed and for brewing. However, little wheat would be grown on the high plateaux of China and Tibet, and much of the (predominantly naked) barley grown there would be eaten as tsampa. Even now, when both types are often grown in the same village in the same season, they are treated as separate crops.

Nevertheless, variation within cultivated barley has been shown to be regionally distributed in the Himalaya (Murphy and Witcombe 1981). Such regional variability could be due to geographic isolation, founder effects and environmental differences between the regions. The possibility that naked barley has arisen independently in several regions has to be rejected; if that were the case, it is likely that the two forms would be much more similar in some regions than in others.

While accepting that barley was almost certainly first domesticated solely in the Middle East and that it does not have a diphyletic origin, we do suggest that there is a great deal more to be learnt about its evolutionary history than is generally supposed. Furthermore, the genetic differences between covered and naked barleys are potentially relevant to breeding programmes in that the variability created through hybridization of the contrasting forms could be exploited.

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