# UNDERSTANDING EPISTASIS IN LINKAGE ANALYSIS: THE *Kap* AND *lks2* LOCI IN THE OREGON WOLFE BARLEY POPULATION

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**Abstract:** An organisms' genotype may not be reflected in its phenotype. For example, epistasis - the interaction of non-allelic genes can cause deviations from expected phenotypic ratios. The epistatic interaction between the hooded (*Kap*) and short-awned (*lks2*) loci in barley (*Hordeum vulgare*) precludes the localization of the *Kap* locus, based on phenotypic data, when alleles at both loci are segregating. In individuals that are homozygous for the recessive allele of *lks2*, the expression of the hooded phenotype is masked, resulting in the expression of the short-awned, rather than the hooded, phenotype. Two strategies were employed to determine the location of the *Kap* locus in an experimental population of barley, the Oregon Wolfe Barleys, and to determine the *Kap* locus genotype of a doubled haploid line in the population. The first strategy capitalized on the availability of molecular marker data in the population and involved inferring the *Kap* locus genotypes of the short-awned individuals based on the genotypes from flanking DNA markers. The second strategy confirmed the predicted genotypes by mapping a barley homeobox gene (*hvknox3*) believed to represent the hooded (*Kap*) locus. The *hvknox3* locus mapped to BIN 4 of barley chromosome 4 (4H), which is the reported map position of the *Kap* locus. The *Kap*-predict and *hvknox3* genotypes were compared and found to be the same, except for OWB line 52. To test the alternative hypotheses regarding the *Kap* genotype of OWB 52, this line was crossed with a known dominant hooded line, OWB 62. Putative F1's were checked with *hvknox3* primers and true F1 heterozygotes were allowed to self-pollinate and to produce seed. F2 seed were planted and the phenotypic assessment of the F2 population will be completed by August 2000.

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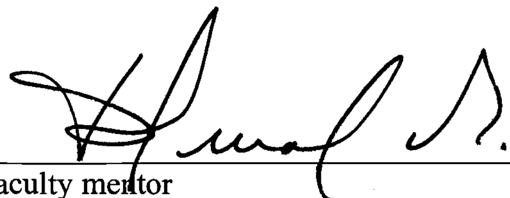

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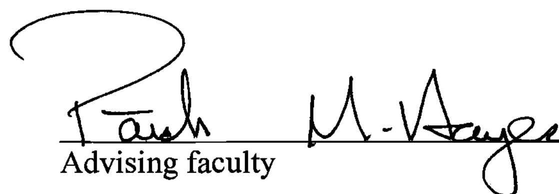

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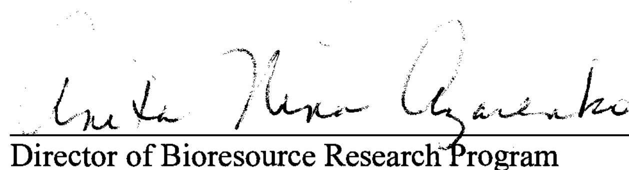

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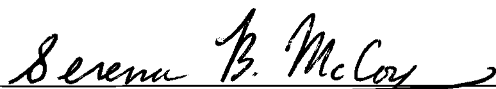

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## Chapter 1

## Introduction

Genetics is the study of the inheritance of traits and the genes that determine these traits. A phenotype is the observable characteristics of an organism and is determined by the organism's genetic constitution (genotype) and its interactions with the environment. A gene is a heritable sequence of DNA bases and can have alternative forms (alleles). An organism with two identical alleles of a gene is said to be homozygous. An organism with different alleles is said to be heterozygous. In the case of complete dominance, the phenotype of a homozygote reflects the genotype of the organism, but the phenotype of a heterozygote may or may not indicate the organisms' genotype. Therefore, genetic analyses can be conducted at complimentary levels. At the DNA (genotype) level, variation can conceivably lead to phenotypic differences, but at the morphological (phenotype) level, differences can be due to changes in the DNA sequence, gene interactions, variation in gene expression and/or variation in structure and function.

Mutations are heritable changes in the DNA sequence of a gene and an organism carrying an altered gene is said to be a mutant. An organism with the unaltered gene is the wild type. Mutants and mutations are very useful in barley genetics research. Spontaneous and induced mutants of barley (*Hordeum vulgare* L.) have been used for the development of cultivars, the study of the inheritance and genetic control of traits, genetic

fine-structure analysis, and the study of the nature of mutations in terms of biochemical, physiological and anatomical features (Hockett and Nilan, 1985). Spontaneous and induced mutations that include morphological variants have been genetically and cytologically mapped on the seven chromosomes of barley (Franckowiak, 1997). However, morphological variants may not be phenotypically neutral. Therefore, there are relatively few morphological trait loci segregating in most mapping populations. Instead, phenotypically neutral DNA markers have been used extensively for linkage mapping in barley and other crops (Kleinhofs et al., 1993; A. Kleinhofs; http://barleygenomics.wsu.edu/).

Efforts are underway to integrate DNA-based marker linkage maps with genetic maps based on mutations and morphological traits. The Oregon Wolfe Barleys are a doubled haploid mapping population that has been used to genetically map 15 morphological traits and about 150 DNA-based markers (Hayes et al., 1998; McCoy et al., 2000; Vales et al., 2000) and could serve as a starting point for map-based cloning of genes determining morphological traits. The Oregon Wolfe Barleys are also a tool for the integration of genetics research and instruction using barley as a model (P.M. Hayes, http://www.css.orst.edu/barley/wolfebar/wolfenew.htm).

The localization of a morphological trait in a mapping population based on phenotype data alone may be difficult due to epistatic interactions between loci. Epistasis is the masking of a gene's phenotypic expression by another gene. The interaction between the hooded (*Kap*) and short-awned (*lks2*) loci is an example of epistasis (Figure 1). Both loci affect the development of the awn, an extension of the lemma believed to be a modified leaf blade (Myler, 1942; Dahlgren et al., 1985) (Figure 2). The hooded

phenotype (*Kap Kap*) is masked when the recessive allele at the *lks2* locus is homozygous (*lks2 lks2*) resulting in an inflorescence with short-awns and without a hood (Figure 1D). The morphological characteristic of individuals with dominant *Kap* and *Lks2* alleles is the formation of a hood structure instead of a normal awn (Figure 1A and 1C).

Morphologically, the hood, which is created by the action of a single *Kap* (from "kapuze" meaning hood in German) gene, contains an extra palea on the distal end of the lemma followed by rudimentary florets with inverse polarities (Figure 3). The change from lemmas ending in awns in wild type barley to lemmas with hoods in hooded barley is an example of a homeotic mutation, where cells have switched from one developmental path (formation of an awn) to another (formation of florets). Linkage relationships indicate that the *Kap* locus is located on chromosome 4 (4H) of barley (Stebbins and Yagil, 1966 and A. Kleinhofs; http://barleygenomics.wsu.edu/). The *lks2* locus affects the length of awns. The long-awned character is dominant (*Lks2*) to the short-awned phenotype (*lks2*) (Figure 1E and 1F, respectively). Linkage relationships indicate that the *lks2* locus is located on chromosome 1(7H) of barley (Takahashi, R. and J. Hayashi, 1959). The epistatic interaction between *Kap* and *lks2* had precluded the localization of the *Kap* locus in the Oregon Wolfe Barleys.

The *Kap* locus was mapped in the Oregon Wolfe Barleys using two strategies. The first strategy was to infer the *Kap* locus genotype using flanking DNA-based markers. The second strategy was to map a barley homeobox gene (*hvknox3*) hypothesized to be the *Kap* locus (Mueller et al., 1995). Both approaches produced the same results, with the exception of the genotype for OWB line 52. The first strategy

indicated that OWB 52 had the *KapKap* genotype while the second strategy indicated that OWB 52 has the *kapkap* genotype. Thus, the objectives of this project were to verify the location of the *Kap* locus in the OWB mapping population and to determine why the predicted genotype of OWB 52 is not the same as its *hvknox3* genotype.

# Chapter 2

# Materials and Methods

## Germplasm

Dr. Bob Wolfe, a Canadian barley geneticist, created the parents of the Oregon Wolfe Barleys (OWB). He developed a set of dominant and recessive marker stocks with easily scored phenotypes over a period of 27 years (Wolfe and Frankowiak, 1990). The dominant parent (Figure 1A) has a two-row, hooded inflorescence and the recessive parent (Figure 1B) has a six-row, short-awned inflorescence. The two parent plants were crossed and the F1 generation was used to develop a population of doubled haploids at Oregon State University (P.M. Hayes; http://www.css.orst.edu/barley/wolfebar/wolfnew.htm). Each doubled haploid (DH) is a completely homozygous genotype that can be repeatedly phenotyped and genotyped. This set of genetic marker stocks contains a minimum of 15 scorable morphological markers. The entire OWB population consists of 94 plants as well as the dominant and recessive parents. A linkage map that integrates 142 morphological and DNA-based markers has been constructed using this population. The map currently includes eleven morphological markers, 80 RFLPs, one IFLP (*hvknox3*), and 50 SSRs (Hayes et al., 1998; McCoy et al., 2000 and Vales et al., 2000).

## Experimental Procedures for the First Strategy

The entire Oregon Wolfe Barley (OWB) population plus the dominant and recessive parents were planted in plastic pots with a commercial potting mix

supplemented with lime and Osmocote slow release fertilizer (The Scotts Company, Marysville, OH). The population was grown in the West Greenhouses on the Oregon State University campus. Plants were provided supplemental light (16 hours light/ 8 hours dark per 24 hour period) from high-pressure sodium lights suspended approximately 2.5 meters above the bench surface. The plants were watered twice daily as needed. After four weeks of growth, the plants were scored for juvenile morphological characteristics. This data were compared with data on the same characteristics that had been previously scored (K. Sato, personal communication). This was done to confirm the identity of each line. Plants were grown to maturity and were scored for the hooded, short-awned, and long-awned phenotypes. The population was then harvested with one inflorescence from each plant kept intact as a reference.

In the OWB population, the phenotypes of individuals with hooded spikes (*KapKapLks2Lks2*) and those without hoods and with long awns (*kapkapLks2Lks2*) should reflect their *Kap* genotypes. On the other hand, non-hooded and short-awned individuals can have two possible genotypes with respect to the *Kap* locus – *KapKaplks2lks2* or *kapkaplks2lks2*. Thus, to determine the approximate linkage map location of the *Kap* locus using phenotypic data, the phenotypes of non-hooded and short-awned individuals were treated as missing (Figure 4). This modified data set was used for genetic linkage analysis using GMendel 3.0 (Holloway and Knapp 1994). Linkage groups were calculated using a LOD score of 2.9 and maximum recombination (rmax) of 0.35. Once a genetic location for *Kap* was found, the genotypes for the missing values were inferred from the genotypes of flanking markers (Figure 5), assuming no double crossovers. When flanking DNA markers (CDO122 and ABG003A) were both “A”, then

*Kap* was predicted to be "A" and when CDO122 and ABG003A were both "B", then *Kap* was predicted to be "B". In cases where there were contrasting alleles at the flanking DNA markers, the *Kap* genotype was not predicted and is indicated as "—".

**Experimental Procedures for the Second Strategy**

The second strategy was to map a barley homeobox gene (*hvknox3*) hypothesized to be the *Kap* locus. The dominance of the *Kap* gene and its close association with the alcohol dehydrogenase gene (*Adh*) locus provided clues to a possible orthology with the Knotted-1 (*kn-1*) gene of maize (Muller et al, 1995). Dominant mutant alleles at the *kn-1* locus cause the formation of ectopic malformations (knots) on the maize leaf blade. The *kn-1* gene also creates dominant mutant alleles and it is believed to produce ectopic meristems on the lemma and awn of the barley floret (Williams-Carrier et al, 1997). The *kn-1* gene is important in meristem maintenance and belongs to the *Knox* gene family. When the maize *kn-1*gene was cloned using cDNA, it was found to encode a homeodomain protein. This information was used to locate a homologous cDNA clone in barley called cHvKnox3. The homeodomain proteins from both *hvknox3* and *kn-1* were compared and found to have an amino-acid sequence homology of 90% (Muller et al, 1995).

The *hvknox3* alleles in barley can be differentiated by an intron fragment length polymorphism (IFLP). The dominant allele at the *hvknox3* locus contains a 305-base pair (bp) tandem duplication in intron 4 (Muller et al., 1995). The primers, HvKnox3B and HvKnox3C (Williams-Carrier et al., 1997) designed to amplify the 5' region of intron 4, were used to genotype the OWB population.

The DNA isolation technique used for the OWB population and the two parents required a leaf sample of 4 cm (40 mg) from two-week old plants. Leaf samples were cut into smaller pieces, and placed in 2-mL collection microtubes in a 96-well plate format. A tungsten carbide bead (Qiagen Co., Valencia, CA 91355) was placed in each tube. About 500 µL of ice-cold isolation buffer [10 mM Tris-HCl (Tris [hydroxymethyl] aminomethane hydrochloride) pH 9.5, 10 mM EDTA (disodium ethylenediamine tetra acetate), 100 mM KCl (potassium chloride), 0.5 M sucrose, 4 mM spermidine, 1.0 mM spermine, 0.1 % (v/v) mercaptoethanol, 2% (w/v) sarkosyl] (modified from Liu and Whittier, 1994) were added to each tube. Leaf samples were disrupted using a Retsch MM 300 mixer mill (F. Kurt Retsch GmbH & Co. KG, Haan, Germany 42781). The samples were ground twice for 1.5 min each time at 30 l/s. The tungsten beads were removed from the tubes using a magnet. About 250 µL of phenol:chlorophorm (1:1) were added and the contents were mixed. The collection microtubes were centrifuged at 12,000 g for 4 minutes in bench-top centrifuges (Eppendorf 5417C) using 2 mL tubes as adaptors. The aqueous layer was transferred to 1.5-mL microcentrifuge tubes. DNA in this aqueous solution was precipitated by adding 50 µL of 3 M sodium acetate (pH 5.0) and 1.25 mL of absolute ethanol. The contents of the tubes were mixed well and centrifuged at 12,000 g for 4 minutes. The liquid solution was discarded. The DNA pellet that remained was rinsed with 70 % (v/v) ethanol following centrifugation at 12,000 g for 2 minutes. After drying, the DNA pellet was dissolved in 400 µL of TE buffer pH 8.0 plus 10 µg $mL^{-1}$ RNase. The average DNA concentration of the samples was 200 ng $\mu L^{-1}$.

The concentration of the DNA was checked in a 1% agarose gel. The gel was made using Gibco/BRL Ultrapure agarose, 1xTBE (0.0045 M Tris-borate and 0.001 M

EDTA pH 8.0) and ethidium bromide. A molecular weight DNA ladder (1Kb ladder-Promega, Madison, WI 53707) was used for comparison. The gel was run for 45 minutes at 59V. The final gel product was observed using ultraviolet light (UV) and photographed with a Polaroid camera. Diluted DNA stocks containing approximately 40 ng $\mu L^{-1}$ DNA were created. The diluted DNA product was used for the Polymerase Chain Reaction (PCR) amplification of intron 4 of the *hvknox3* gene.

The PCR mixture consisted of 0.2 mM each of primers HvKnox3B and HvKnox3C (Williams-Carrier et al., 1997), 0.03 U μL *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1x *Taq* buffer, 40 ng template DNA and nanopure $ddH_2O$. Aliquots of 9.5μL PCR mixture were placed in 96 (200 μL) ultrathin walled PCR tubes to which 0.5 μL of template DNA (40ng $\mu L^{-1}$) were added. The tubes were carefully capped and placed in the Programmable Thermal Controller-100 (MJ Research, Inc., Watertown, MA 02172). PCR amplification occurred using the following program:

- 94°C – 3 minutes
- 58°C – 1 minute
- 72°C – 1 minute

} 1 cycle

- 94°C – 30 seconds
- 58°C – 30 seconds
- 72°C – 30 seconds

} 29 cycles

- Hold 4°C

About 1.5 μL of 6x loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol and 70% $ddH_20$) was added to each tube. The PCR product plus the loading dye was run in a 2% agarose gel (using previously listed ingredients) for 1 hour at 59V. The final gel product was observed under UV light and photographed (Figure 6).

The hooded parent of the OWB population produced a 1,100 bp fragment, while the recessive non-hooded and short-awned parent produced an 800 bp fragment. The data from the gels were coded with either an A for OWB lines showing the dominant *hvknox3* allele (1,100 bp) or B for the OWB's displaying the recessive *hvknox3* allele (800 bp). This data set was then used for linkage analysis and compared with the *Kap*-predict data from the first strategy.

## Experimental Procedure for Testing Hypotheses Regarding the *Kap* Genotype of OWB 52

Three alternative hypotheses were formulated to explain the differences between the *Kap*-predict and *hvknox3* genotypes at the *Kap* locus in OWB line 52:

1) *hvknox3* was misscored.

2) *hvknox3* is the *Kap* gene or is tightly linked to the *Kap* locus and the *kap* allele in OWB 52 resulted from a double crossover between CDO122 and ABG003A.

3) *hvknox3* is linked to *Kap* and the OWB 52 *kap* genotype is the result of a double crossover event involving only the *hvknox3* locus.

The *hvknox3* genotype was scored six times. The full OWB population was scored for *hvknox3* twice (I. Vales-personal communication) and the OWB 52 *hvknox3* genotype was determined four times during the course of this research.

The second hypothesis is based on a double crossover involving the *Kap* locus. The probability of a double crossover occurring between CDO122 and ABG003A and involving the *Kap* locus is low. The recombination distance from CDO122 to *hvknox3* is 0.064 and from *hvknox3* to ABG003A is 0.074 (Figure 11). Multiplying these numbers

together equals 0.0047 or 0.47%, which is the probability of a double crossover occurring between these two loci, assuming no interference.

Double crossovers are rare events, but they do occur. The chances of a double crossover occurring increase with linkage distance between loci. At approximately 10% recombination and below, double crossovers do not occur as often as would be expected (the multiple of two single crossover events) and this is called interference. The distance between the DNA markers flanking the *Kap* locus is 13.8 cM. Accordingly, one would expect nearly complete interference.

The third hypothesis that was formulated to explain the difference between the *Kap*-predict and *hvknox3* genotypes at the OWB 52 *Kap* locus was that the *hvknox3* locus is linked to the *Kap* locus and a double crossover event occurred that only involved the *hvknox3* locus. This possibility seems remote because the probability of a double crossover involving the *hvknox3* locus, but not the *Kap* locus, would have to occur in the short distances between CDO122 and *Kap* or from between *Kap* and ABG003A. In the current map (Figure 11), in which we assume that *hvknox3* = *Kap*, the distance between CDO122 and *hvknox3* is 0.064 and the distance between *hvknox3* and ABG003Ais 0.074. If *Kap* and *hvknox3* are linked loci, the probabilities of double crossovers occurring that would involve only one of the two loci are exceedingly low.

In order to test hypothesis two and three, the *Kap* locus genotype in OWB 52 was further investigated by developing an F2 population from a cross between OWB 52 and a hooded OWB line. Hooded OWB line 62 (*KapKapLks2Lks2*) was chosen for the cross with OWB line 52, which has two possible genotypes, *KapKaplks2lks2* or *kapkaplks2lks2* because the *Kap*-predict genotype is *KapKap* and the *hvknox3* genotype is *kapkap* (Figure

12). OWB 52 is known to be homozygous recessive at the *lks2* locus because it has the short-awned phenotype as opposed to the long-awned phenotype. OWB 62 is known to be homozygous dominant at the *lks2* locus because it is hooded. The F1's from the cross self-pollinated to produce F2 seed and the resulting F2 phenotypic ratios can be used to test alternative hypotheses two and three.

If OWB 52 is *kapkaplks2lks2* (hypothesis 2), then the predicted phenotypic ratio will be 9 hooded: 3 long-awned: 4 short-awned (Table 1).

**Table 1.**—The expected genotypes and phenotypes from a cross between OWB 52 (if OWB 52 = *kapkaplks2lks2* genotype) and OWB 62(*KapKapLks2Lks2*)

| **Genotype** [a] | **Phenotype** | **Genotype** [a] | **Phenotype** |
|---|---|---|---|
| (1) *KapKapLks2Lks2* | Hooded | (2) *Kapkaplks2lks2* | Short-awned |
| (2) *KapKapLks2lks2* | Hooded | (1) *kapkapLks2Lks2* | Long-awned |
| (1) *KapKaplks2lks2* | Short-awned | (2) *kapkapLks2lks2* | Long-awned |
| (2) *KapkapLks2Lks2* | Hooded | (1) *kapkaplks2lks2* | Short-awned |
| (4) *KapkapLks2Lks2* | Hooded | | |

[a] Phenotypic ratios are in parenthesis.

If OWB 52 is *KapKaplks2lks2* (hypothesis 3), then the phenotypic ratio in the F2 population is predicted to be 3 hooded: 1 short-awned (Table 2).

**Table 2.**—The expected genotypes and phenotypes from a cross between OWB 52 (if OWB 52 = *KapKaplks2lks2* genotype) and OWB 62 (*KapKapLks2Lks2*)

| **Genotype** [a] | **Phenotype** |
|---|---|
| (1) *KapKapLks2Lks2* | Hooded |
| (2) *KapKapLks2lks2* | Hooded |
| (1) *KapKaplks2lks2* | Short-awned |

[a] Phenotypic ratios are in parenthesis.

The observed F2 phenotypic ratio, therefore, will distinguish between hypotheses two and three.

To produce the F1 seeds, OWB 52 and OWB 62 were planted into peat pots filled with vermiculite. The pots were placed in a growth chamber for two weeks vernalization of 8 hour days (9° C) and 16 hour nights (6° C). The OWB parents and OWB 52 and OWB 62 are spring habit genotypes. The vernalization treatment was used to ensure uniform germination. The seedlings were transferred into plastic pots (one plant per pot) filled with a commercial soil mix (previously described). The plants were grown under the same conditions as previously described and located in the West Greenhouses on the Oregon State University campus.

After about four weeks, the plants developed inflorescences. In order to cross the two lines, the inflorescences (spikes) were emasculated before pollen shed. Plants were emasculated around 8:00 am in the morning. Emasculation was accomplished by first removing all the "sterile" laterals in the two-row OWB 52 and all the laterals in the six-row OWB 62. Next, the remaining floret tips were carefully cut open with scissors and the three anthers were removed from each floret. Glycine bags were placed over the emasculated barley heads to prevent possible cross-pollination.

Two days later, spikes that were not emasculated and close to pollen shed were trimmed to expose the anthers in each floret. The spike was removed from the plant and placed in a glass of water under a warm lamp. After 10-50 minutes, the pollen ripened and was applied to the emasculated florets. A whole anther was placed into each floret. The cross-pollinated spike was then covered with a glycine bag to prevent contamination. Reciprocal crosses between OWB lines 52 and 62 were carried out to maximize the probability of successful hybridization.

There were 19 putative F1 seeds that developed from crosses between OWB 52 and OWB 62. The seeds were planted into peat pots with vermiculite and vernalized for two weeks (as previously described). When the seedlings were well developed, they were planted into plastic pots with soil mix. The plants were scored for morphological traits to identify plants that resulted from self-pollination. Plants lacking the hairy sheath phenotype, which is a dominant trait, were recognized as the product of self-pollination of OWB 52 because they showed a homozygous recessive trait that could only come from OWB 52, which is homozygous recessive for smooth sheaths. Plants that were a product of OWB 52 selfing were discarded. We could not use morphological characteristics to differentiate products of self-pollination from bonafide crosses resulting from crosses where OWB 62 was the female parent. For this, PCR amplification of intron 4 of *hvknox3* was used to identify true F1s.

A 'mini-prep' extraction technique was used to extract DNA from putative F1's. The DNA extraction procedure was the same as described earlier, except that the F1 leaf tissue was frozen in liquid nitrogen and ground to a powder with a frozen mini pestle. PCR solutions and conditions were those described above. The PCR mixture was pipetted at 9.0 μL aliquots into 13 (200 μL) ultrathin walled PCR tubes. A 1.0 μL of template DNA (40 ng $\mu L^{-1}$) was added to each tube, which was capped and placed into the PTC-100 thermal cycler. The previously described PCR program was used. The resulting product was mixed with 1.5 μL of 6x loading dye and then loaded into a 2% agarose gel and run for 1 hour at 59V. The PCR product was observed under UV light and photographed (Figure 8).

The true F1's, which were heterozygous for the *hvknox3* dominant allele (1,100 bp) and the *hvknox3* recessive allele (800 bp), were allowed to self-pollinate and the resulting F2 seed was collected. One hundred and forty four F2 seeds were planted. No results for the F2 phenotypic analysis are presented because plants are still at the seedling stage.

# Chapter 3

## Results and Conclusion

The entire population of 94 OWB lines was scored for the presence or absence of a hood. Phenotypic ratios were skewed with an excess of putative *kapkap* individuals (61) at the expense of putative *KapKap* individuals (33) (Table 3).

**Table 3.**—Epistatic interaction between the *Kap* and *lks2* loci in *barley (Hordeum vulgare)*

| **Genotype** [a] | **Phenotype** |
|---|---|
| (33) *KapKapLks2Lks2* | Hooded |
| ***(?) KapKaplks2lks2*** | **Non-hooded and Short-awned** |
| (21) *kapkapLks2Lks2* | Non-hooded and Long-awned |
| ***(?) kapkaplks2lks2*** | **Non-hooded and Short-awned** |

[a] Number of individuals in parentheses. A **?** was used where *Kap* genotype is unknown based on phenotypic information only.

If these distorted phenotypic data are used for genetic linkage analysis, the *Kap* locus maps distal to *lks2* on chromosome 1(7H) (Figure 9). However, the *Kap* locus is reported to be on chromosome 4(4H) (Stebbins and Yagil, 1996 and Kleinhofs, A.; http://barleygenomics.wsu.edu/). The epistatic relationship between the *Kap* and *lks2* loci produced an over abundance of the short-awned phenotypes, which the mapping algorithm interpreted as linkage between the two loci. Linkage of *Kap* and *lks2* could occur only in the event of a translocation involving a small segment of chromosome 4(4H), and this is very unlikely. These results demonstrate the risk of using distorted phenotype data for linkage mapping, especially when the distortion involves an epistatic interaction.

In order to overcome this linkage mapping artifact caused by the epistatic interaction between the *Kap* and *lks2* loci, the data on the non-hooded and short-awned phenotypes was removed from the full data set. When *Kap* data minus the non-hooded and short-awned phenotypes and the same markers were analyzed in GMendel 3.0, the *Kap* locus mapped between CDO122 and ABG003A on chromosome 4(4H) (Figure 10). This corresponds to the reported position of the *Kap* locus (Kleinhofs, A.; http://barleygenomics.wsu.edu/).

The genotypic data from the markers flanking the *Kap* locus were used to infer the missing genotypes at the *Kap* locus, assuming there were no double crossovers (Figure 5). The *hvknox3* data were run in GMendel 3.0, in place of the *Kap* data, and *hvknox3* mapped in the same place as the *Kap* locus based on predicted genotypes (Figure 11). Thus, the *Kap* locus position on chromosome 4(4H) is probably well established based on this analysis. These mapping results corroborated the original findings of I. Vales (personal communication). These results support the hypothesis that *hvknox3* is *Kap* or is tightly linked to *Kap.*

The *Kap*-predict and *hvknox3* genotypes were compared and found to be the same, except for OWB line 52 (Figure 12). This difference might be due to mis-scoring the *hvknox3* genotype, a double crossover between the flanking DNA markers and the *Kap* locus, or linkage of *hvknox3* and *Kap*. The first hypothesis was tested by re-genotyping the population with *hvknox3* and the results supported the initial findings of I. Vales (personal communication); therefore, incorrect scoring is very unlikely. Alternative hypothesis two (a double crossover between CDO122 and ABG003A involving *Kap*, where *Kap* and *hvknox3* are the same or tightly linked) seems more likely

than the alternative hypothesis three (a double crossover involving *hvknox3* as a separate locus from the *Kap* locus) due to the distance between CDO122 and ABG003A. The F2 phenotypic data will allow us to distinguish between these alternatives and will set the stage for the next phase of finer structure mapping.

The main objective of this study was to overcome the limitations of phenotype data resulting from an epistatic interaction and to map the *Kap* locus in the OWB population. This was accomplished using strategies including prediction of genotype based on flanking markers and mapping of amplification products of DNA polymorphisms corresponding to *hvknox3*, which may be the *Kap* gene. The use of flanking markers to predict the genotypes at the *Kap* locus of individuals affected by the epistatic interaction with the *lks2* locus was effective. This approach may work for other cases where epistasis interferes with mapping loci based on phenotypic data alone. The results can be seen in Figure 13. This linkage map location corresponds to the reported position of the *Kap* locus (A. Kleinhofs; http://barleygenomics.wsu.edu/) and provides the research community with the first comprehensive integrated molecular and morphological data set based on the same population.

The objective of defining the *Kap* genotype of OWB 52 was not completed during the course of this project. The F2 phenotype analysis will be completed by August 2000. A phenotypic ratio of 9:3:4 would support the hypothesis that OWB 52 resulted from a double crossover between CDO122 and ABG033A and that *hvknox3* is the *Kap* locus, or tightly linked to the *Kap* locus. A phenotypic ratio of 3:1 would support the hypothesis that OWB 52 resulted from a double crossover between CDO122 and ABG003A and that *hvknox3* and the *Kap* are different genes.

The linkage mapping data indicate that *Kap* and *hvknox3* genes are the same gene, or tightly linked. In order to determine whether or not *hvknox3* is *Kap,* one approach would be to transform wild-type barley with the *hvknox3* gene and see if the hooded phenotype is expressed. This complementation test would indicate that *hvknox3* is the *Kap* gene, or that it confers the same function.

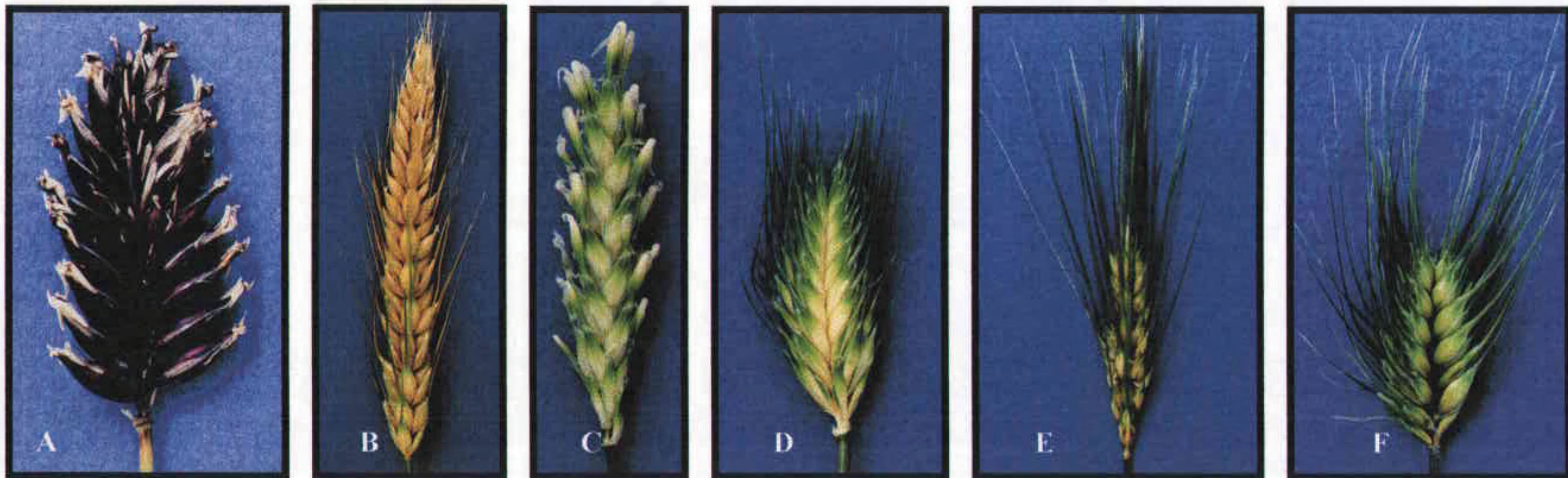


**Figure 1**.—Phenotypes of OWB parents and the four genotype classes at the *Kap* and *lks2* loci.

**A**. Dominant parent – hooded (*KapKapLks2Lks2*)
**B**. Recessive parent – short-awned (*kapkaplks2lks2*)
**C**. OWB #1 – hooded (*KapKapLks2Lks2*)
**D**. OWB #3 – short-awned (*KapKaplks2lks2*)
**E**. OWB #5 – long-awned (*kapkapLks2Lks2*)
**F**. OWB #2 – short-awned (*kapkaplks2lks2*)

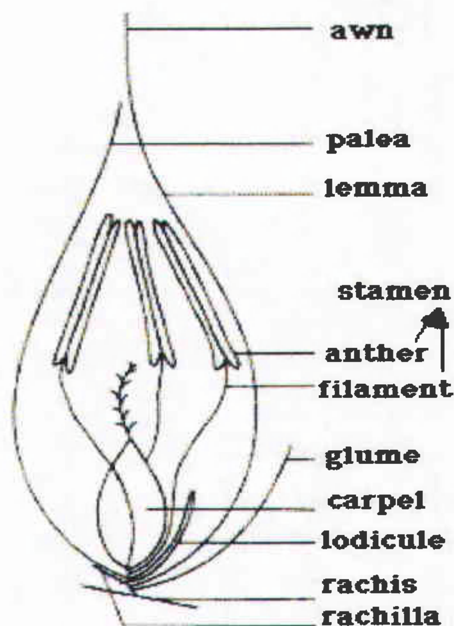


**Figure 2.**--Diagram of a barley floret.
(Taken from website: http://www.mpiz-koeln.mpg.de/~rohde/development.html)

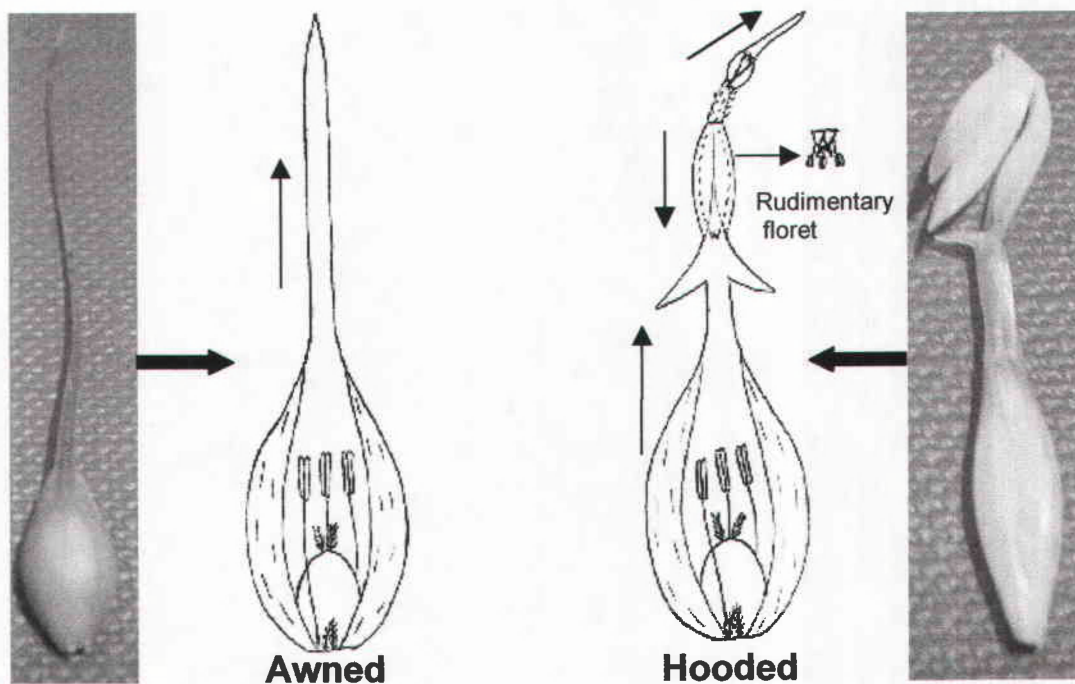


**Figure 3.**--Diagram comparing the structural features of an awned barley floret with a hooded barley floret. The hooded barley floret shows the polarity of its floral structures.

OWB 52

A. ABBABBABAABABABBBBBABBBAAAAAABBBBBBABABBAABBBBBBBBBBAAABBBBBABBBABBBBBABBABABBAABAAABBBBABBAB

B. A--AB-A-AABABAB--B-A---AAAAAA--B--BA-AB-AA---BBBBB---AAA--B--ABBBA--B--A--A-ABBAA-AAA----A--AB

**Figure 4.**-- The phenotypic data for the population of 94 Oregon Wolfe Barleys. In part **A**: A = hooded phenotype, B =non-hooded and long-awned and non-hooded short-awned phenotypes. In part **B**: "–" is in place of B to represent the removal of the non-hooded andshort-awned data.

**A.** OWB 52

```
*HVM40        ABAABAAAAABABABABBBBBBBAAAABBAABBAAABABBAABABBBBBABBBAAA-AAABABBBABABBABBBABA-BAABAAAAABAABAAB
*CDO542       ABAA-BAAAABABABABBBABBBAAAABAAABBBAABABBAABABBBBBABABAAABABBBBBBBABABBABBBABAABAABAAAAABAABAAB
*CDO122       ABAABBAAAABABABABBBABBBAAAABAAABBBAABABBAABABBBBBABABAAABABBBBBBBABABBABBBABABBAABAAAAABAABAAB

*Kap(prelim)  A--AB-A-AABABAB--B-A---AAAAAA--B--BA-AB-AA---BBBBB---AAA--B--ABBBA--B--A--A-ABBAA-AAA----A--AB

*ABG003A      ABAABBAAAABABABABBBABBBABBAAAAABBBBABABBAABABBBABBBABAAABBBBBBBBBABABBBABBABABBAABAAAAABAABAAB
*Dhn6         ABAABBAAAAB-BABABBBABBBABBAAAAA-BBBABABBAABABBBABBBABAAAB-BBBBB-BABABBAABBABABBAABAAAAABAABAAB
*ABC303       ABAABBAAAABABABABBBABBBABBAAAAABBBBABABBAABABBBABBBABAAABAABBBBBBABABBAABBABABBAABABAAABAABAAB
```

**B.** OWB 52

```
*CDO122       ABAABBAAAABABABABBBABBBAAAABAAABBBAABABBAABABBBBBABABAAABABBBBBBBABABBABBBABABBAABAAAAABAABAAB
*Kap(predict) ABAABBAAAABABABABBBABBBA--A-AAABBB-ABABBAABABBB-B-BABAAABABBBABBBABABBA-BBABABBAABAAAAABAABAAB
*ABG003A      ABAABBAAAABABABABBBABBBABBAAAAABBBBABABBAABABBBABBBABAAABBBBBBBBBABABBBABBABABBAABAAAAABAABAAB
```

**Figure 5.**—**A.** *Kap* phenotypes minus non-hooded and short-awned phenotype data placed in map order in linkage group 4(4H). The *Kap* locus is linked to markers - CDO122 and ABG003A. A = hooded phenotype and B = non-hooded and long-awned phenotype **B.** The *Kap* genotypes for the missing data are inferred using flanking markers. When there were contrasting alleles at the flanking markers, the *Kap* locus genotype was scored as missing. A = *KapKap* genotype and B = *kapkap* genotype and "–" = missing data.

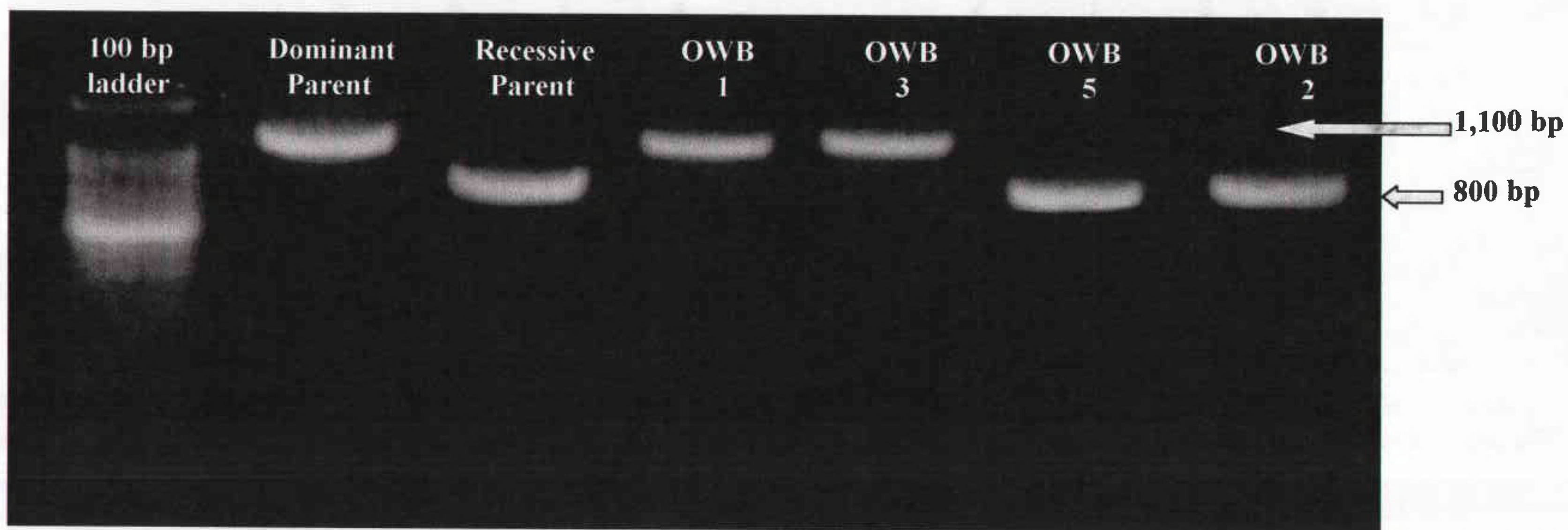


**Figure 6**.-- Photograph of a ethidium bromide-stained agarose gel showing the 1,100-bp fragment from the dominant allele and the 800-bp fragment from the recessive allele in the OWB dominant and recessive parents and a sample of OWB doubled haploid lines. These OWB lines are the same lines whose inflorescence genotypes are shown in Figure 1.

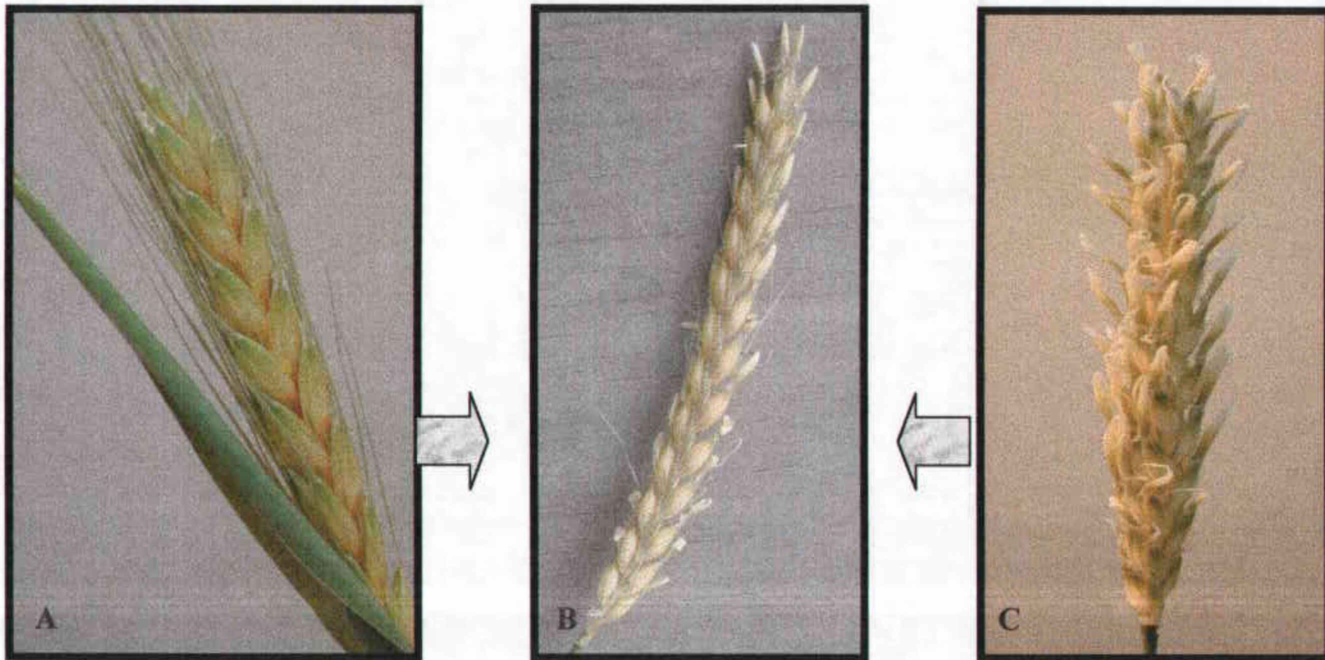


**Figure 7.** –Spike Morphology of OWB 52, OWB 62 and the F1 hybrid.
**A.** OWB 52 (2-row and short-awn ) **B.** F1 hybrid (OWB 52 x OWB 62 = 2-row and hooded)
**C.** OWB 62 (6-row and hooded)

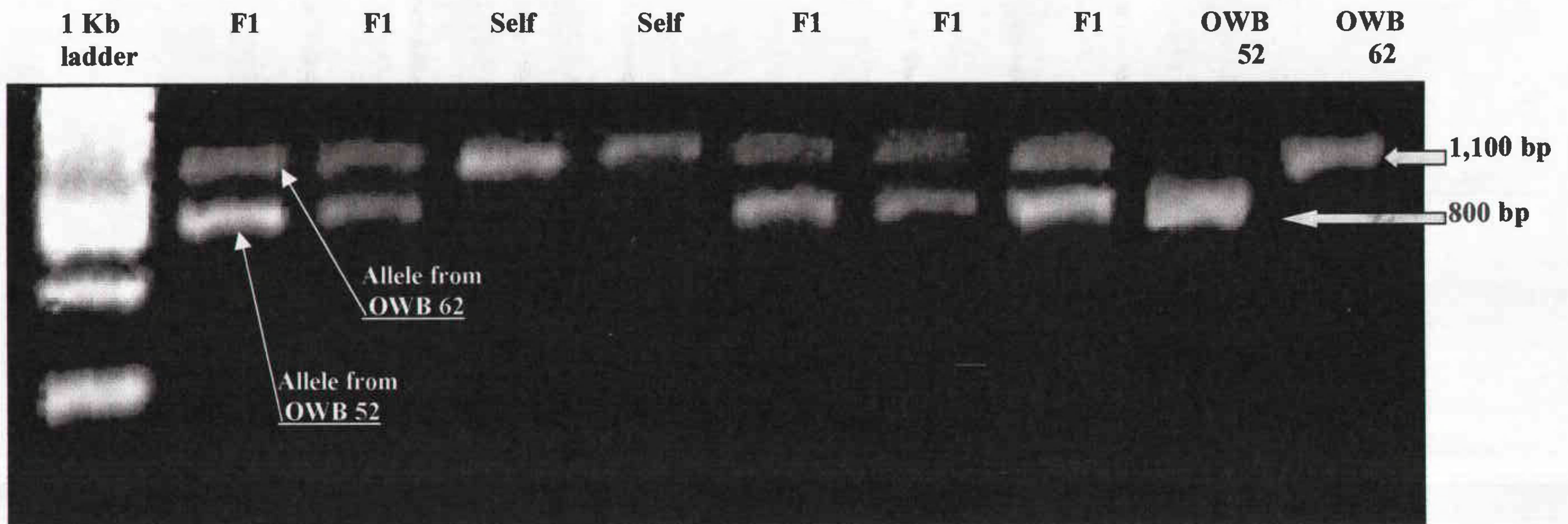


**Figure 8.**—Photograph of a ethidium bromide-stained agarose gel showing F1's with a 1,100-bp fragment from the OWB 62 parent and an 800-bp fragment from the OWB 52 parent. The two selfs are from the OWB 62 parent because they show only the 1,100 bp fragment from OWB 62.

| **Map of group 1** | | | Recombination Fraction | Morgans |
|---|---|---|---|---|
| *ABG601 | -- | *HVM67 | 0.140 | 0.144 |
| *HVM67 | -- | *Hsh | 0.011 | 0.011 |
| *Hsh | -- | *EBMAC0701 | 0.176 | 0.184 |
| *EBMAC0701 | -- | *KFP221 | 0.044 | 0.044 |
| *KFP221 | -- | *MWG652B | 0.054 | 0.054 |
| *MWG652B | -- | *ABG472 | 0.118 | 0.121 |
| *ABG472 | -- | *BMAC0186 | 0.221 | 0.237 |
| *BMAC0186 | -- | *BMAG0353 | 0.058 | 0.058 |
| *BMAG0353 | -- | *BMAC0303B | 0.103 | 0.105 |
| *BMAC0303B | -- | *BMAG0218 | 0.000 | 0.000 |
| *BMAG0218 | -- | *BMAG0384 | 0.000 | 0.000 |
| *BMAG0384 | -- | *HVM03 | 0.000 | 0.000 |
| *HVM03 | -- | *ABC303 | 0.100 | 0.101 |
| *ABC303 | -- | *Dhn6 | 0.022 | 0.022 |
| *Dhn6 | -- | *ABG003A | 0.011 | 0.011 |
| *ABG003A | -- | *CDO122 | 0.096 | 0.097 |
| *CDO122 | -- | *CDO542 | 0.011 | 0.011 |
| *HVM40 | -- | *MWG077 | 0.043 | 0.043 |
| *MWG077 | -- | *MWG634 | 0.194 | 0.204 |
| *MWG634 | -- | *ABG704 | 0.387 | 0.515 |
| *ABG704 | -- | *BMAG0007 | 0.138 | 0.142 |
| *BMAG0007 | -- | *HVWAXY4A | 0.116 | 0.118 |
| *HVWAXY4A | -- | *ABG380 | 0.220 | 0.236 |
| *ABG380 | -- | *HVCMA | 0.308 | 0.359 |
| *HVCMA | -- | *BMAC0187 | 0.136 | 0.139 |
| *BMAC0187 | -- | *BMAC0273A | 0.181 | 0.189 |
| *BMAC0273A | -- | *BMAC047B | 0.039 | 0.039 |
| *BMAC047B | -- | *DAK642 | 0.034 | 0.035 |
| *DAK642 | -- | *MWG808 | 0.021 | 0.022 |
| *MWG808 | -- | *BMAC0303A | 0.058 | 0.058 |
| *BMAC0303A | -- | *Nud | 0.115 | 0.117 |
| *Nud | -- | ***Kap** | 0.298 | 0.343 |
| ***Kap** | -- | ***Lks2** | 0.223 | 0.240 |
| ***Lks2** | -- | *BMAG0120 | 0.109 | 0.110 |
| *BMAG0120 | -- | *WG380B | 0.033 | 0.033 |
| *WG380B | -- | *Ris44 | 0.106 | 0.108 |
| *Ris44 | -- | *ABC253 | 0.149 | 0.154 |
| *ABC253 | -- | *ABG461A | 0.096 | 0.097 |
| *ABG461A | -- | *WG380A | 0.043 | 0.043 |
| *WG380A | -- | *HVM5 | 0.233 | 0.253 |
| *HVM5 | -- | *ThA1 | 0.033 | 0.033 |

Sum of r values = 4.563
Sum of map distances = 4.917

**Figure 9.**—Linkage group calculated with complete *Kap* phenotypic data. This shows a merger of two linkage groups (1(7H) and 4(4H) that are normally separate (See Figure 9 for comparison).

**A.**
**Map of group 1**

| | | | Recombination Fraction | Morgans |
|---|---|---|---|---|
| *ABG704 | -- | *BMAG0007 | 0.138 | 0.142 |
| *BMAG0007 | -- | *HVWAXY4A | 0.116 | 0.118 |
| *HVWAXY4A | -- | *ABG380 | 0.220 | 0.236 |
| *ABG380 | -- | *HVCMA | 0.308 | 0.359 |
| *HVCMA | -- | *BMAC0187 | 0.136 | 0.139 |
| *BMAC0187 | -- | *BMAC0273A | 0.181 | 0.189 |
| *BMAC0273A | -- | *BMAC047B | 0.039 | 0.039 |
| *BMAC047B | -- | *DAK642 | 0.034 | 0.035 |
| *DAK642 | -- | *MWG808 | 0.021 | 0.022 |
| *MWG808 | -- | *BMAC0303A | 0.058 | 0.058 |
| *BMAC0303A | -- | *Nud | 0.115 | 0.117 |
| *Nud | -- | ***Lks2** | 0.181 | 0.189 |
| ***Lks2** | -- | *BMAG0120 | 0.109 | 0.110 |
| *BMAG0120 | -- | *WG380B | 0.033 | 0.033 |
| *WG380B | -- | *Ris44 | 0.106 | 0.108 |
| *Ris44 | -- | *ABC253 | 0.149 | 0.154 |
| *ABC253 | -- | *ABG461A | 0.096 | 0.097 |
| *ABG461A | -- | *WG380A | 0.043 | 0.043 |
| *WG380A | -- | *HVM5 | 0.233 | 0.253 |
| *HVM5 | -- | *ThA1 | 0.033 | 0.033 |

Sum of r values = 2.349
Sum of map distances = 2.474

**B.**
**Map of group 4**

| | | | Recombination Fraction | Morgans |
|---|---|---|---|---|
| *MWG634 | -- | *MWG077 | 0.194 | 0.204 |
| *MWG077 | -- | *HVM40 | 0.043 | 0.043 |
| *HVM40 | -- | *CDO542 | 0.086 | 0.087 |
| *CDO542 | -- | *CDO122 | 0.011 | 0.011 |
| *CDO122 | -- | **Kap-prelim** | 0.093 | 0.094 |
| **Kap-prelim** | -- | *ABG003A | 0.074 | 0.075 |
| *ABG003A | -- | *Dhn6 | 0.011 | 0.011 |
| *Dhn6 | -- | *ABC303 | 0.022 | 0.022 |
| *ABC303 | -- | *HVM03 | 0.100 | 0.101 |
| *HVM03 | -- | *BMAG0384 | 0.000 | 0.000 |
| *BMAG0384 | -- | *BMAG0218 | 0.000 | 0.000 |
| *BMAG0218 | -- | *BMAC0303B | 0.000 | 0.000 |
| *BMAC0303B | -- | *BMAG0353 | 0.103 | 0.105 |
| *BMAG0353 | -- | *BMAC0186 | 0.058 | 0.058 |
| *BMAC0186 | -- | *ABG472 | 0.221 | 0.237 |
| *ABG472 | -- | *MWG652B | 0.118 | 0.121 |
| *MWG652B | -- | *KFP221 | 0.054 | 0.054 |
| *KFP221 | -- | *EBMAC0701 | 0.044 | 0.044 |
| *EBMAC0701 | -- | *Hsh | 0.176 | 0.184 |
| *Hsh | -- | *HVM67 | 0.011 | 0.011 |
| *HVM67 | -- | *ABG601 | 0.140 | 0.144 |

Sum of r values = 1.558
Sum of map distances = 1.605

**Figure 10.**—Linkage groups, 1(7H) in **A** and 4(4H) in **B** calculated with *Kap* phenotypic data minus the short-awned data.

**A.**
**Map of group 1**

| Marker | | Marker | Recombination Fraction | Morgans |
|---|---|---|---|---|
| *ABG704 | -- | *BMAG0007 | 0.138 | 0.142 |
| *BMAG0007 | -- | *HVWAXY4A | 0.116 | 0.118 |
| *HVWAXY4A | -- | *ABG380 | 0.220 | 0.236 |
| *ABG380 | -- | *HVCMA | 0.308 | 0.359 |
| *HVCMA | -- | *BMAC0187 | 0.136 | 0.139 |
| *BMAC0187 | -- | *BMAC0273A | 0.181 | 0.189 |
| *BMAC0273A | -- | *BMAC047B | 0.039 | 0.039 |
| *BMAC047B | -- | *DAK642 | 0.034 | 0.035 |
| *DAK642 | -- | *MWG808 | 0.021 | 0.022 |
| *MWG808 | -- | *BMAC0303A | 0.058 | 0.058 |
| *BMAC0303A | -- | *Nud | 0.115 | 0.117 |
| *Nud | -- | ***Lks2** | 0.181 | 0.189 |
| ***Lks2** | -- | *BMAG0120 | 0.109 | 0.110 |
| *BMAG0120 | -- | *WG380B | 0.033 | 0.033 |
| *WG380B | -- | *Ris44 | 0.106 | 0.108 |
| *Ris44 | -- | *ABC253 | 0.149 | 0.154 |
| *ABC253 | -- | *ABG461A | 0.096 | 0.097 |
| *ABG461A | -- | *WG380A | 0.043 | 0.043 |
| *WG380A | -- | *HVM5 | 0.233 | 0.253 |
| *HVM5 | -- | *ThA1 | 0.033 | 0.033 |

Sum of r values = 2.349
Sum of map distances = 2.474

**B.**
Map of group 4

| Marker | | Marker | Recombination Fraction | Morgans |
|---|---|---|---|---|
| *MWG634 | -- | *MWG077 | 0.194 | 0.204 |
| *MWG077 | -- | *HVM40 | 0.043 | 0.043 |
| *HVM40 | -- | *CDO542 | 0.086 | 0.087 |
| *CDO542 | -- | *CDO122 | 0.011 | 0.011 |
| *CDO122 | -- | ***HVKNOX3** | 0.064 | 0.064 |
| ***HVKNOX3** | -- | *ABG003A | 0.074 | 0.075 |
| *ABG003A | -- | *Dhn6 | 0.011 | 0.011 |
| *Dhn6 | -- | *ABC303 | 0.022 | 0.022 |
| *ABC303 | -- | *HVM03 | 0.100 | 0.101 |
| *HVM03 | -- | *BMAG0384 | 0.000 | 0.000 |
| *BMAG0384 | -- | *BMAG0218 | 0.000 | 0.000 |
| *BMAG0218 | -- | *BMAC0303B | 0.000 | 0.000 |
| *BMAC0303B | -- | *BMAG0353 | 0.103 | 0.105 |
| *BMAG0353 | -- | *BMAC0186 | 0.058 | 0.058 |
| *BMAC0186 | -- | *ABG472 | 0.221 | 0.237 |
| *ABG472 | -- | *MWG652B | 0.118 | 0.121 |
| *MWG652B | -- | *KFP221 | 0.054 | 0.054 |
| *KFP221 | -- | *EBMAC0701 | 0.044 | 0.044 |
| *EBMAC0701 | -- | *Hsh | 0.176 | 0.184 |
| *Hsh | -- | *HVM67 | 0.011 | 0.011 |
| *HVM67 | -- | *ABG601 | 0.140 | 0.144 |

Sum of r values = 1.529
Sum of map distances = 1.576

**Figure 11.**—Linkage groups, 1(7H) in **A** and 4(4H) in **B**, calculated with *hvknox3* allelic data.

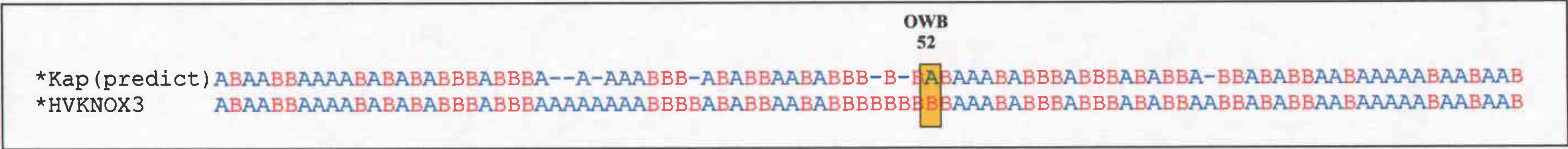


**Figure 12**.--Comparison between the *Kap*-predicted genotypes and the *hvknox3* genotypes. Note OWB 52, where the *Kap*-predicted genotype differs from the *hvknox3* genotype. A = *KapKap* genotype and B = *kapkap* genotype and "–" = missing data

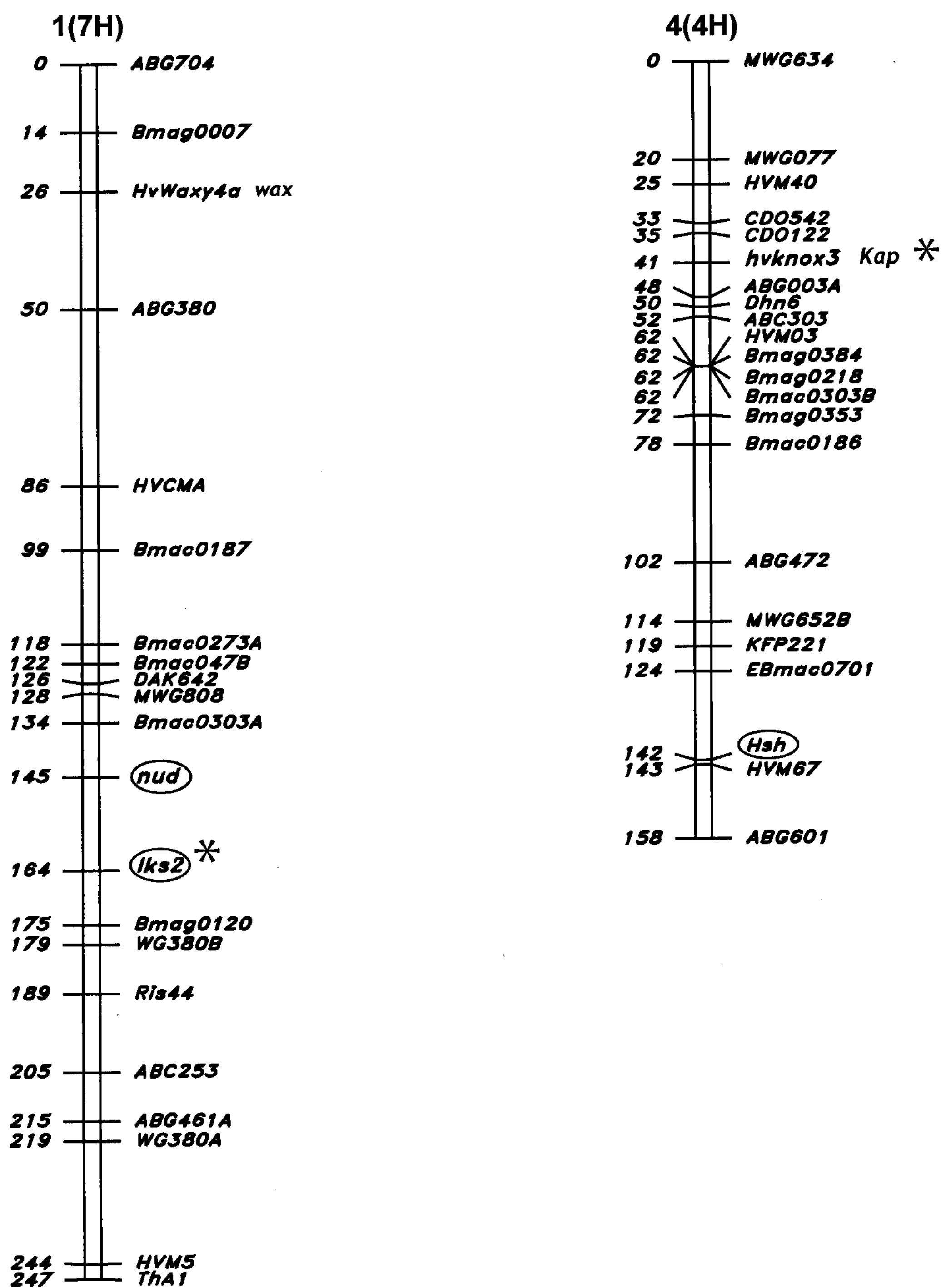


**Figure 13**.--Linkage map of barley chromosomes 1(7H) and 4(4H) with the genetic distances in centiMorgans. * marks the location of the *lks2, Kap* and *hvknox3* loci on their respective linkage groups.

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