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HORDEUM MURINUM IN HOLLAND[1])

J. W. MORRISON
(*Temporary guest worker at Laboratory of Genetics, Wageningen, Netherlands*)

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INTRODUCTION

Hordeum murinum, "Wall barley", "Mäusegerste", or "Kruipertje" is indigenous to Europe. It is a weed of waste ground and is a common grass around vacant buildings, harbours, railways, grain dispersal areas, factory yards, playgrounds etc. Its mode of dissemination and the ability to survive under poor soil conditions enables it to become established quickly on abandoned or disturbed areas. Therefore, although it probably does not originate here, Holland with a network of canals, rivers, and roads has many ideal sites for introduction and the grass is common to most parts of the country (JANSEN, 1951).

The species was first named by Linnaeus in Species Plantarum 1753. Several adequate descriptions have since been published (JANSEN 1951; HUBBARD 1954; or see any other European Flora). Possibly because of conditions under which the plants grow and also because of some innate genetical variability, many morphological forms occur—in height alone the plants may vary from a few cm to almost a meter. The extremes in form have caused some difficulties in classification and at present there are differing opinions as to whether specific or subspecific rank should be used for certain taxa in the genus *Hordeum*. The disorder has been heightened because the morphological features used to delineate species are small and variable characters that often overlap. For example, *H. leporinum* Link was described as a robust plant with large lateral spikelets. The size of the lateral spikelets or the ratio of the laterals to the median has indeed been used as a key for separating this species from *H. murinum*: in *murinum* the median spikelet is longer than the laterals, in *leporinum* the laterals are longer. Problems in classification immediately arise when plants with spikelets of equal size occur. Another species of this group, usually referred to as the murinum complex, has been named *H. glaucum* Steudel. This species was erroneously named *H. stebbinsii* (COVAS 1949) but STEBBINS himself agrees (personal communication) that the correct name is *glaucum*.

Several cytologists have studied specimens from the murinum complex but they have added more confusion because frequently the plants have not been adequately identified. Thus chromosome numbers of 2n = 14 and 2n = 28 have been given to *H. murinum* (see reviews of LÖVE and LÖVE 1948; COVAS 1949; DARLINGTON and WYLIE 1955)

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and a mistaken impression has arisen that there are two chromosome races of the species. CHIN (1941) recognized these difficulties and described two forms of *murinum*. Both had 28 chromosomes. Recently two authoritative publications have appeared that have identified species with chromosome number. COVAS (1949) carefully distinguished all species and gave a key along with a list of characters that can be used to identify the three species *H. stebbinsii* (glaucum), *H. leporinum*, and *H. murinum*. According to this proposal *leporinum* is the tetraploid species and the other two are diploid. Apparently, however, COVAS himself did not count the chromosomes of *H. murinum* (cf. table 1 of COVAS). HUBBARD (1954) stated that *H. murinum* has 14 chromosomes and *H. leporinum* has 28.

Research work on the interspecific hybridization of Hordeum species has been carried on at the Cereal Crops Division, Ottawa, for some time now (HAMILTON *et al.* 1955). Samples sent to Canada as H. *murinum* from European botanical gardens have been identified as such from the key of COVAS or the description of HUBBARD but they invariably had 28 chromosomes. During part of the last year the author has had the opportunity of studying natural populations of *H. murinum* in Holland and some aspects of the study are given here.

MATERIAL

All the material, collected in Holland, will be referred to as *H. murinum* in this paper. In three samples collected in Italy and one sample brought from Canada the laterals are considerably larger than the median spikelets and therefore the specimens are classed as *H. leporinum*. Four samples from the diploid species, brought from Canada and received there as samples under various names will all be considered as *H. glaucum*.

RESULTS

In Table I is a list of locations in Holland where samples of plants were taken. In some areas there were hundreds of plants in which case random samples were taken or a search made for abnormal plants, keeping in mind the various characteristics that are used to distinguish species. Other plants were found growing by themselves. The collections were made at various times from June to November.

All plants had 28 *chromosomes.* It is obvious that the collection has covered the more common sites of introduction into Holland. It is also equally obvious that *H. murinum* (at least in Holland) has 2n = 28 chromosomes. [1])

First metaphase of meiosis was studied in many of the plants to determine chromosome number and to see whether quadrivalents occurred. Not one quadrivalent was seen in any preparation although over 500 cells from ten plants were studied in detail. The chromosomes of samples from many areas were studied in root tip preparations to make certain that there were no differences in karyotype. Most of

[1]) Two samples collected in England also had 28 chromosomes.

the chromosomes of this species, and of this genus for that matter, have median or sub-median centromeres and it is thus not possible to pick out all the individual chromosomes. CHIN (1941) recognized two satellite pairs in his samples of *murinum* from England and America.

TABLE I

Samples of plants of *H. murinum* collected in Holland.

	No. of plants
Wageningen harbour	5
Wageningen sports field	3
Renkum	10
Rhenen	2
Krommenie	1
Bennekom	1
Aalsmeer	1
Rotterdam	1
Zeeland Island (Duiveland)	1
Wageningen [1])	10
Arnhem	9
Zutphen	2
Deventer (Grain silo)	4
Deventer (Pothoofd)	4
Zwolle	2
Wijhe	5
Harderwijk	2
Venlo	3
Gennep	2
Malden	2
Roermond	4
Maastricht	10
Geleen	3
Helmond	6
Nederweert	1
's-Hertogenbosch	4
Total	98

A careful study showed that in the material collected in Holland there are also four chromosomes with satellites (Fig. 1) with each pair distinct from the other because of the ratios of arm length and sizes of the satellites. This number of nucleolar chromosomes agrees with the number of nucleoli found in resting cells. There is also one short chromosome pair with medianly located centromeres, and one pair of long chromosomes with sub-median centromeres (in black, Fig. 1). Thus the karyotype is definitely not that of an autotetraploid. The plants collected in England and both of the plants of *H. leporinum* that were studied in detail had a karyotype identical to that of the Holland material.

When it became clear that the chromosome number of the plants collected in Holland was 28 it was imperative that an accurate analysis be made to be certain that the plants were similar morphologi-

[1]) Collected in October from roadsides, near buildings, etc., with samples taken from 0.5 to 3 km apart.

cally to those of the species *H. murinum* quoted as having 14 chromosomes. At the same time it was possible to determine how much variability existed in the key characteristics that are used to differentiate species. To this end, the variations between spikelets within one spike, the variations between different spikes of the same plant, and

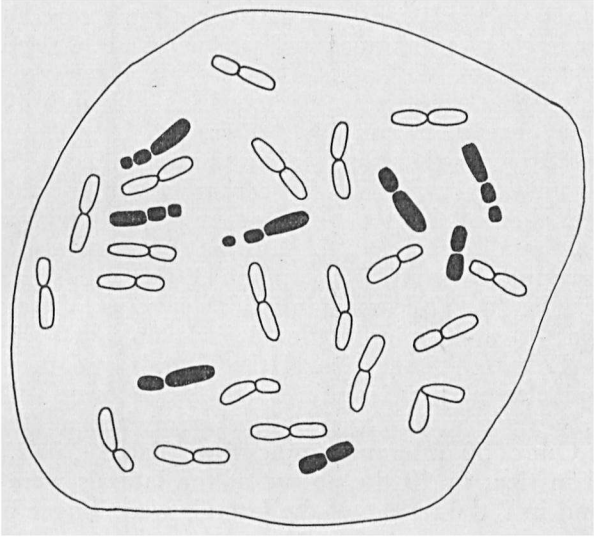

Fig. 1. The chromosomes of *H. murinum*. Root tip mitosis of a plant from Renkum; roots pretreated with monobromonaphthalene before fixation. × 1700.

the variations between plants were assessed as carefully as possible. For a comparison of plants the spikelets were taken from the central part of a normal appearing spike. The spikelets were examined under a dissecting microscope against a grid of mm squares. It was thus possible to give accurate measurements for all floral parts.

In establishing the identity of the specimens collected in Holland, the main features used were taken from the work of Covas (1949) and are listed below. The first four were designed to differentiate *murinum* from both *leporinum* and *glaucum*; the last five and possibly number four, to separate *murinum* and *leporinum* from *glaucum*. Accordingly then, in *H. murinum*:

1. The lemma, palea, and awn of the median spikelet are longer than those of the lateral spikelets.
2. The paleae of the lateral spikelets are almost glabrous.
3. The floret of the central spikelet is sessile or sub-sessile.
4. The inner glumes of the lateral spikelets are narrower than the glumes of the central spikelet.
5. The spike is not dense (3–6 spikelets / cm).
6. The anthers are 0.7–1.0 mm long — in *glaucum* they are shorter.
7. The cilia on the margins of the rachis segments are short.
8. The filaments of the anthers have starch grains.

9. The prolongation of the rachilla of the lateral spikelets is 2–3 mm long, not colored, setaceous—in *glaucum* the rachillae are shorter.

Lemma, palea, and awn of median longer

For brevity in COVAS' key it was stated that the lemma, palea, and awn of the median floret (spikelet) are longer than those of the laterals, but this is somewhat misleading because in a more careful description it can be seen that the lemmas and paleae of the different spikelets may be equal (Table 4, COVAS 1949, or see HUBBARD 1954). The awn on the median floret is always longer. The length of the lemma is difficult to measure accurately because of the extension into an awn. The paleae were therefore measured in all spikelets. The laterals were most uniform in size in the centre of the spike. They were somewhat smaller at the top and bottom so that within one spike there were actual differences in size but the trend was always constant. Thus for example in sample Roermond 3, the ratios (length of the lateral palea: length of the median palea) were as follows: top of the spike, 9 mm: 10 mm; middle 12: 13; and bottom 8: 13. In three spikes from Helmond 5 the size of the laterals and medians of ten different spikelet groups within each spike were of the same tendency, the mean ratios for the three spikes being, 12:12; 11:12; and 11:12. Out of 50 different samples from Table 1, in 25 the paleae were equal in size; in 18 the paleae of the laterals were shorter by 1–2 mm, and in 7 the paleae of the laterals were longer by 1–2 mm. In the four specimens of *leporinum* the mean ratios were, 10:7; 15:10; 13:10; and 15:10; in *glaucum* they were 9:6; 8:6; 10:7, and 9:6. In *glaucum* the spikelets were clearly small in size and the lateral florets as in *leporinum* were definitely larger than the median fertile florets. It is well to note here though that size alone is not a safe criterion because some plants of *H. murinum* with 28 chromosomes had a ratio of 10:8. If we also consider awns we can form a more reliable picture. In samples of *H. murinum* the awns of the median spikelets are always longer than the awns of the laterals, even when the paleae of the laterals exceed the median by 1–2 mm in length. However, in *leporinum* and *glaucum* material the awns of the laterals may extend beyond the awns of the median floret. From the foregoing it is easy to see why there are difficulties in classification between *murinum* and *leporinum* when that classification depends upon the size of the lateral spikelets (Fig. 2A).

Paleae of laterals almost glabrous

The amount of pubescence varied from plant to plant (probably both genetical and environmental as in crop plants). The plants of *H. murinum* usually had some barbs or short hairs on the nerves of the paleae of the lateral florets. There were no big differences within a spike or between spikes of a plant or even between plants. The paleae of the lateral spikelets of the samples of *leporinum* had a similar amount of pubescence; the four samples of *H. glaucum* all had very

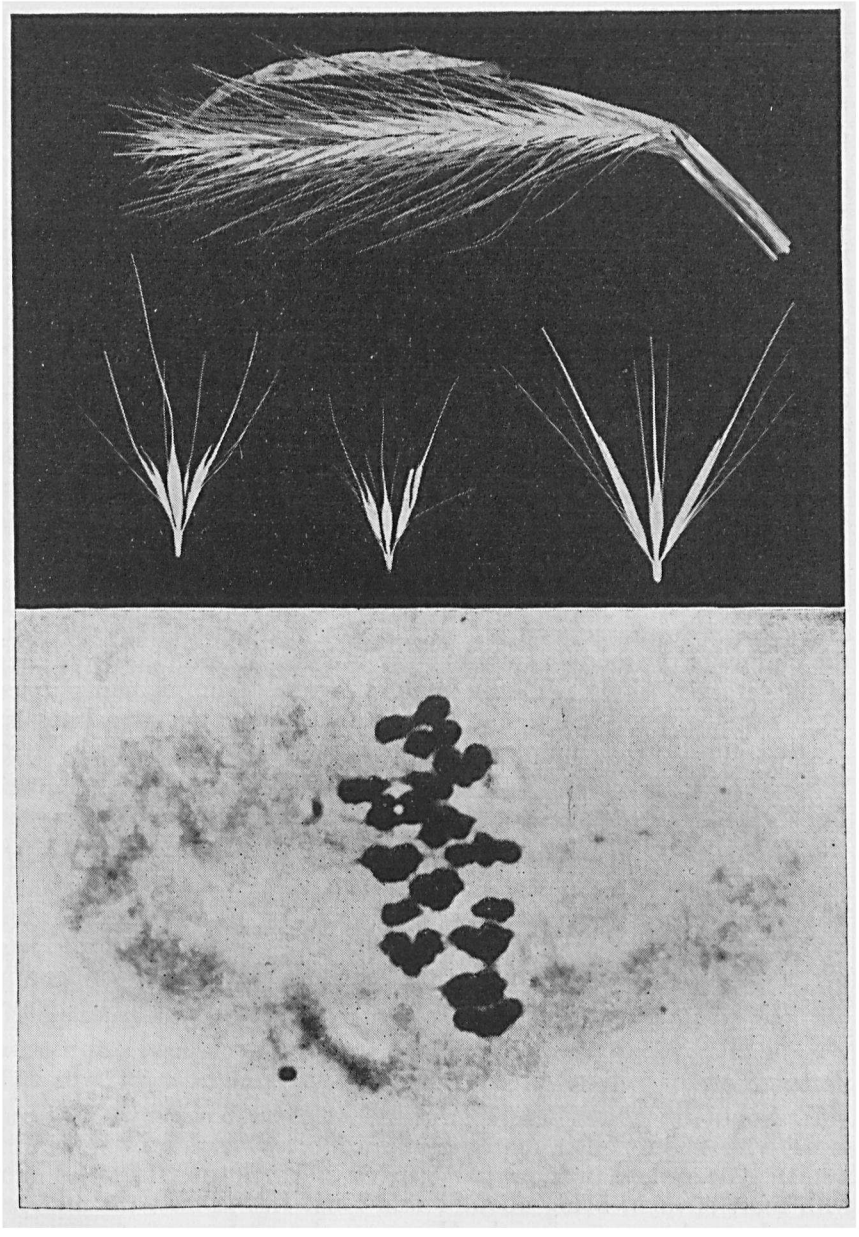

Fig. 2. *A*. Spike of *H. murinum* and groups of three spikelets; from left to right, *H. murinum*, *H. glaucum*, and *H. leporinum*. The awns have been cut off in proportion to normal length and lateral spikelets have been pressed apart to show details in the photograph. Normal size. *B*, The 14 bivalents of *H. murinum* at metaphase of meiosis, with 12 ring and 2 open bivalents. × 1000.

pubescent paleae of laterals. Without a study of a larger sample from *glaucum* it is difficult and somewhat risky to rate this character for distinguishing between diploids and tetraploids. Certainly it is not useful to differentiate *leporinum* and *murinum*. The term "almost glabrous" is rather vague. It does, however, adequately describe the paleae of the lateral spikelets of all *H. murinum* collected in Holland.

Central floret sessile or nearly so

The pedicel length of the floret of the median spikelet was measured in all samples. There was not much variation within a spike or between spikes of a plant. In the sample of 50, the range was 0.25 mm to 1.5 mm with a mean value of 0.6 mm. It is therefore somewhat misleading to class this condition as being sessile. However, in no cases were the pedicels of the median floret as long, or nearly as long, as the pedicels of the lateral spikelets. In both *leporinum* and *glaucum* the pedicels of the median floret measured 1.5–2.0 mm and were nearly as long as the pedicels of the lateral spikelets. Used with caution this character, therefore, seems useful for distinguishing species and once again confirms the samples from Holland as being *H. murinum*.

Inner glumes of the laterals narrower

This character is not certain enough to use for separating *leporinum* and *glaucum* from *murinum*. In all samples of *leporinum* and *murinum*, the glumes of the median spikelet were broader than the inner glumes of the laterals. Along with this general largeness the median glumes were usually more hairy, had longer cilia than the inner laterals. In the four diploid samples the inner glumes of the laterals were equal to or wider than the glumes of the median spikelets.

Spike density

Some major differences were observed in the spike density of *H. murinum* samples but they coincided with vigorous or puny plants. The actual rachis segments attached to the group of three spikelets varied from 2.5 to 4 mm in length, which corresponds to 3–6 spikelets per cm. In the *leporinum* samples the segments were of similar length but in *glaucum* they were as short as 2 mm indicating a more dense spike.

Anther length

It should be noted here that many of the laterals had anthers, some with good pollen, but the condition is variable even within a spike. The anthers in *H. murinum* ranged in length from 0.7 to 1.5 mm with most of them being about 1 mm long. In *leporinum* the anthers were about that same length. However, the anthers of the diploid *glaucum* were never more than 0.5 mm long. Moreover, these anthers are generally not extruded at anthesis. The small size of the anthers can be used as a definite criterion of the diploid material. (This is also a general observation of previous experience in the interspecific crossing program at Ottawa).

Cilia on rachis margins

In all specimens of *leporinum* and *murinum* examined, there were short, stout cilia on the margins of the rachis segments. The length was quite uniform throughout this group. The amount and the length of the cilia varied in the small sample of the *glaucum* group.

Starch in anther filaments

Starch grains were present in the filaments of all samples of *murinum* and *leporinum*. Some had a larger amount than others and as expected this is due in part to the stage of development. The largest amount was present in more mature florets. No starch grains were present in the filaments of the few samples of *H. glaucum*. The small number of diploids examined is not sufficient to establish this as a definite criterion but it does seem useful.

Rachilla length, color and condition

The rachillae of both the laterals and the median spikelets were measured in all samples. No big differences occurred in the samples of *murinum* and *leporinum*: median, 5–6 mm; laterals about 4 mm and, depending much on the general vigor of the laterals, some stout, some slender. In *glaucum* all rachillae were shorter and stout: median 4–5 mm; laterals 2–3 mm. Orange pigmentation was present in all species. Rachilla length, color, and condition are difficult characteristics to use with any certainty for distinguishing species.

There are three other characters that have been used to differentiate species and they will be mentioned here. The amount of pigmentation or the color that develops in the spikes of all species is highly variable. Samples of *H. murinum* collected in Holland were straw-colored, brown, dark brown or purple. Pollen grain size is a useful characteristic of polyploidy in most species. In *Hordeum* it is not so useful (cf. CHIN 1941). In the measurements made for this study, pollen grains of the diploid *H. glaucum* were only a few microns smaller than those of *H. murinum* that were 45 microns in diameter. It has also been claimed that in *leporinum* the inner glumes of the lateral spikelets are ciliated on both sides but in *murinum* only on one side. As mentioned previously, the amount of pubescence is variable. Thus the cilia on the glumes were quite prominent in some plants but much reduced in others. The cilia are especially noticeable in green immature spikes. In some of the Holland plants (murinum) the inner glumes of the laterals were not too well developed and had cilia on one side only but in others there were cilia on both sides.

DISCUSSION

Chromosome number of H. murinum

Some stress has been laid out on the taxonomic features used within the murinum complex to identify *H. murinum*. Some of the characteristics used to separate species were variable and not too reliable; others were considered useful. In no cases were there characteristics that

would place the material collected in Holland in any category other than *H. murinum.* From this, it is obvious that samples picked up in Holland would have to be considered as *H. murinum* and indeed this has been the practice of ecologists, taxonomists, and botanists in Holland.

It is not possible to determine the chromosome number of the type specimen of *H. murinum L.* deposited in the Linnean Society Herbarium in London. Plants having 28 chromosomes are widespread in Holland and indeed were the only plants found. Although the chromosome number was determined for only 98 plants, the plants were selected from thousands. Moreover it is impossible to imagine that this complex stops at Holland's borders—there are some examples to show otherwise. Morphologically, the plants have been identified as *H. murinum.* Therefore, the only conclusion that can be drawn is that *H. murinum* has been erroneously listed as having 14 chromosomes and that we should now regard it as having 28.

Seeds from some of these plants will be grown and specimens preserved in the Botany and Plant Pathology Herbarium, Science Service, Dept. of Agriculture, Ottawa. Plants now growing at the Genetics Department, Wageningen, will later be deposited in the Herbarium at Leyden.

Polyploidy in H. murinum

As long as two different diploid species were supposed to exist it was natural enough to hypothesize that the union of these two species had produced the allotetraploid. Now, unless one diploid species is extinct, a new explanation is needed. In the absence of the second diploid, autopolyploidy is suggested. Much has been written about autotetraploidy (cf. MUNTZING 1936; MYERS 1947; STEBBINS 1950; DARLINGTON 1956) and it need not be repeated here except to list some criteria: 1. Tetrasomic inheritance exists for some features. 2. Autotetraploids are usually morphologically similar to the diploids with perhaps the tetraploids being somewhat larger, and later in maturing. 3. There is reduced fertility, especially in the autogamous crops. 4. Quadrivalents are present at meiosis. Our genetical studies are not far enough advanced to give supporting evidence. It is true that the species are morphologically similar but the other evidence shows that *H. murinum* is *not an autotetraploid.* The fertility of the plants is in no way impaired. Perhaps the strongest evidence against autopolyploidy is that no quadrivalents were found in the many pollen mother cells examined. It has been pointed out that a low frequency of multivalent formation in an autotetraploid may be due: 1. to low chiasma frequency and preference of terminal over interstitial chiasmata; 2. to the small size of the chromosomes; 3. to the excess in numbers which may prevent movement; but none of these restrictions in pairing ability are obvious in *H. murinum* (Fig. 2B). In an autotetraploid there should be a duplication of the karyotype of the diploid chromosomes so that four sets of chromosomes of similar sizes and shapes are present. However, some cytologists assume that inversions,

translocations and deletions could so alter the chromosomes that the ones which were previously similar would now be non-identifiable—a view with which the author does not entirely agree. In another species, *H. bulbosum* (4 x = 28) it is quite evident from the morphology of the chromosomes, that the plants are autotetraploids and this is of course borne out at meiosis. The morphology of the chromosomes of *H. murinum* does not indicate an autotetraploid.

One puzzling feature of polyploidy relationships in this genus is that there are four satellite chromosomes in both diploid and tetraploid species. Some diploid species of *Hordeum* have only one pair of chromosomes with satellites but *glaucum* has two. Both *murinum* and *glaucum* (and of course *leporinum*, if it is considered as a species) have been found in the Mediterranean region and have been cited as originating there. A careful study may reveal some hybrids or it is possible of course that as soon as more investigations have been made in the diploid strains (species?) of *H. glaucum* some plants may be found with a different chromosome karyotype. If this proves to be true we might be further on the road to an answer about the origin of *H. murinum*. However, if another species has combined with *glaucum* to produce *murinum* the second species has contributed little or nothing to the genotype and the resulting phenotype of the plant and it may be difficult to discover it without an extensive crossing program.

Summary and conclusions

Ninety-eight samples of *H. murinum* were gathered from various districts in Holland. They all had 2n = 28 chromosomes.

The morphological characteristics of these plants were examined in details to make certain that the plants conformed to previous descriptions of *H. murinum* because the species has been described as having 14 chromosomes. Some measure of the variability that exists in the features used taxonomically was also made, along with an assessment of the characters that can be used for differentiating species.

Meiosis was studied in detail in many plants. No quadrivalents were observed. The sizes and shapes of the chromosomes in root tip preparations were also studied. The evidence supports the conclusion that *H. murinum* is not an autotetraploid.

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