Distinguishing females of the bumble bees *Bombus ruderatus* (F.) from *Bombus hortorum* (L.) in Britain: a preliminary application of quantitative techniques

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Prepared by: Paul Williams, Luis Hernández

Paul Williams,
Biogeography and Conservation Lab
The Natural History Museum
Cromwell Road
London SW7 5BD.

Tel: 020-7942 5442
Fax: 020-7942 5229
E-mail: paw@nhm.ac.uk
WWW: http://www.nhm.ac.uk/entomology/bombus

EXECUTIVE SUMMARY

This study examined the British bumble bees *Bombus ruderatus* and *B. hortorum* to assess which characters might be used to give the best discrimination between females of the two taxa. The approach taken was to apply quantitative methods, in order to provide explicit and accountable comparisons. The first stage of the analysis examined queens to identify which of 30 morphological and pubescence characters might be most promising. The second stage then sought to assess which of the subset of 13 promising characters worked best with a larger sample of the workers, which are more difficult to distinguish. Although these characters showed statistically significant differences between taxa, many of them showed poor discriminatory power. However, a combination of two morphological characters, the breadth of the head and the length of the malar area, was found to distinguish most queens of the two taxa. This combination has not been used before, and for our sample showed an improved discrimination (90% individuals non-overlapping) compared to some of the most popular traditional characters (39% non-overlapping).

SUMMARY OF RECOMMENDATIONS

1. We recommend that a combination of morphological characters and pubescence characters is needed for separation of *B. hortorum* from *B. ruderatus*. No single character is guaranteed to separate these taxa in every case.
2. We recommend that the ratio between the breadth of the head and the length of the malar area provides the best separation for queens of *B. hortorum* from *B. ruderatus*.
3. We recommend that further study is required to examine quantitatively variation in character 24 (YHAGII), the longitudinal breadth of the band of yellow hairs on gastral segment II in dorsal view.
4. We recommend that further study is required to examine quantitatively variation among a larger sample of female specimens and variation in characters of males.
5. We recommend that further study is required to examine quantitatively variation within colonies of known parentage for comparison of within-colony, between-colony, and between-locality variation.

ACKNOWLEDGEMENTS

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CONTENTS

1. INTRODUCTION

1.1 Aim

The aim of this study was to assess some of the characters used to distinguish between the bumble bees *Bombus ruderatus* and *B. hortorum* in Britain. This is needed for the work of the UK Biodiversity Action Plan *Bumblebee Working Group*. The approach taken was to apply quantitative methods in order to provide explicit and accountable comparisons. The study was budgeted for completion within three weeks, which was sufficient for a preliminary investigation of a small sample of females (queens and workers), but not of males.

1.2 Background

Approximately 239 species of bumble bees (genus *Bombus*) are known in the world (Williams, 1998), of which 23 have been recorded in Britain since 1900. Specialists working with bumble bees use 38 subgenera to group together species that are more similar in morphology and which are likely to be more closely related. One of these subgenera, *Megabombus*, has two taxa in Britain, which have usually been regarded as separate species. The first, *B. hortorum*, remains very widespread and abundant throughout much of the British Isles and is a familiar insect of gardens, even in the inner cities. In contrast, *B. ruderatus* is restricted to central and southern Britain, where it was probably always local and present in only low numbers. Furthermore, *B. ruderatus* appears to have disappeared from many of its former sites in the last few decades (Williams, 1985, 1989; Edwards, 1999). It is important to be able to recognise *B. ruderatus* so that its current distribution and habitat requirements can be ascertained as an aid to conservation.

Since 1900, *B. ruderatus* and *B. hortorum* have been treated consistently as separate species by specialists in the taxonomy of bumble bees, both in Britain and in Europe (e.g. Skorikov, 1922; Richards, 1927; Pittioni, 1937; Tkalcu, 1960; Løken, 1973; Alford, 1975; Reinig, 1981; Rasmont, 1983; Williams, 1998). Despite this, many authors have commented on the difficulty of distinguishing all specimens of these species. Recently, it has been suggested that because of these difficulties, their status as species should be re-assessed (Edwards, 1999; Westrich, 2000).

This study makes no assumptions as to whether these nominal taxa correspond to separate species or not. For present purposes, the names *B. ruderatus* and *B. hortorum* apply to *a priori* groups of individuals, and the aim is to assess variation between these groups in comparison with variation within each group for characterising the groups.

2. METHODS

2.1 Design of the study

This study began with two *a priori* samples: groups of specimens separated and named as *B. ruderatus* and *B. hortorum* by one of the authors (PHW) using the characters listed in the Appendix. All measurements were made by the other author (LMH).

The two taxa co-occur at scattered localities across southern Britain (Alford, 1980). Ideally we would have started with samples from within colonies of known parentage, but these were unavailable. For this analysis, we assume that these populations are geographically relatively homogeneous. In other words, much of the variation would be expected to occur within large samples from any single locality, with relatively weak between-colony and between-locality trends in variation. Although specimens were included from a broad region of Britain, no analysis of geographical trends was made.

The analysis proceeded in two stages. The first was designed to identify which characters might be most promising for distinguishing the taxa; the second then sought to assess this restricted set of promising candidates using a larger and more challenging sample of specimens.

The *first stage* was to measure as broad a variety of characters as time allowed among a small sample of pale queens. Queens are large females, which generally show greater differentiation in their characteristics than workers. The colour pattern characters are visible only for 'pale' queens (individuals with discernible pale thoracic bands), so these were selected preferentially because time was limited (this assumes that morphological characters do not covary with pale/dark differences). Therefore, if any characters were going to discriminate between the taxa, this should be most easily detected among queens. Thus characters of queens were used as a 'training set', to filter potentially useful characters for further investigation.

The *second stage* was to measure the subset of potentially useful characters among as large a sample of workers as time allowed. Workers are small females, which generally show greater size variation (with commensurately greater allometric effects), and weaker differentiation in their characteristics than queens. Therefore characters are usually less distinctive among workers. The problem is that they make up the bulk of most samples and are the caste for which the characters are most urgently needed. Thus worker characters are used as a 'test set', for the assessment of characters selected within the first stage.

2.2 Material examined

Samples of specimens from southern Britain were examined from the collections of The Natural History Museum, London, and of one of the authors (PHW). These consisted of 26 queens (pale colour forms only) and 40 workers (pale and dark forms) for each of *B. ruderatus* and *B. hortorum*.

All material examined was labelled with a number to link specimens to the measurement data. There was insufficient time to record all specimen label data within this study, although this information could be recovered if required at a later date from the numbered specimens (**pale queens**: *hortorum* specimens 1-26,

NHM main coll. drawer 276; *ruderatus* specimens 1-26, NHM main coll. drawer 283; **workers**: *hortorum* specimens 1-13, PHW coll.; specimens 14-25, NHM main coll. drawer 276; specimens 26-40, NHM accessions coll. drawer 'British *hortorum*'; *ruderatus* specimens 1-18, PHW coll.; specimens 19-40, NHM main coll. drawer 284).

2.3 Characters measured

The terminology used for this report follows usage in earlier studies of bumble bees (e.g. Pekkarinen, 1979; Williams, 1991). Most attention was given to characters used previously to identify these taxa (summarised in the Appendix, page 23 of this report; Westrich, 2000). Because the ideal would be to identify useful characters that do not require potentially destructive manipulations of specimens, dissection of mouth parts and of sting bases was not used here. All measurements were taken with an ocular micrometer and calibrated to millimetres, using a Wild stereoscopic microscope (fixed magnifications).

2.3.1 Head morphology

1. HL: head length in frontal view (Figure 1)
2. HW: head breadth across eyes in frontal view (Figure 1)
3. CL: clypeal length, taken from hind line of right ocelli to clypeal margin in frontal view (Pekkarinen, 1979:7, fig. 2, Ch).
4. MALS: malar area length in lateral view (Løken, 1973:8, fig. 3, c-d)
5. MALB: malar area breadth in lateral view (Løken, 1973: 8, fig. 3, a-b)
6. OCW: distance between inner margins of compound eyes (Pekkarinen, 1973: 7, fig. 2, Oc) in frontal view
7. MANDW: distance from right mandible base to left mandible base (Pekkarinen, 1979:7, fig. 2, Cw) in frontal view
8. OR: distance from right ocellus to preoccipital ridge (Pekkarinen, 1979:7, fig. 2, Or) in dorso-frontal view
9. OE: distance from right ocellus to right compound eye (Pekkarinen, 1979:7, fig. 2, Oe) in dorso-frontal view
10. ORL: distance between right to left ocellus in dorsal view (Figure 1)
11. ASC: distance between antennal sockets in dorsal view (Figure 1)
12. ANT1: length of antennal segment I (Pekkarinen, 1979:7 fig. 2, As)
13. ANT2-4: length of antennal segments II, III and IV combined (Figure 3)

2.3.2 Thoracic morphology

14. IWIM: interalar breadth between inner margins of tegula (Løken, 1979:8, fig. 6, a) in dorsal view
15. IWOM: interalar breadth between outer margins of tegula (Pekkarinen, 1979:7, fig. 2, Te) in dorsal view
16. WL: wing length, from wing base to distal margin of radial cell in dorsal view (Figure 3)
17. RA1: distance from the veinlet in the middle of R1 cell (= first submarginal cell) to the distal end of second R1 (= radial cell) (Løken, 1979, pp:8, fig. 5, a) in dorsal view
18. RA2: distance between basal and distal margins of radial cell (Pekkarinen, 1979:7, fig. 2, Ra)
19. HT: length of hind tibia (Pekkarinen, 1979:7, fig. 2, Ti)
20. MT: length of metatarsus (Figure 3)

21. MW: breadth of metatarsus (Figure 3)

2.3.3 Pubescence length and colouration

Both *B. ruderatus* and *B. hortorum* produce dark (melanic and semi-melanic) and pale-banded individuals (reviewed by Alford, 1975; Westrich, 2000), making the identification of these taxa more difficult.

To quantify colour variation, we measured the transverse band of yellow hairs on thorax and the band of pale hairs on gastral tergum III (Appendix) in the pale forms. This pale band is difficult to measure precisely when viewed at high magnification, because: (1) the yellow hairs are mixed with dark hairs near the limits of the yellow band; (2) the hair length varies (uneven); and (3) in some specimens the pubescence is damaged as a result of manipulation during collecting and mounting.

22. WYHC: breadth of yellow hairs band on collar mesally in dorsal view, from tip of yellow hairs on anterior margin to tip of yellow hairs on posterior margin (Figure 2)
23. WYHS: breadth of yellow hairs band on scutellum mesally in dorsal view, from tip of yellow hairs on anterior margin to tip of yellow hairs on posterior margin (Figure 2)
24. YHAGII: yellow hairs on gastral segment II in dorsal view: (p) with a narrow transverse patch ('lunule') of yellow hairs (Appendix 1); (vf) with only few yellow hairs (Appendix 1); a) without yellow hairs (Figure 2)
25. WYHGIII: breadth of pale hairs band on gastral segment III, in dorsal view, from tip of pale hairs to the base of pale hairs (Figure 2)
26. YHCoSc: distance of yellow hairs band in dorsal view, from base of left posterior margin of yellow hairs band on collar to base of left anterior margin of yellow hairs band on scutellum, at level of inner margin of tegula (Figure 2)
27. LYHT: length of yellow hair band below tegula in dorsolateral view (Williams, 2000), from inner margin of tegula to the tip of yellow hairs (Figure 3)
28. WYHT: breadth of yellow hair band below tegula in dorsolateral view (Williams, 2000), from tip of yellow hairs on left margin of yellow hairs band to tip of yellow hairs band on right margin of yellow band (Figure 3)
29. HHF: length of longest hair of hind femur mesally, from base of hair to its tip (femur, ventral margin) (Figure 4)
30. HHT: length of longest hair of hind tibia mesally, from base of hair to its tip (tibia, dorsal margin) (Figure 4).

2.4 Statistical treatment

Two samples from the same population might appear different if, by chance, individuals in the samples differ in their body sizes. The effect of body size on measurements may be reduced (but not removed) by expressing the size of a character relative to body size. In some cases, the relative size of characters may also change substantially with body size, a phenomenon known as allometry. This can be investigated by plotting size ratios (shape) against a representative measure of body size. A disadvantage of this approach is that ratios compound errors in the measurements.

For bumble bees, we follow Pekkarinen (1979) in using the length of radial cell (RA2) as a representative measure of body size. To reduce the effect of variation in body size, measurements have been converted from simple distances to shape ratios, by dividing them by RA2. This was not done for other characters that were already expressed as ratios, such as MALS/MALB and OR/OE (these have been considered diagnostic for some bumble bee taxa: Pekkarinen, 1979).

We believe that the errors associated with measurements of morphological and pubescence characters may differ in size, because of the greater subjective judgement involved in measuring pubescence characters. Consequently, we have treated morphological and pubescence characters separately in the analyses.

To identify which characters give the best discrimination between the two taxa, we used Canonical Correspondence Analysis (CCA), as implemented within the CANOCO 4 software (ter Braak & Šmilauer, 1998). This is a technique for seeking canonical variates, which are linear combinations of the characters that show maximum discrimination among *a priori* groups. Here we have just two groups, the taxa *B. ruderatus* and *B. hortorum,* so the first axis should provide the best separation. We assessed the performance of each character independently of the others, using marginal effect comparisons. The results can be expressed as: (1) *lambda*-1 values, which show the relative strength of the between-group differentiation by each character when each character is considered independently (ter Braak & Šmilauer, 1998); and (2) probability values, which show whether the discrimination provided is better than expected by chance.

3. RESULTS

3.1 Results for queens (stage 1)

Absolute measurements for morphological characters and colour characters of pale queens are presented in Tables 1-4).

The results of the Canonical Correspondence Analysis (CCA) for morphological characters of queens are presented in Table 5. The marginal effect scores show that characters HW, CL, MALS, ANT2-4, MALSB and WL (all relative to RA2) give significant separation between pale queens of *B. hortorum* and *B. ruderatus*. The best separation is provided by HW/RA2 and MALS/RA2.

Table 6 shows that all of the pubescence characters give significant separation between pale queens of the two taxa, with the exception of WYHT/RA2. The length of longest hair on the hind tibia (HHT/RA2) was statistically significant in the CCA for the separation of *B. ruderatus* and *B. hortorum*, although we consider measurements of this character to be difficult and unreliable.

3.2 Results for workers (stage 2)

Using only those characters that differed significantly between the two groups of pale queens, we assessed whether they might prove useful for distinguishing the two groups of workers. Absolute values for morphological and pubescence characters of workers are shown in Tables 7-10.

Results of the CCA in Table 11 showed that none of the morphological characters gave significant separation between workers of *B. hortorum* and *B. ruderatus*. However, the CCA results in Table 12 showed that pubescence characters WYHT, WYHC, LYHT, HHT and WYHS (all relative to RA2) did provide significant separation.

4. DISCUSSION

4.1 Statistical tests

The statistical tests applied in CCA are very liberal for problems of the kind where we wish to discriminate taxa. These tests were designed originally to answer questions concerning whether two samples (here taxa) were likely to have been drawn from the same population, which is assessed by comparing the central tendencies (means or medians) between the two groups. For the taxonomic problem, on the other hand, we would prefer to ascribe *all* individuals to one or other taxon, without overlap or uncertainty. Consequently, a significant CCA result does not necessarily imply that a character will be useful for discriminating all individuals of the two taxa.

4.2 Characters for discriminating taxa

Figure 5 shows the variation in some of the higher-scoring characters from Tables 5, 6, and 12. The first plot (Figure 5a) uses two of the morphological characters with the highest *lambda* values in Table 5, the breadth of the head, in combination with the length of the malar area. This distinguishes most queens of the two taxa, with no more than 5 of 52 individuals (9.6%) lying within the region of overlap (with a minimum misclassification rate of 2/52 individuals). The relationship between these characters and body size is shown in Figure 5b (the ratio between the measurements is used to show discrimination between the two groups of bees because in Figure 5a they occupy the upper left and lower right quadrants of the graph). Figure 5b shows that the shape ratio between these characters does increase (allometrically) with body size. Furthermore, there is greater overlap for workers than for queens on the *y* axis, so this shape ratio is generally less successful for distinguishing small individuals. Nonetheless, the region of overlap is still sufficiently small that these characters may prove useful. This particular ratio has not been used before, and gives considerably better discrimination between *B. ruderatus* and *B. hortorum* queens than some of the most popular traditional morphological and colour characters (Løken, 1973; Prys-Jones & Corbet, 1987: Figure 6: 60.7% overlap in individuals between taxa).

The second row of Figure 5 shows some of the colour characters that are most successful (Table 6) in separating the taxa for queens (Figure 5c). The overlap (20/52) is greater than for the morphological characters in Figure 5a. The relationship between these shape characters and body size is shown in Figure 5d (the product of the two measurements relative to body size is used to show discrimination between the two groups of bees in this and Figures 5f,h because in Figures 5c,e,g they occupy the lower left and upper right quadrants of the graphs). However, for workers (Figure 5d) there is strong overlap in these characters (overlap on the *y* axis).

The third row of Figure 5 (e, f) shows some of the colour characters that are most successful (Table 12) in separating the taxa for workers. The extensive overlap in this example shows clearly that statistically significant differences in central tendency between groups does not necessarily result in high discriminatory power. In this particular case, these characters are both likely to be associated more with the degree of darkening of the colour pattern, rather than with any more useful differences between taxa. Although darkening of the colour pattern is more common in *B. ruderatus*, it does occur in both taxa.

The last row of Figure 5 (g, h) shows two characters that have been used before to separate the taxa. Again, these work better for queens than for workers. It is notable that, according to these results, the scutellar band shows a broader range of breadth variation in *B. ruderatus* than in *B. hortorum*. Therefore, while it may sometimes be broader than for *B. hortorum* as described in some keys (Løken, 1973; Prys-Jones & Corbet, 1987), it may also be narrower. Again, the darkening of the colour pattern in semi-melanic individuals (more frequent in *B. ruderatus*) confounds any differences between the taxa.

4.3 Discriminating and recognising taxa

Unfortunately, the characters studied here provide no simple, easy solution to discriminating *B. ruderatus* from *B. hortorum.* This is perhaps not surprising, because it supports the comments of earlier, experienced taxonomists on the difficulty of discriminating all specimens of these taxa. One value of studies such as this is that they quantify how well the various characters perform, so characters can be compared in an explicit and rigorous way.

More encouragingly, some of these characters do discriminate most of the larger specimens (Figure 5a,b). It is important to note that these particular characters (Figure 5a,b) were not used to make the initial discrimination between the groups before the analysis. Therefore they do provide additional support for the *a priori* distinction recognised between *B. ruderatus* and *B. hortorum*, irrespective of whether these taxa are easily discriminated in every case, and irrespective of whether they are interpreted as separate species or not.

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6. Tables and figures

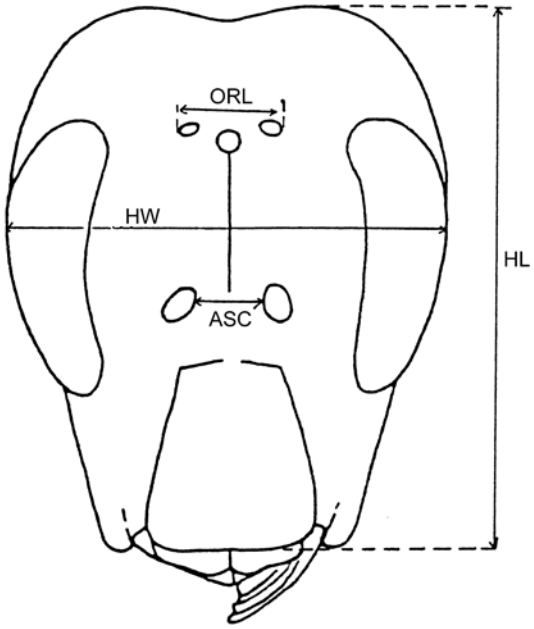

Figure 1. Frontal view of the head of a bumble bee. For character abbreviations see the text.

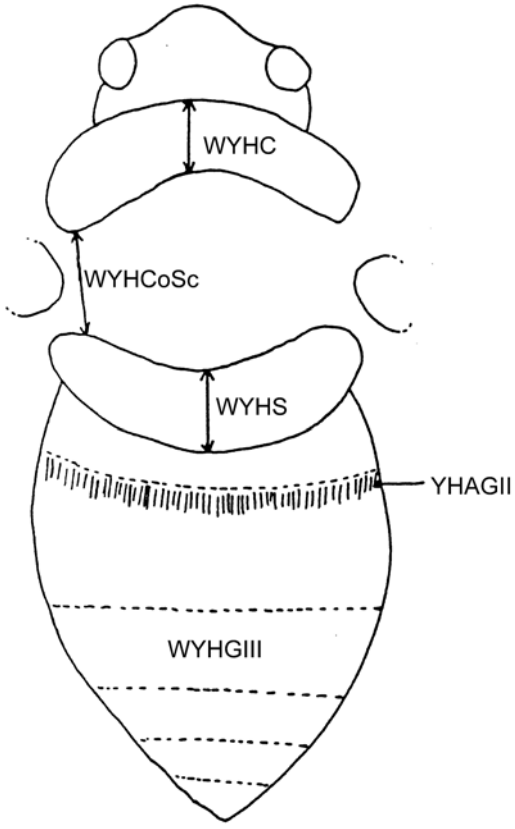

Figure 2. Dorsal view of a bumble bee showing characters of the colour pattern and pubescence examined in this study. For character abbreviations see the text.

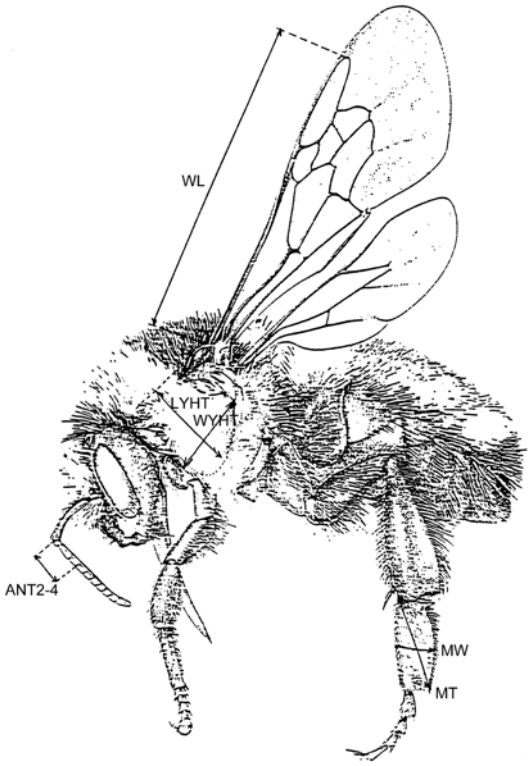

Figure 3. Lateral view of a bee (after Goulet & Huber, 1993). For character abbreviations see the text.

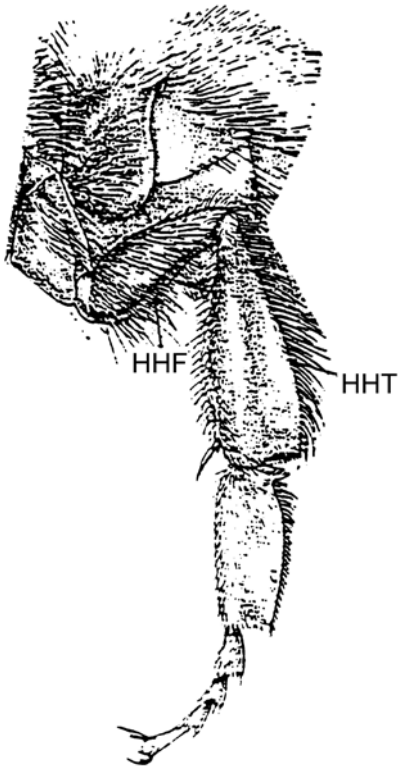

Figure 4. Lateral view of the hind leg of a bee (after Goulet & Huber, 1993). For character abbreviations see the text.

Table 1. Measurements of morphological characters of pale *B. ruderatus* queens (n = 26) in millimetres.

1 HL	*2 HW*	*3 CL*	*4 MALS*	*5 MALB*	*6 OCW*	*7 MANDW*	*8 OR*	*9 OE*	*10 ORL*
6.308	5.395	5.312	1.60	1.04	2.76	3.24	1.04	0.6	1.50
6.225	5.229	5.146	1.44	1.14	2.72	3.12	1.30	0.66	1.40
6.474	5.561	5.312	1.64	1.08	2.92	3.48	1.30	0.68	1.40
6.391	5.478	5.312	1.54	1.10	2.84	3.40	1.18	0.68	1.50
5.976	4.980	4.897	1.42	0.98	2.64	3.16	1.12	0.70	1.24
6.474	5.229	5.478	1.58	1.16	2.88	3.40	0.98	0.70	1.52
6.059	5.146	5.063	1.52	1.06	2.80	3.36	0.88	0.70	1.36
6.059	5.146	4.897	1.48	1.10	2.76	3.12	1.16	0.68	1.44
6.474	5.395	5.312	1.54	1.00	2.84	3.40	1.14	0.66	1.50
6.391	5.395	5.395	1.50	1.06	2.88	3.32	1.14	1.14	1.40
6.474	5.395	5.312	1.50	1.10	2.88	3.40	1.20	0.66	1.46
6.308	5.478	5.395	1.60	1.18	2.80	3.40	1.12	0.70	1.40
6.391	5.395	5.395	1.52	1.14	2.80	3.28	1.12	0.70	1.50
6.557	5.395	5.229	1.54	1.10	2.76	3.28	1.14	0.66	1.42
6.972	5.644	5.395	1.44	0.98	2.92	2.80	1.26	0.72	1.40
6.474	5.395	5.312	1.54	1.10	2.88	3.40	1.12	0.72	1.46
6.225	5.312	5.312	1.52	1.04	2.76	3.28	1.04	0.66	1.48
5.976	5.146	5.063	1.48	1.04	2.64	3.20	0.94	0.66	1.40
6.142	5.229	5.229	1.54	1.14	2.72	3.24	0.98	0.66	1.36
6.308	5.395	5.312	1.56	1.16	2.84	3.40	1.18	0.68	1.50
6.474	5.478	5.395	1.58	1.10	2.88	3.40	1.08	0.70	1.52
6.391	5.395	5.312	1.58	1.08	2.80	3.44	1.06	0.68	1.50
6.308	5.312	5.146	1.42	1.10	2.76	3.32	1.16	0.66	1.46
6.640	5.229	5.478	1.64	1.18	2.92	3.52	1.12	0.70	1.52
5.810	5.395	4.980	1.60	1.06	2.76	3.28	1.16	0.68	1.46
6.391	5.478	5.312	1.60	1.10	2.80	3.28	1.04	0.68	1.44

11 ASC	*12 ANT1*	*13 AN2-4*	*14 IWIM*	*15 IWOM*	*16 WL*	*17 RA1*	*18 RA2*	*19 HT*	*20 MT*	*21 MW*
0.62	2.44	1.40	6.142	8.549	14.442	5.229	4.48	6.557	4.40	1.64
0.60	2.48	1.68	6.474	8.217	14.774	5.146	4.40	5.893	4.64	1.64
0.70	2.52	1.48	7.055	9.213	10.126	5.229	4.44	6.640	4.60	1.86
0.74	2.56	1.36	6.806	9.047	14.774	5.561	4.72	6.640	4.64	1.70
0.62	2.36	1.20	6.142	7.968	13.778	4.980	4.28	6.059	4.00	1.54
0.80	2.36	1.40	7.221	8.881	14.940	5.312	4.48	6.640	4.36	1.60
0.66	2.40	1.28	6.806	8.300	13.778	5.229	4.52	6.225	3.96	1.60
0.64	2.28	1.28	6.308	8.217	13.944	5.146	4.36	6.308	4.20	1.52
0.66	2.40	1.52	6.806	8.881	14.774	4.814	4.60	6.889	4.60	1.70
0.64	2.48	1.28	6.806	8.549	14.442	5.146	4.32	6.059	4.40	1.70
0.70	2.44	1.40	7.304	8.881	14.774	5.312	4.52	6.640	4.60	1.62
0.66	2.48	1.32	7.055	9.047	15.438	5.478	4.60	6.640	4.04	1.62
0.68	2.44	1.40	7.055	8.466	14.442	5.229	4.44	6.474	4.12	1.62
0.64	2.48	1.28	7.138	8.549	14.110	4.980	4.20	6.723	4.20	1.72
0.70	2.52	1.28	6.972	8.881	14.774	5.312	4.52	6.474	4.80	1.70
0.70	2.44	1.28	6.889	8.964	14.276	5.146	4.40	6.059	4.64	1.76
0.72	2.40	1.32	6.723	8.300	14.442	5.478	4.44	6.225	4.44	1.58
0.62	2.20	1.28	6.806	8.383	14.110	4.980	4.36	6.059	4.36	1.58
0.62	2.44	1.32	6.806	8.549	13.778	4.980	4.28	6.474	4.64	1.60
0.68	2.52	1.40	6.972	8.798	13.778	5.312	4.48	6.640	4.64	1.70
0.62	2.48	1.40	7.221	9.047	14.774	5.561	4.68	6.557	4.20	1.70
0.62	2.44	1.24	6.972	8.383	15.272	5.395	4.64	6.557	4.40	1.70
0.66	2.40	1.36	6.640	8.715	14.940	5.063	4.36	6.557	4.04	1.72
0.66	2.48	1.32	7.055	8.881	14.276	5.146	4.36	6.474	4.48	1.72
0.66	2.48	1.40	6.557	8.466	15.272	5.229	4.44	6.723	4.36	1.66
0.62	2.52	1.28	7.138	8.964	15.272	5.229	4.52	6.391	4.40	1.74

Table 2. Measurements of morphological characters of *B. hortorum* queens (n = 26) in millimetres.

1 HL	2 HW	3 CL	4 MALS	5 MAlB	6 OCW	7 MANDW	8 OR	9 OE	10 ORL
5.28	4.56	4.80	1.48	1.10	2.44	3.16	0.92	0.64	1.34
5.76	4.88	4.96	1.44	0.96	2.60	3.16	0.90	0.66	1.36
5.60	4.16	4.72	1.66	1.04	2.56	3.00	1.16	0.60	1.32
5.84	4.80	4.80	1.50	1.04	2.68	3.16	0.84	0.66	1.36
5.36	4.40	4.64	1.36	0.96	2.40	2.80	0.98	0.62	1.24
5.28	4.16	4.72	1.54	1.00	2.48	2.88	0.90	0.64	1.30
5.52	4.56	5.20	1.44	0.98	2.40	2.92	0.94	0.62	1.28
5.84	4.72	4.96	1.44	1.02	2.64	3.04	0.96	0.64	1.36
5.60	4.56	4.72	1.40	1.00	2.48	2.92	0.98	0.56	1.28
5.68	4.24	4.80	1.36	1.00	2.52	2.88	0.92	0.60	1.36
6.00	4.80	5.04	1.66	1.10	2.68	3.12	0.88	0.64	1.36
5.76	4.64	5.12	1.42	1.10	2.44	3.04	0.84	0.62	1.32
5.76	4.80	5.04	1.52	1.14	2.60	2.92	0.92	0.66	1.36
5.68	4.72	4.80	1.50	1.00	2.48	2.96	1.08	0.56	1.38
5.60	4.64	4.80	1.44	1.04	2.48	2.84	0.90	0.62	1.30
5.68	4.64	4.96	1.50	1.12	2.44	3.16	1.04	0.60	1.20
5.76	4.88	4.72	1.58	0.84	2.52	2.60	1.10	0.60	1.40
5.76	4.72	4.88	1.46	1.02	2.52	3.00	1.04	0.62	1.32
6.00	4.72	5.12	1.56	1.00	2.64	2.92	1.04	0.64	1.44
6.24	4.88	5.12	1.52	1.04	2.60	3.00	1.12	0.60	1.36
5.84	4.72	5.04	1.42	0.90	2.52	2.96	1.00	0.62	1.32
5.20	4.72	4.80	1.56	1.02	2.64	3.00	1.04	0.58	1.38
5.76	4.72	4.88	1.52	1.02	2.48	2.84	1.00	0.64	1.34
5.60	4.64	4.64	1.40	0.86	2.52	2.96	0.88	0.66	1.30
5.60	4.48	4.80	1.36	1.04	2.48	3.00	0.90	0.64	1.30
5.84	4.72	4.96	1.58	1.08	2.48	3.00	0.96	0.64	1.32

11 ASC	12 ANT1	13 AN2-4	14 IWIM	15 IWOM	16 WL	17 RA1	18 RA2	19 HT	20 MT	21 MW
0.62	2.16	1.28	6.225	7.885	13.944	4.897	4.16	5.976	3.60	1.60
0.64	2.08	1.28	6.308	7.885	13.778	4.814	4.08	6.225	4.00	1.44
0.62	2.36	1.32	6.225	7.802	13.944	4.980	4.32	5.727	3.92	1.60
0.62	2.24	1.36	6.225	7.885	13.778	4.316	4.08	5.810	3.96	1.62
0.60	2.08	1.20	5.561	7.387	12.616	4.316	3.72	5.727	3.56	1.36
0.62	2.24	1.32	5.976	7.968	13.446	4.814	4.08	6.225	3.64	1.50
0.52	2.08	1.28	6.308	7.636	12.948	4.565	4.00	5.478	3.80	1.30
0.60	2.24	1.40	6.225	8.134	13.944	4.980	4.20	6.142	3.68	1.40
0.62	2.16	1.24	5.810	7.387	12.782	4.482	3.92	5.810	3.80	1.50
0.54	2.04	1.20	6.225	7.470	13.280	4.648	3.96	5.561	3.88	1.48
0.62	2.20	1.32	6.308	8.134	15.438	4.980	4.20	6.225	3.68	1.56
0.58	2.24	1.32	6.225	7.719	12.948	4.399	4.00	5.395	3.96	1.50
0.62	2.24	1.40	6.059	7.636	13.446	5.063	4.08	5.976	4.12	1.48
0.60	2.20	1.24	5.976	7.719	12.948	4.731	3.92	5.810	3.84	1.52
0.60	2.16	1.24	6.059	7.553	12.782	4.316	3.76	5.727	4.08	1.56
0.64	2.20	1.36	6.142	7.968	13.280	4.814	3.96	5.727	3.92	1.50
0.64	2.24	1.40	5.976	7.968	13.944	5.063	4.24	6.474	3.80	1.56
0.64	2.32	1.36	6.474	8.051	13.778	5.063	4.28	6.225	4.00	1.56
0.62	2.28	1.28	6.806	8.466	14.774	5.063	4.24	6.391	3.88	1.70
0.64	2.40	1.36	6.806	8.383	14.608	5.146	4.40	6.474	4.00	1.54
0.52	2.32	1.32	6.640	8.134	13.778	4.731	4.08	6.059	4.00	1.52
0.58	2.12	1.32	6.225	8.051	13.280	4.731	4.04	5.810	3.80	1.58
0.66	2.24	1.32	6.391	8.051	13.778	4.648	4.08	5.976	3.84	1.54
0.66	2.28	1.28	5.976	7.470	13.778	4.897	4.08	5.478	3.96	1.56
0.60	2.20	1.32	6.640	7.553	13.446	4.731	4.08	5.893	3.68	1.46
0.66	2.04	1.24	6.225	7.636	13.446	4.565	3.84	5.644	4.08	1.56

Table 3. Measurements of pubescence characters of pale *B. ruderatus* queens (n = 26) in millimetres.

22 WYHC	*23 WYHS*	*24 YHAGII*	*25 WYHGIII*	*26 YHCoSc*	*27 LYHT*	*28 WYHT*	*29 HHF*	*30 HHT*
1.76	3.16	a	1.44	3.56	2.60	2.12	0.84	1.00
2.60	3.40	a	1.28	3.64	2.56	2.44	1.64	1.02
2.60	3.40	a	1.20	4.00	2.20	2.80	1.80	2.12
3.44	2.80	a	1.28	3.80	2.40	1.92	2.26	1.82
1.44	2.28	a	1.20	3.44	2.04	1.60	1.80	1.72
2.08	2.04	a	1.48	4.00	2.36	2.24	1.88	2.26
1.96	2.64	a	1.00	3.96	2.24	2.40	2.06	2.20
2.08	2.44	a	1.20	4.20	2.40	1.80	1.90	2.20
1.60	2.52	a	0.84	4.00	2.24	1.92	2.10	2.10
2.20	1.96	a	1.40	4.00	2.20	2.20	1.70	1.94
1.52	1.56	a	1.16	4.12	2.00	1.80	1.44	2.38
1.00	2.24	a	1.20	4.68	1.80	1.92	2.28	1.34
1.68	2.44	a	1.16	3.60	3.00	2.00	1.96	2.00
2.12	2.00	a	1.32	4.08	2.20	2.48	2.12	2.00
3.00	2.44	a	1.36	4.00	3.00	2.68	2.28	2.12
1.60	2.84	a	1.24	3.64	2.88	2.44	2.60	2.40
2.56	1.84	a	1.24	4.08	2.24	2.48	2.32	2.36
2.60	2.76	a	1.32	3.80	2.88	1.80	2.00	1.76
1.96	1.56	a	1.16	3.96	2.36	1.80	2.24	2.28
2.00	2.40	a	1.28	4.68	2.52	2.00	2.36	2.16
2.88	2.80	a	1.40	4.48	2.40	2.20	1.60	2.12
2.28	2.88	a	1.44	4.72	2.60	2.24	2.52	2.40
2.20	1.44	a	0.76	4.40	3.16	2.24	2.04	2.40
1.96	2.20	a	1.16	4.52	2.68	2.00	2.24	2.00
3.24	3.44	a	1.16	3.96	3.00	2.68	2.28	1.64
2.44	3.00	a	1.28	4.20	2.48	2.44	1.80	2.00

Table 4. Measurements of pubescence characters of *B. hortorum* queens (n = 26) in millimetres.

22 WYHC	*23 WYHS*	*24 YHAGII*	*25 WYGIII*	*26 YHCoSc*	*27 LYHT*	*28 WYHT*	*29 HHF*	*30 HHT*
2.40	3.40	p	1.68	3.40	3.12	1.96	2.68	1.96
3.36	3.28	p	1.28	3.16	2.80	2.36	1.64	1.80
2.80	2.40	p	1.52	3.16	3.00	1.92	2.00	2.40
3.16	3.00	p	1.40	3.40	2.20	2.24	1.80	2.00
1.80	3.00	p	1.40	3.28	2.60	1.80	1.76	2.00
2.72	2.20	p	1.60	3.12	2.72	1.60	2.04	1.84
2.00	1.56	p	1.52	3.40	2.76	1.80	2.24	1.72
2.88	2.76	p	1.40	4.40	2.68	2.24	1.80	2.00
3.80	3.68	p	1.32	3.00	3.32	2.04	1.84	1.96
3.56	3.20	p	0.80	3.40	3.20	2.28	1.72	1.72
2.60	1.96	p	1.28	3.60	2.80	2.16	1.76	2.16
2.96	2.72	p	1.40	3.20	3.40	2.00	2.20	2.32
2.16	2.72	p	1.56	3.28	3.76	2.12	2.12	2.36
3.56	3.52	p	1.60	3.00	3.00	1.72	2.48	2.32
2.60	2.44	p	1.80	3.12	2.84	2.28	1.68	2.28
2.32	2.76	p	1.48	3.16	3.00	1.60	1.48	1.64
3.20	2.80	p	1.40	3.20	2.56	1.96	1.80	2.04
2.40	2.24	p	1.32	3.68	3.20	2.04	1.80	1.96
4.40	3.68	p	1.56	3.12	3.64	2.60	2.60	1.88
3.52	3.20	p	1.52	3.52	2.92	2.92	2.08	2.00
4.00	3.40	p	1.60	3.00	2.48	2.68	2.00	1.92
2.56	2.80	p	1.40	3.16	2.88	2.76	1.80	2.24
2.80	2.12	p	1.68	2.88	2.48	1.72	1.96	2.04
3.20	3.44	p	1.20	3.28	2.80	2.92	2.52	2.40
3.40	2.60	p	2.00	3.20	2.20	2.20	1.88	2.04
2.24	2.20	p	1.28	3.76	2.64	2.60	2.00	1.68

Table 5. CCA results for marginal effects of all morphological characters (relative to RA2) of pale queens, *B. ruderatus* and *B. hortorum*. For character name abbreviations see the character list. *Lambda* represents the eigenvalue for each character independently. The contribution of each character is assessed using a Monte Carlo test, which provides the test statistic (*F*) and a probability estimate (*P*).

Character number	**Character**	***Lambda***	***F***	***P***
2/18	HW/RA2	0.29	20.12	0.005
4/18	MALS/RA2	0.27	18.94	0.005
13/18	ANT2-4/RA2	0.20	12.79	0.005
16/18	WL/RA2	0.12	6.73	0.001
20/18	MT/RA2	0.09	5.19	0.02
4/5	MALS/MALB	0.07	3.96	0.04
3/18	CL/RA2	0.05	2.72	0.11
8/9	OR/OE	0.04	2.21	0.13
6/18	OCW/RA2	0.04	2.13	0.13
1/18	HL/RA2	0.04	2.00	0.18
5/18	MALB/RA2	0.02	1.12	0.30
7/18	MANDW/RA2	0.02	1.21	0.24
12/18	ANT1/RA2	0.02	1.19	0.24
10/18	ORL/RA2	0.02	0.78	0.42
15/18	IWOM/RA2	0.02	0.76	0.40
21/18	MW/RA2	0.01	0.61	0.51
11/18	ASC/RA2	0.01	0.31	0.66
19/18	HT/RA2	0.01	0.28	0.57
14/18	IWIM/RA2	0.00	0.04	0.88

Table 6. CCA results for marginal effects of all pubescence characters (relative to RA2) of pale queens, *B. ruderatus* and *B. hortorum*. For character name abbreviations see the character list. *Lambda* represents the eigenvalue for each character independently. The contribution of each character is assessed using a Monte Carlo test, which provides the test statistic (*F*) and a probability estimate (*P*).

Character number	**Character**	***Lambda***	***F***	***P***
30/18	HHT/RA2	0.64	89.16	0.005
25/18	WYHGIII/RA2	0.44	38.57	0.005
27/18	LYHT/RA2	0.44	38.61	0.005
22/18	WYHC/RA2	0.42	35.57	0.005
26/18	YHCoSc/RA2	0.31	22.46	0.005
23/18	WYHS/RA2	0.21	13.61	0.005
29/18	HHF/RA2	0.20	12.30	0.005
28/18	WYHT/RA2	0.07	3.80	0.07

Table 7. Measurements of morphological characters of *B. ruderatus* workers (n = 40) in millimetres.

2 HW	*4 MALS*	*5 MAlB*	*13 ANT2-4*	*16 WL*	*18 RA2*
4.32	1.18	0.94	1.00	11.79	3.24
4.32	1.08	0.78	1.20	11.62	3.52
3.90	1.10	0.78	1.00	10.29	2.84
3.90	1.02	0.82	0.84	9.79	2.84
4.57	1.24	0.82	1.12	10.13	3.44
3.98	1.04	0.74	1.12	9.79	2.88
4.15	1.20	0.82	1.16	11.29	3.24
3.65	0.94	0.80	1.12	9.13	2.60
3.82	1.06	0.86	0.96	9.79	2.68
4.32	1.16	0.84	1.24	11.62	3.40
3.74	1.08	0.82	0.96	9.30	2.84
3.57	0.98	0.74	1.00	9.13	2.52
4.23	1.14	0.88	1.12	11.45	3.32
3.98	1.04	0.70	1.00	9.79	2.88
4.07	1.06	0.78	1.12	11.12	3.04
4.32	1.22	0.84	1.16	10.79	3.08
3.74	0.96	0.76	1.04	9.30	2.72
3.74	1.00	0.80	1.12	9.96	2.80
4.23	1.06	0.80	1.12	10.62	3.24
4.40	1.26	0.82	1.16	11.45	3.40
3.57	1.00	0.74	1.04	10.29	2.92
4.23	1.22	0.68	1.04	11.45	3.28
4.57	1.32	0.94	1.20	11.79	3.32
4.65	1.34	0.86	1.16	13.11	3.64
4.32	1.20	0.86	1.08	10.62	3.32
4.32	1.22	0.86	1.20	11.45	3.44
3.98	1.10	0.80	1.00	9.96	3.00
3.98	1.04	0.80	1.16	10.46	3.20
4.23	1.16	0.90	1.04	10.62	3.24
4.23	1.06	0.86	1.16	10.46	3.24
3.65	0.96	0.70	1.00	8.80	2.52
3.98	1.06	0.72	1.04	9.79	3.00
4.23	1.20	0.80	1.12	10.96	3.32
4.15	1.14	0.70	0.96	10.96	3.28
4.15	1.10	0.82	1.12	9.96	2.96
4.73	1.32	0.98	1.24	12.78	3.76
4.07	1.16	0.76	1.16	10.46	3.24
4.32	1.24	0.86	1.24	10.79	3.36
4.65	1.26	0.86	1.16	11.79	3.64
4.48	1.16	0.86	1.16	11.79	3.56

Table 8. Measurements of morphological characters of *B. hortorum* workers (n = 40) in millimetres.

2 HW	*4 MALS*	*5 MAlB*	*13 ANT2-4*	*16 WL*	*18 RA2*
4.233	1.24	0.84	1.00	10.956	3.32
4.316	1.32	0.88	1.24	11.620	3.52
3.984	1.16	0.84	1.24	10.956	3.24
3.486	1.00	0.72	1.04	9.960	2.92
4.150	1.24	0.92	1.24	11.786	3.52
4.233	1.24	0.84	1.28	11.786	3.52
4.233	1.22	0.80	0.96	11.786	3.52
4.233	1.26	0.94	1.08	11.786	3.48
3.984	1.06	0.80	1.08	10.292	3.16
3.818	1.12	0.82	1.12	10.458	3.12
4.316	1.24	0.90	1.28	11.952	3.56
4.067	1.26	0.80	1.12	11.454	3.20
4.150	1.28	0.84	1.24	11.454	3.36
3.569	0.98	0.72	1.00	8.798	2.52
3.569	0.88	0.68	0.80	10.292	2.72
3.901	1.04	0.74	1.16	10.292	3.08
3.735	1.04	0.70	0.92	9.628	2.84
3.735	1.06	0.76	1.04	9.794	2.92
3.652	1.02	0.70	1.00	8.964	2.76
3.735	0.96	0.72	1.00	10.126	3.04
3.569	1.00	0.72	1.00	9.462	2.80
3.569	0.92	0.70	1.00	8.632	2.68
3.320	0.96	0.64	0.92	7.968	2.44
3.237	0.56	0.52	0.8	7.470	2.00
3.486	0.60	0.66	0.84	8.134	2.32
3.652	1.00	0.72	0.92	10.126	2.84
3.403	0.92	0.70	0.92	8.798	2.72
3.569	0.98	0.72	1.00	9.794	2.80
3.403	0.84	0.64	0.84	8.632	2.44
4.316	1.28	0.84	0.92	11.122	3.40
4.067	1.24	0.88	1.16	11.122	3.52
3.486	0.92	0.76	0.88	8.798	2.60
3.735	1.04	0.78	0.92	10.126	3.04
3.237	0.80	0.64	0.88	7.802	2.28
3.818	1.20	0.82	1.04	9.628	2.84
3.818	1.10	0.76	1.08	10.458	3.12
3.818	1.10	0.76	1.04	10.126	3.00
3.320	0.84	0.64	0.88	7.802	2.08
3.652	1.08	0.76	1.08	9.628	2.76
3.320	0.96	0.66	0.92	8.300	2.36

Table 9. Measurements of pubescence characters of *B. ruderatus* workers (n = 40) in millimetres.

22 WYHC	*23 WYHS*	*25 WYHGIII*	*26 YHCoSc*	*27 LYHT*	*29 HHF*	*30 HHT*
1.80	1.92	1.00	3.48	1.84	1.44	1.24
1.92	2.00	0.80	2.80	1.96	1.64	1.68
2.36	2.24	0	2.76	2.00	1.56	1.04
2.00	2.08	0.88	2.56	1.84	1.20	1.40
1.68	2.04	0.72	2.96	2.36	1.92	1.28
1.40	1.56	0	3.00	1.44	1.40	1.52
1.92	1.96	0.48	1.80	1.56	1.08	1.68
1.80	1.56	0.52	2.40	2.00	1.28	1.32
2.00	1.88	0.60	2.60	1.60	1.20	1.20
2.04	2.00	0.92	2.72	2.20	1.64	1.64
0	0	0	0	0	1.28	1.32
2.00	1.44	0.60	2.12	1.44	0.92	1.32
0	0	0	0	0	0.92	1.32
0	0	0	0	0	1.28	1.40
1.40	2.36	0.64	2.72	1.64	1.32	1.20
1.68	1.44	0.60	2.72	2.12	1.44	1.00
0	0	0	0	0	1.04	1.16
0	0	0	0	0	0.80	1.60
0	0	0	0	0	1.32	1.44
0	0	0	0	0	1.20	1.72
0	0	0	0	0	1.44	1.24
0	0	0	0	0	1.68	1.44
1.20	1.80	0	3.00	2.40	1.40	1.52
2.08	2.00	0	3.60	2.36	1.40	1.36
1.12	1.40	0	2.88	1.60	1.28	1.16
0	0	0	0	0	1.24	1.56
0	0	0	0	0	1.40	1.48
0	0	0	0	0	1.56	1.32
1.12	0.80	0.40	3.20	1.20	1.16	1.60
1.00	1.12	0.48	3.20	1.64	1.28	1.52
0.60	1.16	0.40	2.40	1.40	1.52	1.36
1.04	1.36	0.44	2.52	1.60	1.32	1.36
1.24	1.32	0	3.24	1.60	1.04	1.32
1.00	0.96	0	2.84	1.96	1.40	1.44
1.56	2.00	0	3.20	1.64	0.80	1.72
1.40	1.40	0	3.56	1.40	1.76	2.04
1.68	1.60	0	3.28	2.00	1.16	1.80
1.40	1.52	0	3.00	2.00	1.68	1.48
1.00	2.00	0	3.56	2.00	1.36	1.84
0	0	0	0	0	1.20	2.00

Table 10. Measurements of pubescence characters of *B. hortorum* workers (n = 40) in millimetres.

22 YHC	*23 WYHS*	*25 WYHGIII*	*26 YHCoSc*	*27 LYHT*	*29 HHF*	*30 HHT*
1.56	2.00	0.64	2.48	2.52	1.48	1.80
2.24	1.80	0.80	2.76	2.20	1.32	1.76
1.24	1.20	0	3.04	2.36	1.04	1.64
2.40	2.00	0	2.60	1.60	1.20	1.56
2.36	2.40	0.80	2.76	2.00	1.04	2.16
1.16	1.60	0.40	3.24	2.00	1.40	1.64
2.08	1.80	0.84	3.04	2.12	1.40	1.80
2.28	1.80	1.08	2.80	2.56	1.40	1.60
1.52	1.12	0.80	2.56	1.96	1.12	1.44
1.16	1.24	0.80	2.80	1.68	1.12	1.28
2.48	1.84	0.52	1.60	2.44	1.00	1.40
0	0	0	0	0	1.24	1.24
2.20	2.00	1.00	2.84	2.24	1.16	1.96
2.32	1.60	0	2.60	1.60	0.80	1.40
2.68	1.80	0.68	2.40	2.08	2.44	1.64
2.20	1.60	0.40	2.36	2.08	1.60	1.60
2.08	1.00	0	2.72	1.76	1.48	1.60
1.00	1.52	0	2.60	1.12	1.32	1.68
1.64	1.40	0	2.76	1.00	1.40	1.28
2.00	1.72	0.60	2.60	1.80	1.04	1.24
1.80	1.40	0	2.08	2.20	1.48	1.60
2.20	1.28	0	2.44	1.88	0.96	1.52
1.80	0.92	0	2.20	1.72	1.20	1.28
0.88	1.00	0	2.04	1.80	1.12	1.28
1.44	1.40	0	2.08	1.44	1.20	1.48
1.96	1.40	0	2.60	1.96	1.24	1.32
1.28	1.56	0	2.20	1.72	1.40	1.60
2.36	1.16	0	2.44	1.80	0.96	1.44
1.68	1.40	0	2.40	1.60	0.88	1.28
1.64	1.44	0	1.28	2.00	1.80	1.88
1.84	1.32	0	2.48	2.20	1.20	1.80
1.52	1.12	0	2.08	1.64	1.00	1.28
1.40	1.48	0	2.40	2.04	1.56	1.24
1.56	1.16	0	2.12	1.28	0.92	1.56
2.04	1.84	0	2.48	1.88	1.44	1.52
1.40	1.76	0	2.52	2.04	1.40	1.56
2.24	1.60	0	2.40	2.36	1.28	1.76
0.84	1.24	0	2.04	1.44	1.24	1.56
1.80	1.96	0	2.40	1.76	1.20	1.44
1.72	1.80	0	2.20	1.48	1.28	1.12

Table 11. CCA results for marginal effects of selected morphological characters (relative to RA2) of workers, *B. ruderatus* and *B. hortorum*. For character name abbreviations see the character list. *Lambda* represents the eigenvalue for each character independently. The contribution of each character is assessed using a Monte Carlo test, which provides the test statistic (*F*) and a probability estimate (*P*).

Character number	**Character**	***Lambda***	***F***	***P***
2/18	HW/RA2	0.02	1.36	0.26
4/18	MALS/RA2	0.00	0.27	0.62
4/5	MALS/MALB	0.00	0.18	0.64
16/18	WL/RA2	0.00	0.03	0.87
13/18	ANT2-4/RA2	0.00	0.01	0.93

Table 12. CCA results for marginal effects of selected pubescence characters (relative to RA2) of workers, *B. ruderatus* and *B. hortorum*. For character name abbreviations see the character list. *Lambda* represents the eigenvalue for each character independently. The contribution of each character is assessed using a Monte Carlo test, which provides the test statistic (*F*) and a probability estimate (*P*).

Character number	**Character**	***Lambda***	***F***	***P***
22/18	WYHC/RA2	0.26	26.79	0.005
27/18	LYHT/RA2	0.23	22.84	0.005
30/18	HHT/RA2	0.16	14.37	0.005
23/18	WYHS/RA2	0.11	10.01	0.005
26/18	YHCoSc/RA2	0.09	7.34	0.01
29/18	HHF/RA2	0.00	0.33	0.56
25/18	WYHGIII/RA2	0.00	0.07	0.78

Figure 5 (next page). Scatterplots showing (left) variation in shape, relative to RA2 as a simple adjustment to compensate in part for variation in size, and (right) allometry, variation in shape with size, for selected high-scoring characters from Tables 4, 5, and 12. Figures c-h are for pale females only. The lines on the scatterplots are minimum convex polygons to include all data points for the two taxa separately.

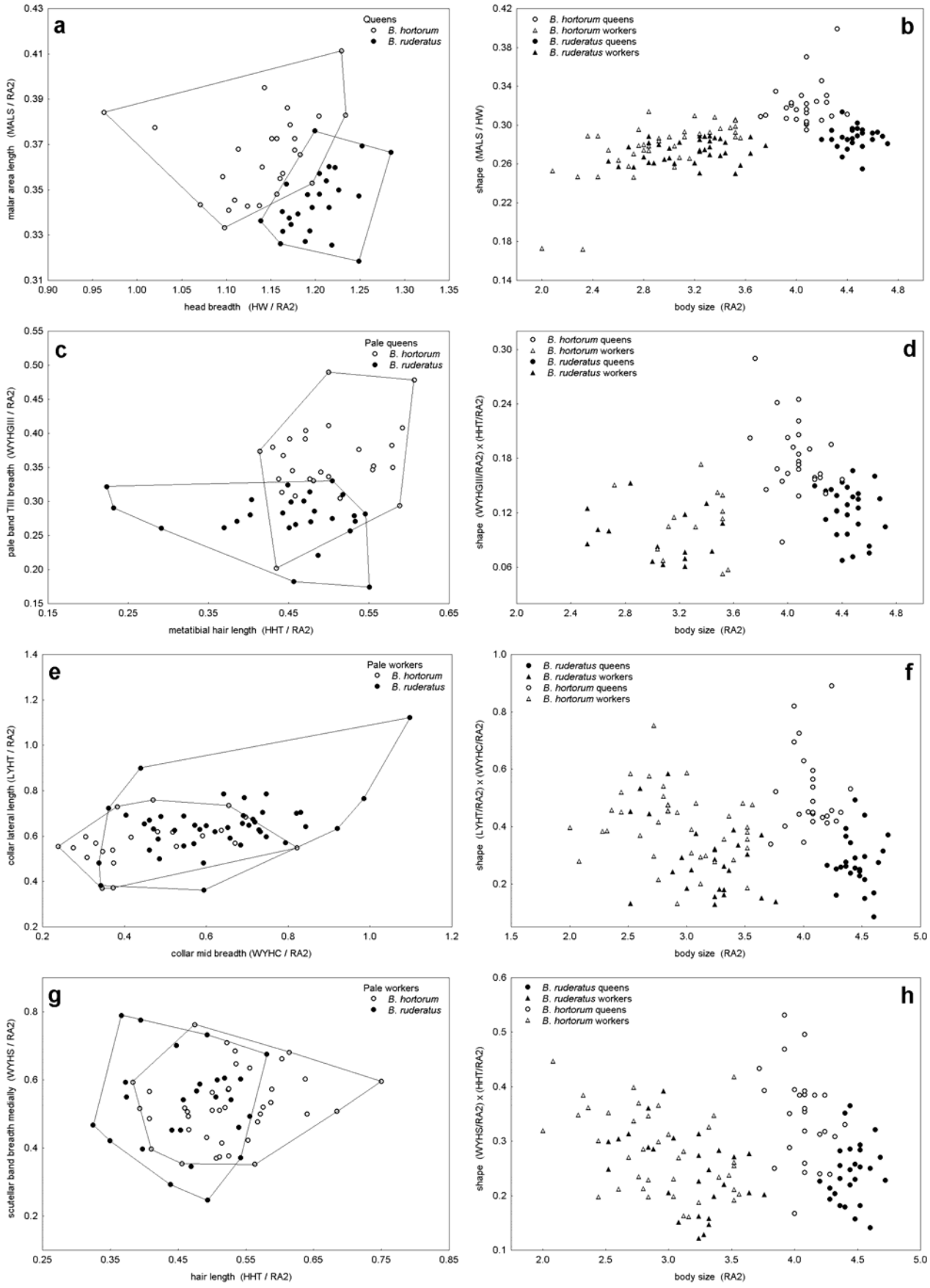
a
Queens
B. hortorum
B. ruderatus
malar area length (MALS / RA2)
head breadth (HW / RA2)
b
B. hortorum queens
B. hortorum workers
B. ruderatus queens
B. ruderatus workers
shape (MALS / HW)
body size (RA2)
c
Pale queens
B. hortorum
B. ruderatus
pale band TIII breadth (WYHGIII / RA2)
metatibial hair length (HHT / RA2)
d
B. hortorum queens
B. hortorum workers
B. ruderatus queens
B. ruderatus workers
shape (WYHGIII/RA2) x (HHT/RA2)
body size (RA2)
e
Pale workers
B. hortorum
B. ruderatus
collar lateral length (LYHT / RA2)
collar mid breadth (WYHC / RA2)
f
B. ruderatus queens
B. ruderatus workers
B. hortorum queens
B. hortorum workers
shape (LYHT/RA2) x (WYHC/RA2)
body size (RA2)
g
Pale workers
B. hortorum
B. ruderatus
scutellar band breadth medially (WYHS / RA2)
hair length (HHT / RA2)
h
B. ruderatus queens
B. ruderatus workers
B. hortorum queens
B. hortorum workers
shape (WYHS/RA2) x (HHT/RA2)
body size (RA2)

Figure 5.

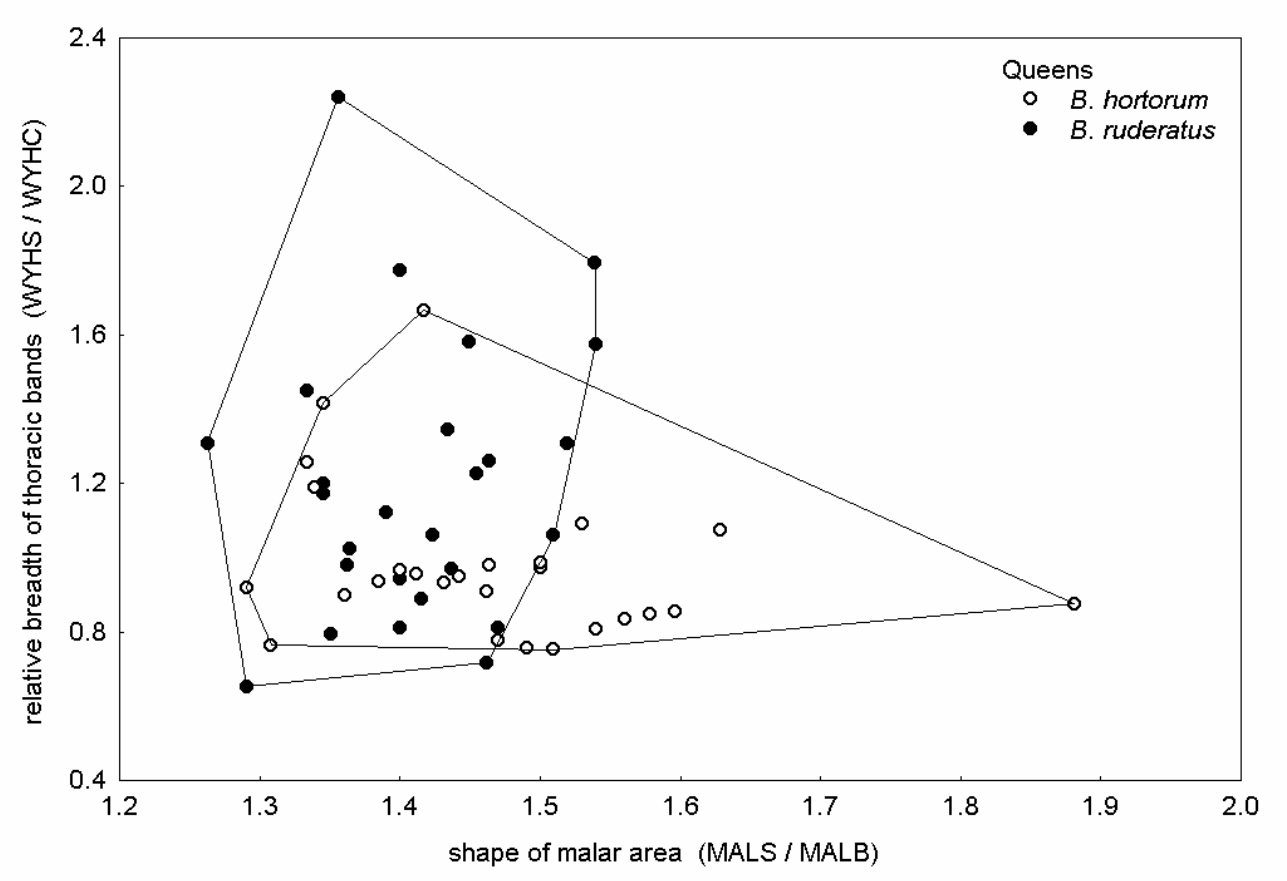

Figure 6. Scatterplot showing some of the most popular, traditional characters for separating *B. ruderatus* and *B. hortorum*. The lines on the scatterplot are minimum convex polygons to include all data points for the two taxa separately.

7. Appendix

This table was prepared by PHW and distributed to the UK Biodiversity Action Plan *Bumblebee Working Group* in December 1998 as a summary of some of the characters used to separate *B. hortorum* and *B. ruderatus*. (females: queens and workers).

	Bombus hortorum	***Bombus ruderatus***
	Female morphology *(NB all characters clearer in larger individuals)*	
1	**Centre of clypeus evenly curved and shining, with only a few scattered medium and fine punctures**	**Centre of clypeus uneven with patchy reflections, with scattered large, medium and many small punctures**
2	Ratio of oculo-malar distance to basal breadth of mandible said to be >1.5 (yet to be measured for a representative sample)	Ratio of oculo-malar distance to basal breadth of mandible approximately 1.5 (yet to be measured for a representative sample)
3	Pubescence long and uneven	Pubescence short and even
	Female colour pattern of pubescence *(NB melanics and semi-melanics occur in both species)*	
1	Band of yellow hairs on scutellum medially narrower, and often much narrower, than yellow band of anterior thoracic collar	Band of yellow hairs on scutellum medially nearly equal in breadth to yellow band of anterior thoracic collar
2	Anterior of gastral tergum 2 usually with a narrow transverse patch ('lunule') of yellow hairs	Anterior of gastral tergum 2 usually with only a very few yellow hairs
3	**Gastral tergum 3 with pale hairs laterally extending about half of the distance from posterior to anterior margins**	**Gastral tergum 3 with pale hairs laterally extending the entire distance from posterior to anterior margins** (often for a breadth nearly equal to the length of the tergum, albeit mixed with black hairs)

Notes.
This list is not exhaustive. Hopefully it includes some of the characters that may be easiest to use.
No single character is guaranteed to work in every case.