A morphological and experimental investigation of the *Pulvinaria vitis* complex in Europe

by

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Abstract

Multivariate analysis and host transfer experiments were used to demonstrate that the complex of nominal species associated with Pulvinaria vitis (L.) consists of a single biological species in Britain. Confusion in the literature has resulted from uncertainty as to the number of species present and the description of nominal species according to their host plant. The morphological characters used in the taxonomy of the complex were found to show considerable plasticity varying with host and deme.

Principal components analysis of morphological data obtained from field-collected specimens grouped individuals according to their host plant. These groupings were confirmed with canonical variates analysis. Host transfer experiments were used to determine whether these groupings were caused by genetic or environmental factors.

Specimens from a single population on the same host-plant species were reared on a variety of hosts. Analysis of data from the resulting adults formed groups according to the host plant on which they were reared. In addition, specimens from separate populations on different host-plant species were reared on a single host species. Analysis of the data from the resulting adults formed groups according to their original parental population. The enumerate characters responsible for the grouping in each case were identified. The structure and function of some of the wax-producing structures that were influenced by host-plant species were investigated by scanning electron microscopy.

Gel electrophoresis produced results with 4 enzyme systems, each of which showed inter-population variation, but relatively little intra-population variation.

Methods of reproduction were investigated. P. vitis is a biologically plastic species using three chromosome systems; deuterotoky, thelytoky and diploid arrhenotoky. The sexual function of adult males is uncertain. The gross morphology of sperm bundle, endosymbionts and karyotype (2n = 16) were examined.

P. vitis is redescribed and illustrated and the considerable morphological, ecological, cytogenetic and enzyme variability discussed.

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Pulvinaria vitis (L.) adult females with ovisacs on Ribes nigrum (above) and on Vitis vinifera (below)(Photographs: RHS, Wisley)

1 Introduction

1.1 Scale insects

Scale insects are plant sap-feeding bugs related to aphids, whiteflies and psyllids. They attack all parts of the plant including roots, stems, leaves, buds and fruit. Many are notorious pests of agricultural crops, especially in warmer climates, and particularly on forestry, fruit and nut trees, woody ornamentals and indoor plants. Agricultural losses and extra production costs attributed to scale insects have been estimated to exceed $500 million annually in the U.S.A. (Kosztarab, 1977) and $5 billion annually worldwide (Kosztarab & Kozár, 1988). The economic importance of the group, however, is probably considerably underestimated.

Female and immature scale insects look very different from most insects as they are wingless, scale- or wart-like, and their legs are hidden beneath their bodies. The winged males look more like typical insects, but are very small, short lived and rarely noticed.

1.2 Woolly scales in Britain

Scale insects are widely distributed and common in Britain, living on many kinds of plants, but are one of the most overlooked and neglected group of animals; perhaps because most are small, sessile, well-camouflaged and thus not easily noticed. Others are quite conspicuous because their colour or the white, woolly ovisac produced by the females contrasts with the host plant. There are 89 indigenous and 92 exotic species of scale insect recorded in Britain belonging to 10 families; 48 species belong to the family Coccidae or 'soft scales'. Some of the exotic species have become naturalised and wide spread outdoors, but most have only rarely been recorded and only on imported plants or plant produce. The numbers of indigenous and exotic species in each scale-insect family recorded in Britain, are listed in Appendix 1.1. The British Coccidae are listed, together with keys for their identification, in Appendix 1.2.

Woolly scales, referred to as cottony scales in the U.S.A., belong to the soft-scale genera Pulvinaria, Neopulvinaria and others, all in the tribe Pulvinariini. The genus Pulvinaria was subdivided into several genera by Borchsenius (1956, 1957, 1958), but these generic concepts are not universally accepted. The reasons why they are not accepted by some

coccidologists are discussed in Section 2.3. The number of genera recognised in the Pulvinariini therefore differs between authors. Adult females of the genus Pulvinaria can usually be distinguished in the field in Britain by a characteristic elongate, white, waxy ovisac produced under the body. This ovisac pushes the female's body forwards and upwards, so that she rests at an angle to the surface of the host.

The commonest woolly scale in Britain is the horse-chestnut scale, P. regalis Canard. This was first recorded in Britain in 1964 and since then has become widespread throughout southern England and Wales, mainly on limes (Tilia spp.), sycamore (Acer pseudoplatanus), maples (Acer spp.), horse-chestnut (Aesculus hippocastanum), elms (Ulmus spp.), sweet bay (Laurus nobilis), Skimmia spp. and Cornus spp. It is polyphagous and has been recorded from well-over 100 species of plant. It is not known from which geographical area of the world P. regalis originates. In Britain, it usually attacks urban trees and it was suggested that such trees are more susceptible to infestation due to being stressed by pollution, lack of water and by being cramped by pavements and buildings (Speight & Matthew, 1984). I have personally observed, however, enormous populations of P. regalis on field maples (Acer campestre), sycamores, elms and horse-chestnuts in natural situations in The Netherlands. In these situations, it was unlikely there could be stress stress due to the above causes.

There are two other exotic species of woolly scale that have become well-established outdoors in southern Britain. One is the iceplant scale, P. mesembryanthemi (Vallot), which is found on iceplants (Carpobrotus spp.), and the other is the cottony camellia scale, P. floccifera (Westwood), found on camellia (Camellia spp.) and holly (Ilex aquifolium). P. theae Froggatt from Australia, recorded in Britain on camellia has recently been synonymised with P. floccifera by Qin (1990).

Two other exotic species of woolly scale are recorded here for the first time, from Britain. Cottony orchid scale, P. phaiae Lull, was found on orchids in greenhouses and was previously misidentified as P. floccifera. Gill (1988) provides a key for their separation, but some authors (Borchsenius, 1957; Kosztarab & Kozár, 1988) consider P. phaiae to be a synonym of P. floccifera. The second species recorded for the first time in Britain is the cottony hydrangea scale, P. hydrangeae Steinweden. It was found outdoors on apple (Malus sylvestris) and sycamore in London during the

summer of 1990. Records of insect pests in Britain obtained from the Royal Horticultural Society, Wisley, indicate that the cottony hydrangea scale has been a well-established pest of outdoor hydrangea (Hydrangea spp.) in London since 1987 (Andrew Halstead, pers. comm.). Cottony hydrangea scale is a pest of ornamental plants in Belgium, France and the Netherlands.

The other exotic species of woolly scale recorded in Britain, P. horii (Kuwana) and green-shield scale, P. psidii (Kuwana), are only rarely found either in glasshouses, or on imported plants or plant produce. Some authors (Takahashi, 1955; Kawai, 1980) place P. horii in the genus Lecanium Burmeister, but this generic name has been invalidated by the International Commission on Zoological Nomenclature (I.C.Z.N.), opinion 1303 (Melville & Smith, 1987).

In addition to the 8 exotic species of woolly scale discussed above, there is the indigenous woolly vine scale P. vitis complex, which is the focus of the present study. This complex is the most economically important of the woolly scales in Britain because its host range includes grapevine (Vitis vinifera), currants (Ribes spp.) and peach (Prunus persica). The economic importance of the complex is discussed in Section 1.3.2 below.

1.3 Woolly vine scale: the Pulvinaria vitis (L.) complex

The name "P. vitis complex" in this study refers to all the nominal taxonomic species that are morphologically very similar to what is accepted by coccidologists as P. vitis (L.). There are 47-55 species names and synonyms associated with the complex, which are listed in Table 1.1. The names of the nominal species that belong to the complex are referred to below within quotation marks due to the uncertainty of their validity as species.

The adult female morphologies of the nominal taxa within the P. vitis complex have been described many times (Borchsenius, 1957; Gill, 1988; Kosztarab & Kozár, 1988; Leonardi, 1920; Newstead, 1903; Paramonova & Saayan-Baranova, 1984; Steinweden, 1946). Adult females of the P. vitis complex are distinguished from other species of Pulvinaria recorded from Britain by the presence of simple spine-like marginal setae, 8-segmented antennae, multilocular pores each with 7-13 (mean 10) peripheral loculi, dorsal submarginal tubercles usually present, ventral submarginal tubular duct band

Table 1.1 Synonymy of the Pulvinaria vitis complex

Coccus betulae Linnaeus, 1758: 455, Ref. to De Geer. Syn. by Newstead, 1903.
Coccus carpini Linnaeus, 1758: 455. Syn. by Newstead, 1903.
Coccus oxyacanthae Linnaeus, 1758: 456, Ref. to Réaumer, 1738. Syn. by Newstead, 1903.
Coccus vitis Linnaeus, 1758: 456, Ref. to Réaumer, 1738.
* Chermes carpini serico albo Geoffroy, 1762: 508. Syn. with P. carpini by Fernald, 1903.
* Chermes mespili Geoffroy, 1762: 508. Syn. with P. carpini by Fernald, 1903.
* Chermes vitis oblongus Geoffroy, 1762: 506. Ref. Réaumer, 1734. Syn. by Fernald, 1903.
Chermes carpini Fourcroy, 1785: 228. Syn. with P. betulae by Lindinger, 1912.
Chermes vitis Fourcroy, 1785: 228. Syn. by Fernald, 1903.
Chermes betulae Olivier, 1792: 442. Syn. with P. betulae by Fernald, 1903.
Chermes crataegi Olivier, 1792: 442. Syn. with P. oxyacanthae by Fernald, 1903.
Calypticus spumosus Costa, 1835: 10. Syn. by Fernald, 1903.
Lecanium carpini Ratzeburg, 1843: 194. Syn. with P. carpini by Fernald, 1903.
Lecanium salicis Bouché, 1851: 112. Syn. by Newstead, 1903.
Coccus innumerabilis Rathvon, 1854: 256-258 (numerous misidentifications: Fernald, 1903; Sanders, 1909).
Lecanium vitis (L.) Fitch 1856. Syn. by Fernald, 1903.
Lecanium vitis Walsh, 1868: 14. Syn. by Leonardi, 1920.
Pulvinaria evonymi Goureau 1869: 47. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria vitis (L.) Targioni Tozzetti, 1866: 34.
Pulvinaria betulae (L.) Signoret, 1873: 31. Syn. by Newstead, 1903.
Pulvinaria carpini (L.) Signoret, 1873: 34. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria fraxini (Lichtenstein) Signoret 1873: 36. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria oxyacanthae (L.) Signoret, 1873. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria piri Signoret 1873. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria populi Signoret, 1873: 42 (218). Syn. with P. betulae by Newstead, 1903.
Pulvinaria ribesiae Signoret, 1873: 43. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria tremulae Signoret, 1873: 45. Syn. with P. betulae by Lindinger, 1912.
Pulvinaria salicis (Bouché) Signoret, 1873: 44. Syn. by Newstead, 1903.
Lecanium betulae Kaltenbach, 1874: 610. Syn. with P. betulae by Fernald, 1903.
Lecanium oxyacanthae Kaltenbach, 1874: 213. Syn. with P. oxyacanthae by Fernald, 1903.
Coccus (Lecanium) vitis Kaltenbach, 1874: 95. Syn. by Fernald, 1903.
Pulvinaria innumerabilis (Rathvon) Putnam, 1880: 339. (numerous misidentifications: Fernald, 1903; Sanders, 1909).
Pulvinaria betulae alni Douglas 1891: 100. Syn. with P. betulae by Fernald, 1903.
Pulvinaria persicae Newstead, 1892: 142. Type, England NM(NH). Syn. by Newstead, 1903 [examined].
Pulvinaria amygdali Cockerell, 1896: 225. (numerous misidentification: Harmon, 1923).
Pulvinaria innumerabilis occidentalis Cockerell, 1897: 12-13. Syn. by Sanders, 1909.
Lecanium coryli Vejdovsky, 1898 (misidentification of male according to Lindinger, 1912).
Pulvinaria ehrhorni King, 1901: 145. Syn. with P. occidentalis by Steinweden, 1946.
Pulvinaria mespili Cockerell, 1901: 90. Syn. with P. carpini by Fernald, 1903.
Pulvinaria vitis ribesiae Newstead, 1903. Syn. with P. betulae by Lindinger, 1912.

Table 1.1 continued

Pulvinaria goethei King, 1903, Syn. with P. betulae by Lindinger, 1912.
Pulvinaria rehi King, 1903, Syn. with P. betulae by Lindinger, 1912.
Pulvinaria simplex King, 1903: 475, Syn. by Sanders, 1909.
Pulvinaria vinifera King, 1903: 481, Syn. with P. betulae by Lindinger, 1912.
Pulvinaria vitis opacus King, 1903, Syn. with P. betulae by Lindinger, 1912.
Pulvinaria vitis sorbusae King, 1903, Syn. with P. betulae by Lindinger, 1912.
Pulvinaria vitis verrucosae King, 1903, Syn. with P. betulae by Lindinger, 1912.
Pulvinaria coulteri Cockerell, 1905: 514-515, Syn. with P. occidentalis by Steinweden, 1946 (type material from Rosa sp., Colorado, USA, which is an unusual host).
Pulvinaria occidentalis subalpina Cockerell, 1910: 428, Syn. with P. occidentalis by Steinweden, 1946.
Lecanium vitis ribesiae Wolff, 1911, Syn. with P. betulae by Lindinger, 1912.

* The work of Geoffroy (1762) has been rejected by the I.C.Z.N., opinion 228.

Relationship between the P. vitis complex and the following species is uncertain:
Lecanium acerella Rathvon, 1876: 101
Pulvinaria cockerelli King, 1899: 417
Pulvinaria innumerabilis betheli King, 1899a: 142, description fits the P. vitis complex
Pulvinaria populeti Borchsenius, 1953: 289, Probable paratype material examined which fit into the morphological range of the P. vitis complex.
Pulvinaria salicicola Borchsenius, 1953: 289
Pulvinaria terrestris Borchsenius, 1953: 290
Pulvinaria vini Hadzibejli, 1960, Specimens seen which fit into the morphological range of the P. vitis complex.
Pulvinaria ellesmerensis Richards, 1964, Paratype examined which fits into the morphological range of the P. vitis complex.

Incorrect Synonyms
Pulvinaria hunteri King, 1901: 144 Syn. by Sanders, 1909; original description mentions 'large marginal spines are practically the same as those of innumerabilis'. It was recorded on maple and honey locust, I consider that this is likely to be a synonym of N. innumerabilis Rathvon and not P. vitis.
Coccus innumerabilis Rathvon, 1854 Syn. by Sanders, 1909 = N. innumerabilis (Rathon)
Lecanium maclurae Fitch, 1855 Syn. by Sanders, 1909 = ? N. innumerabilis (Rathon)
Lecanium acericorticis Fitch, 1859: 775, Syn. by Sanders, 1909 = N. innumerabilis (Rathvon)
Pulvinaria innumerabilis tiliae King & Cockerell, 1898 Syn. by Sanders, 1909 = N. innumerabilis (Rathon)
Coccus pyri Shrank, 1801: 144/ Lecanium pyri Fitch, 1855. Syn. by Sanders, 1909 = Eulecanium tiliae (L.), 1758.
Coccus fagi Baerensprung (preoccupied), 1849: 174, Syn. by Newstead, 1903 = Cryptococcus fagisuga Lindinger

not extending around head region, and only 1 long seta situated at the base of each front coxa (see Section 2.2 and Figs. 2.3 and 2.4).

The immature stages of "P. vitis (L.)" were described by Phillips (1962) and Leonardi (1920); "P. betulae (L.)" by Řeháček (1960) and "P. populi Signoret" by Borchsenius (1957). The adult male of "P. betulae" was described by Giliomee (1967) and Řeháček (1960); "P. ribesiae Signoret" by Newstead (1903) and "P. populi" by Borchsenius (1957). The male test of P. betulae was described by Borchsenius (1957) and Řeháček (1960); "P. betulae/vitis" and "P. ribesiae" by Paramonova & Saakyan-Baranova (1984) and "P. populi" by Borchsenius (1957).

1.3.1 How many species are there?

All the nominal—taxonomic species in the P. vitis complex are morphologically very similar and it is not known how many evolutionary species there are, or how each may be recognised (see Section 3.1.2 for the definition of a species). There has consequently been much confusion in the literature. Soft scales belonging to the P. vitis complex were originally considered to be different taxa when found on different host-plant species and were named accordingly. For example, "P. vitis (L.)" on grapevine, "P. ribesiae Signoret" on currants, "P. betulae (L.)" on birch (Betula spp.), "P. populi Signoret" on poplar (Populus spp.), "P. oxyacanthae (L.)" on hawthorn (Crataegus spp.), "P. salicis (Bouché)" on willow (Salix spp.), "P. persicae Newstead" on peach and "P. carpini (L.)" on hornbeam (Carpinus betulus). New Pulvinaria species, possibly belonging to this complex, are still being described from Europe (Săvescu, 1983: 1985), adding to the confusion.

The keys that are available to the P. vitis complex do not work as there are too many intermediate forms which do not fit any of the present morphological descriptions. For example, individuals of woolly scale collected from a single grapevine in Devon, England, key out as six different species using recently published keys (Kostarab & Kozár, 1988; Danzig, in Bei-Bienko, 1987). These keys, and the key by Borchsenius (1957) to the Palaearctic Pulvinaria, are given in Appendix 1.3. The range of morphological variation of a single population of woolly vine scale and the species names that they key out to are given in Appendix 1.4. The main morphological differences and diagnostic characters of the nominal taxa involve the size of the body and appendages, the number and arrangement of marginal setae and

numbers of spiracular pores present. Borchsenius (1957) also used the ratio of marginal seta length to interval size between setae as a diagnostic character for individual species. This is a very unreliable character, however, as it varies considerably with the age of the specimen and between individuals from the same population. Listed in order of size and pore number, with the largest first, the main nominal species in the complex are: "P. populi", "P. betulae", "P. vitis", "P. ribesiae" and "P. oxyacanthae".

Why is it necessary to know how many species there are and how to distinguish between them? Common host plants, such as hawthorn or birch, may act as reservoirs of woolly scale that could cause repeated reinfestation of crops (such as grapevines and currants) after spraying with insecticides. The scale insect's life cycle must be known in order to find the optimum spraying time for maximum control and this may vary on different host-plant species. Parasitoids have been used successfully for biological control of scale insects and these may differ for different woolly scale species.

The first names associated with the P. vitis complex to be published after the starting point of zoological nomenclature (1 January 1758, decided by the I.C.Z.N.) were "Coccus betulae", "C. carpini", "C. vitis" and "C. oxyacanthae" by Linnaeus (1758). Linnaeus did not give morphological descriptions but gave references to previous publications, such as Réaumur (1734), who had produced excellent illustrations of unnamed woolly scales on grapevine and hawthorn from France. Similar unnamed scale insects on grapevine had also been illustrated by Galeatii (1746). The gross morphology of adult female woolly scales and their ovisacs were described by Geoffroy (1762), using the names "Chermes vitis oblongus", "C. carpini serico albo" and "C. mespili". The work of Geoffroy, however, has been rejected by the I.C.Z.N. opinion 228 (Hemming, 1958) for not applying the principals of binominal nomenclature.

Several catalogues were produced at the end of the eigthteenth and the beginning of the nineteenth centuries, listing these names and proposing new combinations (Boyer de Fonscolombe, 1834; Fabricius, 1775: 1776: 1781: 1787: 1794: 1803; Fourcroy, 1785; De Villiers, 1789; Modeer, 1778; Olivier, 1792). The most significant of the early works was a series of papers by Signoret (1873) which described all the important nominal species belonging to the complex: "P. betulae (L.)", "P. carpini (L.)", "P. fraxini (Lichtenstein)",

"P. oxyacanthae (L.)", "P. piri Signoret", "P. populi Signoret", "P. ribesiae Signoret", "P. tremulae Signoret", "P. salicis (Bouché)" and "P. vitis (L.)".

Numerous papers were published in Europe during the first half of the twentieth century, briefly describing taxa in the complex and giving biological observations (Green, 1920: 1928; Leonardi, 1920); but there was little significant original research until the 1950's. Borchsenius (1957) produced a major revision, describing all the taxa as well as some new species, and provided a key to the complex. Much of the subsequent work in Eastern Europe, Russia and China has been based on his contribution.

The number of species currently recognised in the complex depends on the authority, but there are five names in common use; "P. vitis", "P. betulae", "P. ribesiae", "P. oxyacanthae" and "P. populi". Much of the taxonomic uncertainty has resulted from inadequate morphological descriptions dating from the end of the eig hteenth to the beginning of the twentieth centuries. Most of these descriptions only describe the gross morphology of the adult female and ovisac, and give host-plant species. Even with more recent, detailed descriptions there have often been inconsistencies in the characters used so that comprehensive direct comparisons are not possible. There is usually no indication of the degree of morphological variation that can occur, even within a single population on the same host. This is because descriptions were often made from an inadequate number of specimens.

No holotypes were designated prior to about the beginning of the twentieth century and syntypic material has often been lost, or is of such poor quality as to be of little use. There is no type material of P. vitis from Linnaeus and it appears that there has been no type material designated subsequently. It is therefore impossible to be certain of the true identity of all the names involved. When the identities are uncertain, there is no designated type material avaliable, the names have not been widely used and the original descriptions fit "P. vitis", it is more useful to synonymise the names than to leave them as valid. The first published synonymy of the names of Linnaeus (1758) was produced by Newstead (1903) who synonymised "P. betulae (L.)", "P. carpini (L.)" and "P. oxyancanthae (L.)" with "P. vitis (L.)". The name "P. vitis (L.)", therefore, takes priority over the other names published at the same date.

Another difficulty presented to workers on the P. vitis complex has been that most of the publications on the complex have been in Russian or other

Eastern European languages. They are consequently less accessible to English-speaking workers, who do not have the facilities to translate large works.

There has been considerable confusion with the taxonomy of species of woolly scale in North America, as well as in Europe, since the introduction of the P. vitis complex at the end of the nineteenth century; this is discussed in detail in Appendix 1.5.

Although "P. vitis" and "P. betulae" cannot be separated consistently by any available key, the former name is used in Western Europe, North America, North Africa and New Zealand, and "P. betulae" is used in Eastern Europe, U.S.S.R. and Japan. There are also inconsistencies with the common names used. In Europe, Pulvinaria spp. are called woolly vine, grape, birch, currant or poplar scale according to the host plant from which they are collected. In North America, "P. vitis" is called the cottony vine scale; it was also referred to as the cottony maple scale (Essig, 1958) when confused with Neopulvinaria innumerabilis (Rathvon), and cottony peach scale (Harmon, 1923) when confused with P. amygdali Cockerell. Neopulvinaria innumerabilis is also some times known as cottony vine scale in North America (Stafford & Doutt, 1974).

1.3.2 Economic importance

The P. vitis complex is the most economically important of the woolly scales in Britain. It has been recorded as a pest of grapevine, currants, quince (Cydonia oblonga), peach, apricot (Prunus armeniaca) and pear (Pyrus communis) especially under glass and in sheltered situations outdoors. It also occurs on other economically important plants, such as cherry (Prunus cerasus) and plum (Prunus domestica). Although the complex has been recorded as a serious pest of grapevine in southern and central Europe (Kosztarab & Kozár, 1988), it does not appear to be a widespread pest on grapevine in Britain, but where it does occur it is very difficult to eradicate. Levels of infestation may be related to the age of the vines and, as the modern English grapevine growing industry is still only a recent innovation, the woolly vine scale may become more signficant as the vines mature. "P. vitis" is a pest of peach in Canada and U.S.A., where two serious outbreaks were recorded in Ontario and New York state during 1926-1929 and 1946-1954 (Phillips, 1963).

"P. ribesiae" is recorded as a pest of redcurrants (Ribes sylvestre), blackcurrants (R. nigrum) and gooseberries (R. uva-crispa). Outbreaks have been recorded on blackcurrants in Britain in Gloucestershire and Somerset during 1981 and 1988 respectively. It may, however, be more of a problem in gardens and allotments which are not sprayed as often as commercial farms. "P. ribesiae" has also been reported as a serious pest of blackcurrants and gooseberries in Byelorussia (Paramonova & Saayan-Baranova, 1984) and Siberia (Babenko, 1974) in the U.S.S.R.

The P. vitis complex can produce heavy infestations, completely encrusting the bark. This has a debilitating effect on the host and, if the grapevines or currants are not treated, the crop is significantly reduced. Damage is caused by depletion of plant sap, which reduces the vigor of the plant and may cause premature leaf fall. Unchecked heavy infestations kill young plants, but most isolated infestations are overlooked and the death of the plant is attributed to some other cause. A secondary effect is the contamination of fruit and foliage with sticky, sugar-rich honeydew excreted by the scale insects. This serves as a medium for the growth of sooty-mould fungi that turns the foliage black and unsightly and can seriously lower the market value of fruit. Photosynthesis by the plant is also reduced, resulting in lower sugar levels in the fruit. Ornamental plants, such as pyracanthas (Pyracanthus spp.) and spindle (Euonymus europaeus), lose their aesthetic value if coated with black mould. The woolly ovisac produced by the adult female scale insect may also contaminate fruit and be a nuisance during picking (Alford, 1984).

1.3.3 Distribution

The P. vitis complex is widespread throughout the United Kingdom but appears most common in southern England and Wales. It apparently originated in Europe, but is now found throughout the Palaearctic and has been introduced to the Nearctic and Neotropical regions and New Zealand. It has been recorded in 38 countries: Algeria, Austria, Brasil, Bulgaria, Canada, China, Czechoslovakia, Denmark, Eire, Finland, France, Germany (including both the former West and East Germany), Greece, Holland, Hungary, Iran, Israel, Italy, Japan, Jordan, Luxemburg, Malta, Mongolia, Morocco, Netherlands, New Zealand, Norway, Poland, Portugal, Romania, Spain, Sweden, Switzerland, Turkey, United Kingdom, U.S.A., U.S.S.R. and Yugoslavia. It was recorded from Jordan

for the first time; an adult female was collected from grapevine by T. Mustaba in May, 1986.

1.3.4 Host plants

The complex is broadly polyphagous, being recorded on 94 host-plant species belonging to 17 families and 4 subclasses of the Dicotyledonae. The plant families that contain the majority of host species are the Rosaceae, Salicaceae and Betulaceae. The complex is most commonly found on the following host plants: grapevine, currants, birches, hawthorns, willows, poplars, pyracanthas, Sorbus spp., and Prunus spp. Both "P. vitis" and "P. betulae" are recorded as highly polyphagous, but "P. ribesiae" is usually only recorded from currants, "P. oxyacanthae" only from hawthorn and "P. populi" only from poplars. A systematic host-plant list and summary table is given in Appendices 1.6 and 1.7.

Many host plants such as saxifrage (Saxifraga sp.), horse-chestnut, lime and sycamore, have only been reported once or a couple of times. The complex has also been reported as common on some hosts in one country but as rare or absent from the same hosts in neighbouring countries. For example, it has been reported many times on hazel (Corylus avellana), walnut (Juglans regia), beech (Fagus sylvatica) and ash (Fraxinus excelsior) in Eastern Europe, but never on these hosts in Britain. Many host records are unreliable and are probably the result of misidentifications. Neopulvinaria innumerabilis and P. regalis are sympatric with the P. vitis complex and can easily be confused in the field. Host-plant species in common between N. innumerabilis and the P. vitis complex are dogwood (Cornus spp.), hawthorn, false acacia (Robinia pseudoacacia), lime and grapevine. Host-plant species in common between P. regalis and the P. vitis complex are Norway maple (Acer platanoides), sycamore, horse-chestnut, hornbeam, dogwood, hazel, Prunus spp., willows, whitebeam (Sorbus aria) and lime.

1.3.5 Life cycle

The life cycle reported for the P. vitis complex varies in different parts of its geographical range and for the different nominal species. In particular, the overwintering stage, presence or absence of males and time of adult male emergence vary. In Britain, the life cycle reported is as follows: adult scale insects appear in September to October. The adult males are

short lived and die soon after mating, but the adult females overwinter and resume growth in the following spring, becoming more convex and darker brown. In May, they begin to produce their ovisac of white waxen threads and oviposit. Between 1,000 to 5,000 eggs are laid over 2-5 weeks. The first instars, or 'crawlers', hatch in June and disperse over the young shoots, feeding from the phloem. In the first half of July, they moult into second instars and migrate to the older wood where, in August, they moult into third instars and eventually become adults three weeks later.

The life cycle reported for "P. ribesiae" in parts of U.S.S.R. is considerably different. In Byelorussia, it is normally the second instars and a significant number of fully grown, first instars which overwinter (Paramonova & Saayan-Baranova, 1984). Adult males emerge, mate and die in the beginning of June, but occasionally some also appear in October. Second instars are also the usual overwintering stage in Moscow province, but in mass outbreaks all stages can overwinter (Drozdovsky, 1959; Danzig, 1972). Scale insects are also reported to overwinter in various stages in Siberia (Babenko, 1974). In North America, "P. vitis" is reported to overwinter as immatures in California (Gill, 1988), as adult females in Ontario (Phillips, 1963) and in various stages in the Canadian arctic (Richards, 1964).

Distinct sexual and parthenogenetic races have been reported to occur in Europe by several authors (Danzig, 1986; Jablonowski, 1916; Schmutterer, 1952). Males are completely unknown from North America (Phillips, 1963; Gill, 1988; Richards, 1964) and parts of Europe and Russia (Danzig, 1972). The complex is univoltine, although a few individuals of "P. ribesiae" have been reported producing a partial second generation in Byelorussia (Paramonova & Saayan-Baranova, 1984). This suggests that the second instar diapause reported in Byelorussia is easily broken by environmental conditions.

1.3.6 Karyotype

Karyotype analysis has provided some evidence for the presence of distinct taxa within the P. vitis complex. Drozdovsky (1966) reported that large and small specimens, identified as "P. betulae" and "P. ribesiae", have a diploid chromosome number of 16 and 18 in each somatic cell, respectively. They were found on birch and blackcurrant in Moscow province, U.S.S.R. These results have not been repeated and there is no cytogenetic work published on

the other nominal species in the complex. The karyotype of British specimens is determined in Chapter 8 and the morphological variation of Drozdovsky's specimens reported to have different karyotypes is compared to the variation of British field-collected specimens in Section 3.3.2.

1.3.7 Natural enemies and associated insects

There is an extensive list of natural enemies and associated insects recorded for the P. vitis complex, which are discussed in detail in Chapter 7. The most important natural enemies are parasitoids, which normally keep populations of woolly scale at low numbers, particularly in the Palearctic where the P. vitis complex originated. The relative importance and effectiveness for scale-insect control of the different parasitoid species is also discussed in Chapter 7. I have personally found the most common parasitoid of the P. vitis complex in southern Britain to be Coccophagus lycimnia (Walker). Two other parasitoid species of the P. vitis complex which were found to be locally common are recorded here for the first time from Britain; Metaphycus melanus Sugonjaev and Coccophagus semicircularis (Förster). The latter species was previously misidentified numerous times in the literature as C. scutellaris Dalmon (A. Polaszek, pers. comm.).

1.4 Aims of the present study

The principal aim is to determine the diagnostic morphological characters and their ranges of variation for each of the species in the P. vitis complex in Britain in order to separate each species by means of a key. The confusion in the literature can then be rectified and life cycle, host range, distribution and natural enemies determined for each of the species. Pulvinaria specimens from other geographical regions, recorded as belonging to the P. vitis complex, can be compared to the European specimens in order to elucidate their true identity. Only when the identity of all the taxa involved has been determined can their true economic importance be assessed.

Another objective is to improve the methodology for the resolution of complexes of sibling species. Morphological variation within a complex is studied, with particular reference to the effects of host-plant species, parasitism and locality. Methods to distinguish genetic from environmental morphological variation are also investigated.

For practical use by field workers, morphological differences need to be

determined between the distinct taxa in order to produce keys. Biological differences, such as host-plant species or life cycle, are also useful. Cytological, electrophoretic, endosymbiont and serological differences are useful only to confirm the findings from morphological studies. They are of little practical use to field workers who do not have the facilities, expertise, time or funds to use such methods.

2 Classification and morphology

2.1 Superfamily Coccoidea

The higher classification of scale insects is controversial at present. Some workers regard the scale insects as a superfamily, the Coccoidea, within the Homoptera (Balachowsky, 1942; Boratynski, 1970; Miller, 1984; Miller & Kosztarab, 1979; Morrison, 1928) while other workers regard them as a suborder, the Coccinea (Borchsenius, 1950: 1958; Bodenheimer, 1952; Danzig, 1986; Koteja, 1974b). Phylogenetic trees for the two basic classifications are presented in Figs. 2.1 and 2.2. The former classification with the scale insects regarded as a superfamily is used in this present study. Scale insects are generally regarded as being the sister group to the aphids (Aphidoidea), the ancestral group being termed the Aphidiformes (Schlee, 1969b) or Aphidomorpha (Hennig, 1981). Both superfamilies are monophyletic as a whole with whitefly (Aleyrodoidea) and psyllids (Psylloidea) (Schlee, 1969a: 1969b; Theron, 1958). These four superfamiles comprise the Sternorrhyncha which are distinguished from the Auchenorrhyncha by being opisthognathus, meaning that the labium arises from almost between the front coxae and is directed backwards.

All scale insects were originally assigned to the family Coccidae by Linnaeus (1758), but now this taxon is restricted to only those species resembling Coccus hesperidum L. There are currently about 6,000 described species of Coccoidea worldwide, in about 800 genera placed in between 15 and 22 families (Miller & Kosztarab, 1979).

2.2 Family Coccidae

Classification

The Coccidae is the third largest family of scale insects after the armoured scales (Diaspididae) and mealybugs (Pseudococcidae) respectively. It contains about 1,000 species in approximately 140 genera and the number of described species is increasing annually.

The Coccidae has been divided into subfamilies or groups of genera, based either on the morphology of adult females (Bodenheimer, 1953; Borchsenius, 1957; Tang et al., 1990) or on the morphology of adult males (Giliomee, 1967). Borchsenius recognised three subfamilies, the Filippinae, Ceroplastinae and the Coccinae. The Coccinae were further divided into two

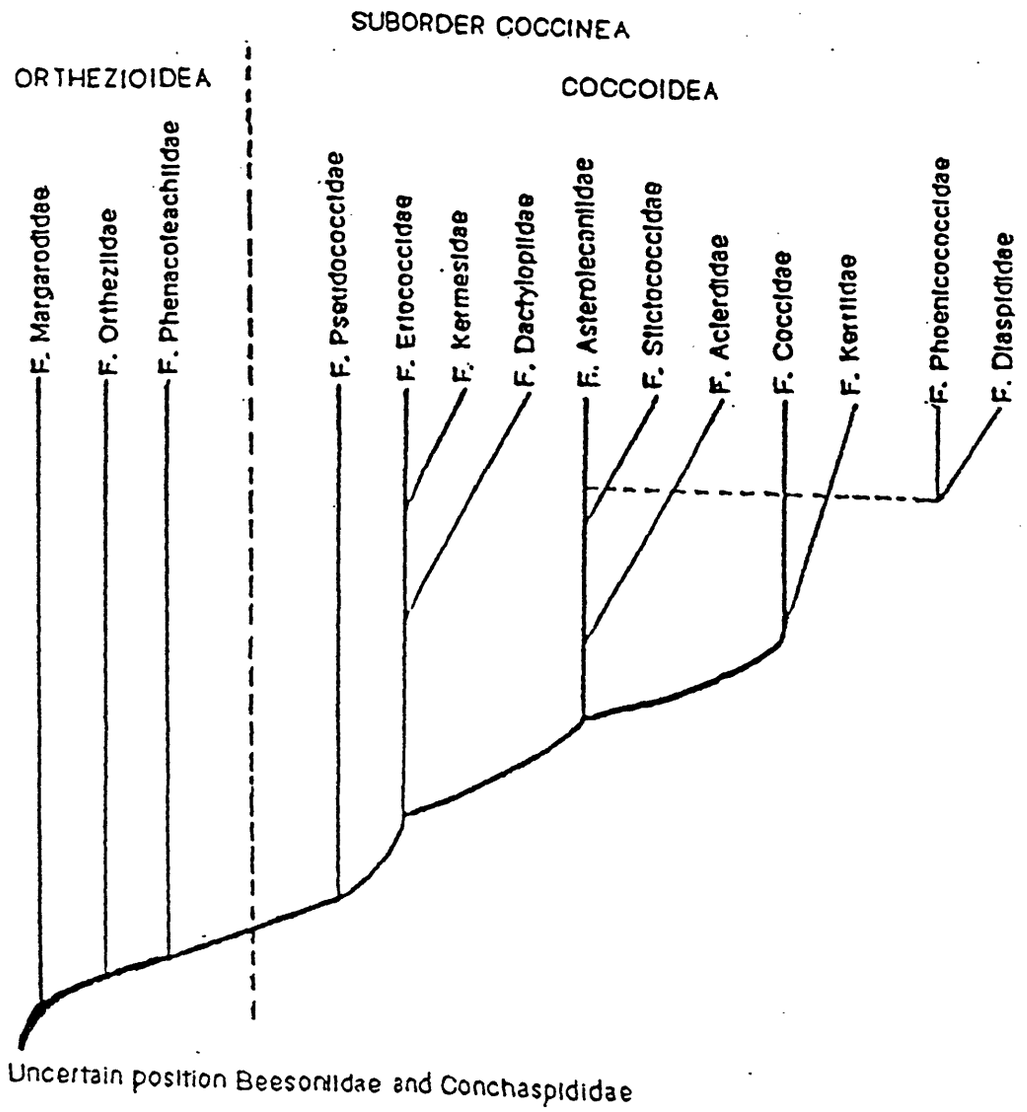


Fig. 2.1 Phylogenetic tree of the scale insects according to Danzig (1986)

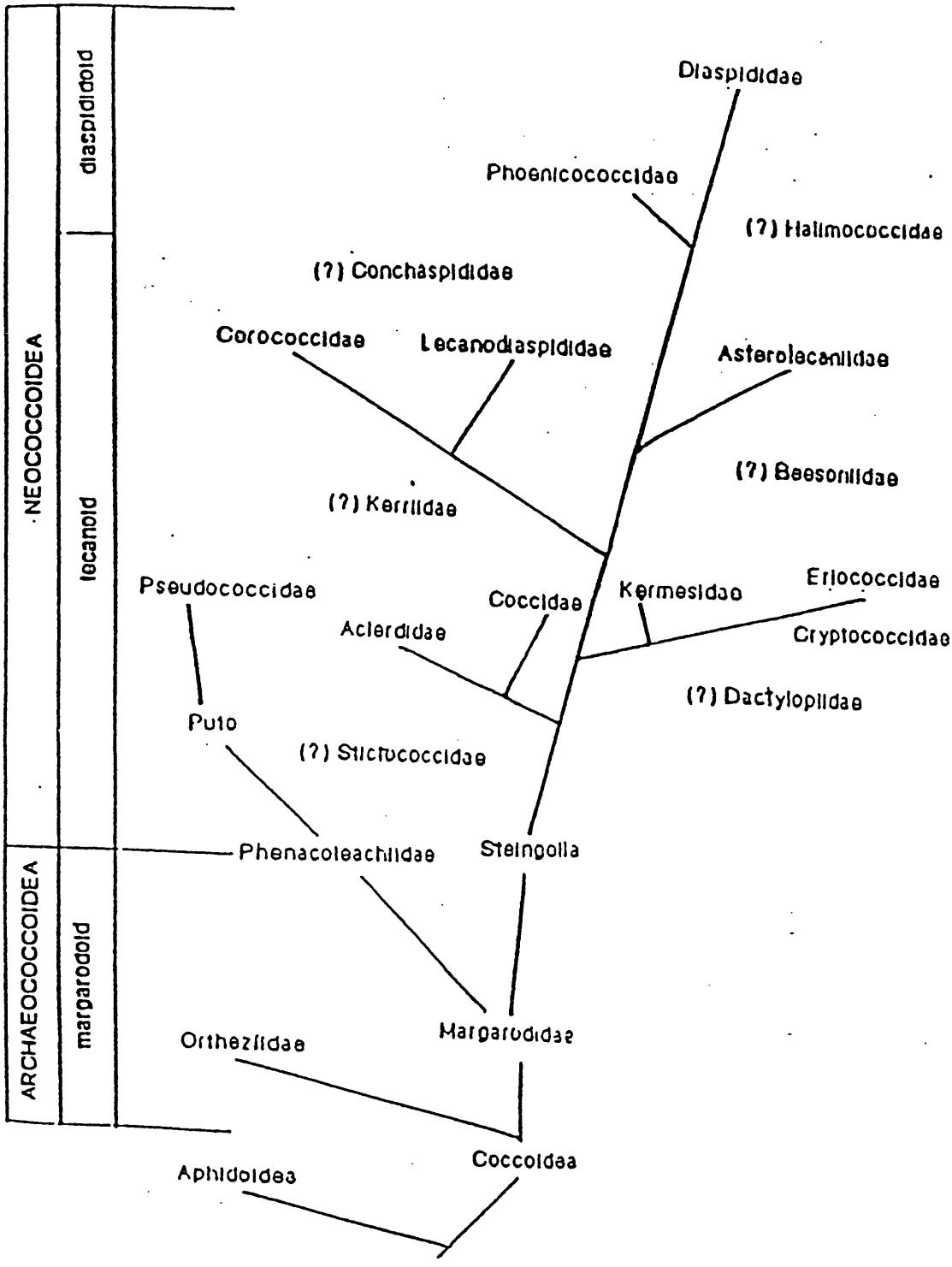


Fig. 2.2 Phylogenetic tree of the scale insects according to Miller and Kosztarab (1979)

tribes, the Coccini and Pulvinariini. The Filippinae form part of the Eriopeltis-group of Giliomee (1967) and are currently termed the Eriopeltinae. Tang et al. added a fifth subfamily to the system of Borchsenius, the Pseudopulvinariinae. Giliomee recognised four natural groups of genera; the Eriopeltis-group, Coccus-group, Eulecanium-group and the Inglisia-groups. Ray and Williams (1983) added a fifth natural group to the system of Giliomee, the Toumeyella-group.

Before the 1920's, Coccidae species descriptions consisted of little more than the gross appearance of the adult female and mention of the host-plant species. From the beginning of the twentieth century, up until 1930's, there was a reliance on leg and antennal-segment lengths of adult females for identification (King, 1903; Brain, 1915; Hollinger, 1917; Shinji, 1935). It was gradually appreciated that these characters are unreliable due to interspecific variation (Ferris, 1918). Coccidae identification and classification has been based, almost exclusively, on the external morphology of adult females subsequently to detailed descriptions and drawings of adult females published in the late 1920's onwards (Ferris, 1937-55; Balachowsky 1948-53; Morrison, 1925, 1928; Steinweden, 1929). Adult females are normally the stage most frequently encountered and usually have the largest number of morphological characters potentially useful for identification. Today, identification of Coccidae species is based mainly on secondary characters (Williams, 1985), such as the shapes and numbers of setae, wax-producing pores and ducts.

Coccidae classification at the present time, based almost entirely upon the morphology of the degenerate-adult females, is somewhat inadequate. There is much disagreement of the classification, particularly at generic and familial levels, between coccidologists. Many authors have commented that it could be improved if other instars were considered, in particular the adult males, which may retain primitive characters after adult female characters have converged as a result of adapting to similar habitats (Williams, 1985). Unfortunately, adult male Coccidae are rarely collected, as they are ephemeral and usually live only from a few hours to a day. Work on adult male morphology of Diaspididae have shown them to be potentially very useful for classification and particularly for studying evolutionary relationships (Afifi, 1968; Beardsley, 1960: 1962; Boratynski, 1970; Boratynski & Davies, 1971; Giliomee, 1961; Theron, 1958). The morphology of immature instars has

also been found to be useful in classification for the Diaspididae (Takagi, 1969), and the Coccidae (Řeháček, 1960). The wax test of male Coccidae has also been used for identification (Řeháček, 1960; Miller & Williams, 1990).

Many species of Coccidae are difficult to identify because they are polymorphic. Discrepencies in the classification are also partially a result of lack of original research and literature, insufficient collaboration between workers and inadequate descriptions and keys.

New taxonomic techniques have been employed, where separation of sibling species based only on morphology is unreliable. These methods include studies of endosymbionts (see Section 8.5), chemotaxonomy (Banks, 1976; Brown, 1975), cytology (see Sections 8.2 and 8.3), sperm bundle morphology (see Section 8.4), enzyme electrophoresis (see Chapter 9) and serology (Rotundo & Tremblay, 1976).

Morphology

The general morphology of the family Coccidae has been comprehensively described numerous times and the taxonomic significance of various characters discussed (Dziedzicka, 1977; Gill, 1988; Hamon & Williams, 1984; Koteja, 1974a: 1974b; Koteja & Liniowska, 1976; Williams & Kosztarab, 1972). Adult females are larviform and neotenic. The taxonomically important morphological structures of adult female Coccidae are shown in Figs. 2.3 and 2.4 and are discussed in more detail in Section 3.2.5. Adult males possess a single pair of well-developed mesothoracic wings, the metathoracic wings being absent or reduced to minute hamulohalteres. All adult male Coccidae lack functional mouthparts.

Immature and adult female Coccidae are distinguished from the other families of Coccoidea by the presence of a pair of triangular opercula covering the anal opening at the base of the anal cleft. These are absent only in the adult females of the genus Physokermes Targioni Tozzetti (Schmutterer, 1956). Other distinctive characters are the enlarged frontal part of the head, the obscurely two-segmented labium and the glass-like, waxy male puparium. On the ventral surface are four spiracular furrows, each extending from a spiracle to the margin and terminating with a group of spiracular setae.

Characters of taxonomic value for determining species include the number, form and arrangement of setae, particularly the marginal and spiracular

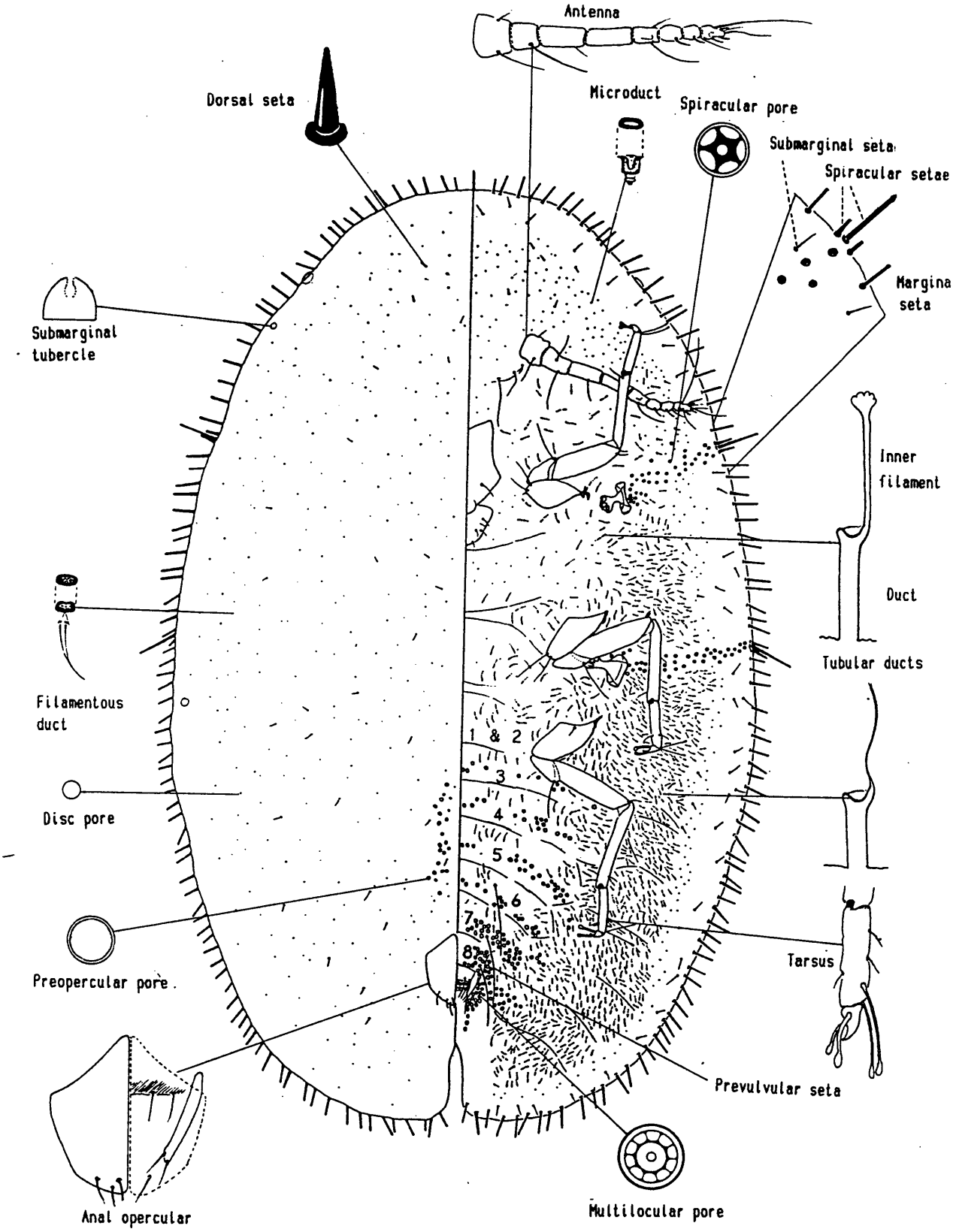
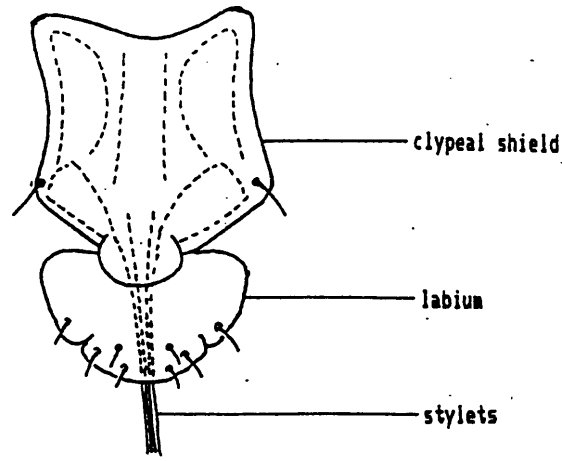
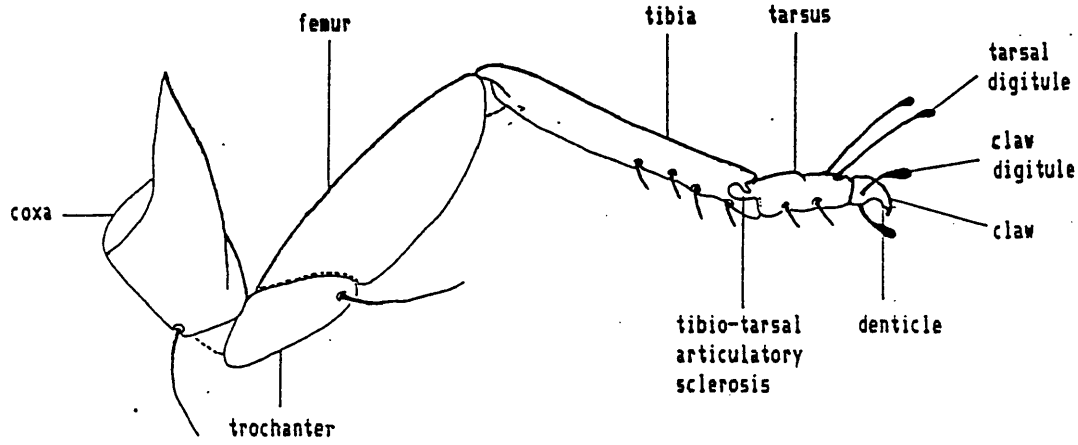


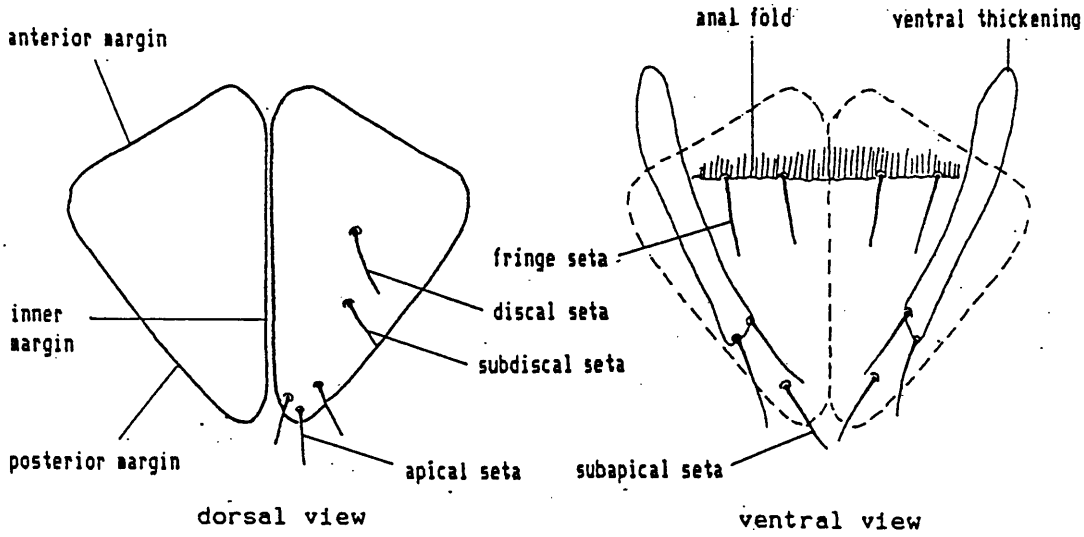
Fig. 2.3 General morphology of adult female Coccidae



Mouth parts



Leg structures



Opercular structures

Fig. 2.4 Details of the morphology of adult female Coccidae

setae; wax-producing structures such as the pores and tubular ducts; the relative development of appendages such as legs, antennae and opercula; and the texture and pattern of the dorsal derm.

Lack of standardisation of morphological terminology has resulted in some discrepencies in terms used for particular characters. This is more noticable between families in the Coccoidea than within families. The terminology used in this work follows Gill (1988) with a few exceptions. Appendix 2.1 lists the terminology used, along with variations of terms which have been recently published (Danzig, 1986; De Lotto, 1979; Hodgson, 1967, 1968; Williams & Kosztarab, 1972).

2.3 Genus Pulvinaria Targioni Tozzetti

The type-species of the genus Pulvinaria is Coccus vitis L., 1758 by original designation. The Pulvinaria is placed in the tribe Pulvinariini by Borchsenius (1957), along with 25 other genera and more than 200 species (T. Qin, pers. comm.). Pulvinaria is a large, widely-distributed genus which was subdivided into several subgenera by Cockerell (1894: 1896a: 1898), which were later raised to generic level (Cockerell, 1899). Borchsenius (1952: 1953) divided the Pulvinaria of the Palaearctic region into 8 genera and later recognised 11 Palaearctic genera in the Pulvinariini (Borchsenius, 1957). Borchenius's generic concepts of the Pulvinariini, however, are not universally accepted by coccidologists, especially North American authors (Williams & Kosztarab, 1972; Hamon & Williams, 1984; Gill, 1988), because his concepts are based partly on field characteristics, and the morphological characters between the genera intergrade when considered on a world basis (Gill, 1988). Currently, the number of taxa recognised vary, but there are approximately 150 described species worldwide. The approximate numbers of Pulvinaria species recorded from each zoogeographical region are given below in Table 2.1, together with references to the most important sources. Some cosmopolitan species are recorded from more than one region.

The genus Pulvinaria can be recognised by the presence of a ventral submarginal band of tubular ducts, numerous tubular ducts in the median area of the venter, tibio-tarsal articulatory scleroses (absent in P. ericicola McConnell and often absent in P. urbicola Cockerell) and spiracular setae usually in groups of three with the central seta the longest (absent in P.

bigeloviae Cockerell). A detailed morphological description of the genus Pulvinaria is given in Appendix 2.2.

Table 2.1

Approximate numbers of Pulvinaria species recorded from each zoogeographical region

Zoogeographical region	No. of Spp.	References
Afrotropical	20	Brain, 1920; Hodgson, 1967: 1968; De Lotto, 1979.
Australian	14	Froggatt, 1915; Qin, 1990.
Austro-Oriental	5	Williams & Watson, 1990.
Malagasian	6	Williams, 1982.
Nearctic	16	Steinweden, 1946; Williams & Kosztarab, 1972; Hamon & Williams, 1984; Gill, 1988.
Neotropical	18	Hempel, 1900; Fonseca, 1969: 1972: 1973.
New Zealand & Polynesian	3	Williams & Watson, 1990.
Oriental	14	Green, 1896-1922; Tao et al., 1983.
Palaearctic	48	Borchsenius, 1957; Canard, 1965; Kosztarab & Kozár, 1978: 1988; Danzig, 1980; Ezzat & Hussein, 1969; Hadzibejli, 1955: 1977; Kanda, 1960; Kawai, 1980; Kuwana, 1902, 1907: 1914; Takahashi, 1955: 1956; Tereznikova, 1981; Wang, 1982.

3 Investigation of morphological variation

3.1 Introduction

The principal aim of the present study is to determine the number of species within the Pulvinaria vitis (L.) complex in Britain and to discover the diagnostic morphological characters and range of variation for each species.

Initially, the morphological variation of field-collected specimens from different localities and host-plant species can be investigated, to see if they can be segregated into natural groups. If such groups do occur, they would be the result of genetically- and/or environmentally-induced variation. However, since only genetic variation is indicative of possible species differences, it is necessary to determine if the observed (phenotypic) variation is environmentally or genetically induced. In order to determine genetically-induced variation, the effects of the environment on phenotypic variation must first be identified and quantified. This may be achieved by rearing genotypically identical individuals under different environmental conditions; resulting variation would be environmentally induced. Alternatively, individuals with different genotypes may be reared under identical environmental conditions and resulting variation would be genetically induced. When the environmentally induced and genetically induced variation have been identified and separated, it should be possible to form an opinion as to the number of species occuring in the P. vitis complex in Britain.

This chapter investigates the morphological variation of field-collected specimens and Chapters 4 and 5 examine some affects of the environment and genotype on the phenotype. Chapter 4 studies the effect of parasitism and Chapter 5 the effect of host-plant species, by means of host-transfer experiments, on morphological variation.

Only when the limits of genetic variation have been determined can useful comparisons be made between material originating from different geographical areas and host-plant species. Such injudicious comparisons, without adequate consideration of environmentally-induced variation, result in new taxa being erected for different morphs of the same species.

Correlation of variation between morphological characters is also examined as an understanding of correlation is useful in the interpretation of the

variation observed in future field-collected material and when investigating similar species complexes of Coccidae.

3.1.1 Environmentally-induced variation in Coccidae

Intraspecific-morphological variation is widely prevalent amongst the Coccidae. This presents problems to the taxonomist, as it is often difficult to conclude whether a variable, cosmopolitan and polyphagous entity represents one or several species. There are numerous examples of what is currently regarded as a single polymorphic species, where each form was initially described as distinct. For example, there are 130 synonyms for Parthenolecanium corni (Bouché) (Lindinger, 1934) and 25 synonyms for Coccus hesperidum L. (Gill et al., 1977). Conversely, there are morphologically very similar but distinct species, such as the Pseudococcidae, Planococcus citri (Risso) and Planococcus minor (Maskell), which were considered the same until studied in detail (Cox, 1981).

Environmentally- and genetically-induced variation maybe influenced by, or associated with, the same factors. For example, climatic conditions, and consequently environmentally-induced variation, are strongly influenced by geographical location. Genetic variation is also associated with geographical distribution in many widely distributed demes. Other factors which can influence environmentally-induced variation of Coccidae are host-plant species and parasitism. Genetic variation may also be influenced by host species, as different biotypes of scale insect may be better adapted for different host species.

Temperature was reported to have an effect on the morphological variation of a number of species of Pseudococcidae by Cox (1983). She reported that there was an inverse linear relationship between temperature and the numbers of wax-producing pores and the lengths of appendages and setae. Specimens grew more slowly at lower temperatures and were consequently larger when they reached maturity. Other characters were influenced by size or by a combination of size and environmental factors acting independently.

Morphological variation of Coccidae has also been reported to be influenced by host-plant species (see Chapter 5). Danzig (1970: 1986) found that "P. betulae" were on average smaller on currant and Spiraea sp. than on birch and other species of tree in the same locality. However, no reliable microscopic differences were found. The colour and size of "P. vitis" were

also reported to vary with host species in the U.S.A. (Sanders, 1909). Body shape, size and number of tubular ducts of Parthenolecanium corni (Bouché) varied according to the species of host from which it was collected (Ebeling, 1938; Saakyan-Baranova et al., 1971).

Host-plant species has also been reported to influence the life cycle and sex ratio of populations of "P. betulae" (Danzig, 1986) and Parthenolecanium corni (Saakyan-Baranova et al., 1971). Danzig found the overwintering stage for "P. betulae" on Spiraea sp. was the second instar, but on birch, poplar and ash in the same locality in the U.S.S.R., it was the adult female. Males were very rare and formed separate colonies from the females, except on Spiraea sp. where the colonies were mixed. Fecundity has also been reported to be affected by host-plant species. "P. ribesiae" has been recorded laying 610-1,657 (mean 1,025) eggs on blackcurrant, a maximum of 1,240 on gooseberry and a maximum of 2,100 on redcurrant (Babenko, 1974; Paramonova & Saayan-Baranova, 1984). "P. vitis" has been reported to lay 1,238-5,108 (mean 3,486) on hawthorn (Schmutterer, 1952), and a mean of 4,000 on peach (Phillips, 1963).

Danzig (1966) found parasitism to have a great influence on morphological variation of Coccidae (see Chapter 4). Parasitised individuals of "P. betulae" were characterised by a distinct reduction in the number of wax-producing structures, numbers of setae and size of the spiracles. Danzig suggested that parasitism caused a reduction in the wax-producing glands associated with reproduction which resulted in sterility.

3.1.2 The species concept used in this study

An individual 'species' is a group of life-forms which can be consistently distinguished from other groups of life-forms based on a suite of characteristics, at least some of which are unique. An individual species can be defined in different ways depending on the purpose of the definition and the species concept used. For practical reasons, taxonomists have traditionally attempted to distinguish species based on genetically-determined morphological traits, but recently species have been defined on a combination of behavioural, physiological, ecological and/or cytogenetic characters.

The concept of a species differs between biologists, taxonomists, geneticists and palaeobiologists. The widely accepted biological species

concept, given by Mayr (1969), of 'groups of interbreeding natural populations, reproductively isolated from other such groups', does not take into consideration unisexual (all female) populations which occur widely amongst scale insects. Such unisexual life-forms have been excluded from biological species concepts in the past, and labelled 'pseudospecies' by Dobzhansky (1972), 'agamospecies' (Blackman & Day, 1981) or 'microspecies' by botanists.

The most significant limitation of a biological species concept, however, is the absence of a time dimension (Blackman & Day, 1981). Species are the essential units of evolution distinct from one another as each species occupies a separate evolutionary role or niche in the economy of Nature (Blackman & Day, 1981). Simpson (1961) conceived an evolutionary species concept as an ancestor-descendant sequence of populations evolving separately from others and with its own unitary evolutionary role and tendencies. Simpson, however, had a problem of delimiting a species in time and suggested that an evolutionary lineage would have to be divided arbitarily into a succession of species in the fossil record. Phylogeneticists have no conceptual problem in delimiting evolutionary species as they believe that each species exists as a single lineage spanning the time interval between two speciation events. The modes of speciation have been reviewed extensively by White (1978) who emphasised that there is a multiplicity of events that contribute to the evolutionary phenomenon whereby a species splits into two.

In this study, a species concept involving an evolutionary role is used following that given by Meglitsch (1954). A species is a natural population, evolving as a functional unit, or retaining the capacity to evolve as a unit if physical barriers are removed. A species population is the visible manifestation of a pool of genes which retain its character as a unified pool because, in theory, any allele present may eventually come to replace all the allelomorphic factors in the pool, either as a result of interbreeding or as a consequence of simple differential survival in the case of uniparental organisms. Thus, a species is an independent and distinctive region of gene flow, regardless of the mechanisms involved in the distribution of these genes, and is applicable equally to organisms which reproduce sexually and asexually.

3.1.3 Selection of analytical methods

An analytical method is required to determine if groups of field-collected specimens of the P. vitis complex can be consistently segregated morphologically. Multivariate analysis is a useful discriminating method to apply where two or more closely-related groups cannot be segregated consistently by conventional-taxonomic methods. Multivariate analysis is useful for comparing ranges of variation between two or more closely-related groups and for providing graphical representations of such relationships which are easier to interpret than numerical tables. This method is particularly useful when comparing relationships between and within species groups or genera, rather than at higher levels of classification. An important advantage of a multivariate approach, over the analysis of isolated variables, is that it permits an integrated assessment of variation in the life-form in which due regard is given to covariation between the variables.

Principal components analysis, or the R Method, is a method of multivariate analysis that is commonly used for investigating species complexes. Principal components analysis depends on the relationships between variables and is used when no prior interrelationships can be suggested or are suspected. An example of the application of principal components analysis is a study for the resolution of a difficult species complex of medically-important biting midges (Lane, 1981). Davies and Boratynski (1979) carried out one of the most comprehensive taxonomic applications of principal components analysis and principal coordinates analysis, or the Q Method, on 24 species of male armoured scales (Diaspididae).

When interrelationships can be suggested or are suspected prior to analysis, the most appropriate multivariate method is canonical variates analysis. This is used to study interrelationships between a number of groups simultaneously, with the end view of representing the interrelationships between groups graphically in only a few dimensions. It also shows the relative importance of character variation causing the separation. Each canonical-variate axis is evaluated as a linear combination of the original variables, chosen to maximise the segregation between populations relative to the variation between individuals within each population. Successive axes are chosen to discriminate well between groups and to satisfy the additional requirement that each new canonical variate is

uncorrelated to the other variates. A more detailed discussion of the theory of canonical variates analysis is given by Ashton et al. (1957).

In this study, the morphological data was first analysed with principal components analysis to investigate the relationships between variables. Specimens from each geographical area formed groups according to the host-plant species or genus from which they are collected using principal components analysis (see Section 3.3.1). The morphological data was then analysed with canonical variates analysis grouping the specimens, prior to analysis, according to host-plant species or genus from each geographical area. The results of the principal components and canonical variates analyses were compared and the morphological characters causing the separation of specimens according to host plant were examined.

There have been numerous recent publications where multivariate analysis has been applied to entomological and taxonomic problems. Examples of the application of canonical variates analysis include an investigation of races of honey bee (Louis & Lefèbore, 1971), behavioural studies on closely related species of Hawaiian Drosophila (Ringo & Hodosh, 1978) and an investigation on post-embryonic growth of the water bug Notonecta maculata (Cuzin-Roudy & Laval, 1975). Canonical variates together with discriminant function analysis have also been used to separate pairs of closely related species of aphid in the genera Amphorophora and Myzus (Blackman et al., 1977; Blackman & Paterson, 1986; Blackman, 1987).

3.2 Methods

3.2.1 Museum material examined

Depositories

Slide-mounted, field-collected specimens were examined from the following depositories; the collection data for the specimens is listed in Appendix 3.1. Abbreviations for the depositories used in the Appendix are given below:

CDFA Californian Department of Food and Agriculture, Sacramento, U.S.A.

IPP Institute of Plant Protection, Budapest, Hungary

IZAS Institute of Zoology, Academy of Sciences, Lenningrad, U.S.S.R.

MNHN Muséum National d'Histoire Naturelle, Paris, France

NHM Natural History Museum, London, U.K.

NZAC New Zealand Arthropod Collection, Entomology Division, D.S.I.R., Auckland, New Zealand

Material used for analysis

A large amount of slide-mounted material was available from the above depositories (see Appendix 3.1), but only a small proportion of the specimens could be included in the analysis. This is because most preparations were of heavily sclerotised, post-reproductive females whose bodies were distended due to the development of eggs and infested with fungi. It was not possible to record all the morphological characters used in this investigation from such specimens. Post-reproductive females are collected more frequently than teneral females as they are more conspicuous, especially with their large, white ovisacs. Only one or two individuals had been mounted from each locality, which did not give a representative sample. Most of the material used in this study, therefore, had to be freshly collected or reared.

3.2.2 Field collection of Pulvinaria

Collection localities

The majority of samples were collected from Britain, although a small number were freshly collected from France by V. Gavroche, from Hungary by F. Kózar and C. Malumphy and from the Netherlands by C. Malumphy. A large proportion of those collected in Britain were obtained from the London area. A list of collecting sites and reported infestations in Britain during 1987-90, host-plant species, relative population sizes and reference numbers to slides are given in Appendix 3.2.

Timing of collection

For the morphological studies in this work, specimens of the P. vitis complex were collected as teneral adult females which occur from October to April in Britain and Western Europe. For rearing in the laboratory, material was collected as eggs within the ovisacs or as mature females about to oviposit. The ovisacs and mature females occur from the end of April to the end of July depending on the average temperatures of the year and whether they are outdoors or under glass.

Recognition of infestations

Teneral adult females are often extremely difficult to find, due to their small size and cryptic colour and habits. Field descriptions of the adult female and ovisac are given in Appendix 2.2. They usually occur on sheltered

parts of the host plant such as the undersides of branches, in cracks or crevices, under peeling bark or at the base of stems. Large populations of the P. vitis complex were found in Hungary completely hidden beneath the peeling bark of mature vines. The presence of the scales was betrayed by large numbers of ants that attended the infestation.

Populations of the P. vitis complex were more easily found during the spring and summer when their conspicuous ovisacs were present. Once a population was found, a detailed map was drawn showing the precise location of each host plant so that it could be revisited later in the year to collect teneral females. During the winter, ovisacs remaining from the previous generation were usually the first indication of the presence of the P. vitis complex. When collecting from mature birch trees, however, it was found productive to pull off small twigs sprouting from the main trunk and to search the bases for teneral females, regardless of the presence or absence of ovisacs.

Methods of collection and preservation

Specimens of the P. vitis complex were usually collected by taking cuttings of infested parts of the host plant and returning them to the laboratory. Specimens were then carefully transferred to glass vials containing 70% ethanol, or were allowed to dry naturally. Scales preserve better and for longer periods when dried naturally than when kept in alcohol, so long as mould growth is prevented. Alternatively, mature adult females and ovisacs were kept alive for rearing. If it was not possible to take cuttings, scales were carefully placed directly into vials containing 70% ethanol in the field. Host-plant species, locality, date, collector's name, associated insects etc. were recorded at the time of collection. Labels were written in water- and alcohol-insoluble pencil or Indian ink. Gill (1988) reported that soft scales become very difficult or impossible to clear properly when preserved in alcohol, but this was not found to be the case with the P. vitis complex.

A minimum of 10 individuals was collected from each host-plant species at each locality, whenever possible. This was often not achieved, however, as the populations were too small and well dispersed. Usually only a single plant species and often only an individual plant was infested in each locality, despite other potential-host plants being present in the vicinity.

When heavy infestations of the P. vitis complex were found, a large number of specimens (about 50) was collected in order to provide a more representative sample. It was also necessary to collect a large number as there were usually more than one species of scale present, and a high percentage were parasitized. Parasitism could render specimens less suitable for taxonomic purposes (see Section 4.1). Moulting or newly moulted specimens also made poor slide preparations as the appendages were easily distorted and, occasionally, some characters such as wax pores did not take up the stain.

3.2.3 Slide preparation

Fresh or preserved material

Scale insects were examined microscopically for accurate identification and morphometric analysis. They therefore were preserved by mounting on glass microscope slides. Before being mounted, they first needed to be macerated, dewaxed, dehydrated, stained and cleared. Maceration dissolves the body contents, leaving only the exoskeleton. This requires staining to show the microscopic cuticular structures clearly. All specimens examined in this investigation were preserved in Canada balsam, which is currently regarded as the most stable permanent mounting medium.

The preparation technique given below is modified from Martin (1987). The procedure is not rigid and can be readily modified or adapted to suit a particular sample. Specimens had been initially preserved dry or in 70% ethanol (see Section 3.2.2 above). Specimens were manipulated and mounted on microscope slides with the aid of a Vickers binocular dissecting microscope. The pyrex tubes and watch-glasses containing specimens and solutions were heated, where necessary, with a dry heating block. Care was necessary to keep individual samples accurately labelled throughout.

Many of the reagents used in this preparation are potentially dangerous and needed to be treated with care. The health and safety precautions necessary when using the chemicals listed below are given in the HMSO Compendium of Product Safety Data Sheets, according to the Control of Substances Harzardous to Health (COSHH) Regulations.

Slide preparation of Coccidae

1. Specimens were placed in 70-90% ethanol in a pyrex tube which was

gently heated to simmering point for a few minutes. Fixation in hot alcohol made the specimens less fragile so they lost fewer setae during mounting. The alcohol was then decanted using a fine teat pipette, taking care not to accidently suck up the specimens.

2. Approximately $1cm^3$ of 10% potassium hydroxide (KOH) was added and heated to simmering point for approximately 5-10 minutes, or until the specimens lost all or most of their body colour. The length of time required varied, depending on how long the specimens had been preserved in alcohol, the instar and their maturity. Early instars required less time than adults. Large, heavily sclerotised adult females took much longer and it was best to initially make a small dorsal incision above and between the antennal bases, to ensure complete maceration. Alternatively, mature adult female specimens were left in cold KOH for about 24 hours; this method was also better for the more delicate species. Where necessary, as much wax as possible was teased away from the specimens using fine needles. Excess KOH was then decanted.
3. Specimens were soaked in about $2cm^3$ of cold distilled water or 70% ethanol for a minimum of 10 minutes (up to several hours if possible). This rinsed out the remaining KOH and cleared the specimens. The specimens were then tipped into a watch-glass and the liquid decanted.
4. The specimens were rinsed with about $2cm^3$ cold glacial acetic acid which was then decanted.
5. A few drops of chloral-phenol, a wax solvent, were added to the watch-glass, and the specimens were examined under a binocular microscope. Any remaining body contents were expelled by making a small dorsal incision above and between the antennal bases, and pumping the liquified body contents out, using two very fine spatulas. When possible, the main tracheal branches were teased from the spiracles and removed through the dorsal incision, using mounted micropins with curved points. Parasitoid larvae and pupal cases and fungal hyphae were also removed. Parasitoid larvae were retained with the host specimen. The watch-glass was covered with a glass square and gently heated for 10-15 minutes, depending upon how waxy the specimens were. Waxier specimens required longer. Females with ovisacs were often associated with large quantities of wax. In such cases, the chloral-phenol had to be changed one or more times until all the wax was dissolved. The chloral-phenol was then

decanted.

6. Fresh glacial acetic acid was added, together with a drop of acid fuschin stain. The watch-glass was agitated until the acid was a uniform pink colour. The stain was left for about two minutes or until the specimen acquired a pink tinge. The liquid was then decanted. If the specimen was over-stained, it was soaked in 70% ethanol until the excess stain dissolved, then returned to glacial acetic acid.
7. To remove excess stain, the specimens were rinsed with fresh glacial acetic acid, which was then decanted.
8. Fresh glacial acetic acid was added and the specimen were left for at least 5 minutes to completely dehydrate. This was again decanted.
9. A few drops of clove oil were added, enough to allow the specimens to float freely, and left for at least 10 minutes while the specimens cleared. Whilst in clove oil, any remaining body contents were removed in the same way as described in stage 5 above. More care had to be taken, however, not to knock setae off after the specimens had been dehydrated.
10. A single specimen with its ventral surface upwards, was transferred with a fine spatula on to a clean 16 or 18mm diameter coverslip. Parasitoid larvae were usually mounted with their host. Specimens were always mounted separately unless it was absolutely certain that they were the same species, in which case, up to four individuals of the same species were mounted together on the same slide. The specimens had to be evenly spaced on the coverslip to avoid it tilting when mounted. Each scale body was spread out and the appendages arranged. Excess clove oil was absorbed with the rolled corner of a tissue. Care was taken not to leave fibres from the tissue on the slide. A drop of dilute Canada balsam was applied to a glass slide and the slide quickly inverted. The droplet of balsam on the slide was then carefully placed onto the specimen and coverslip. Care was taken to ensure that air was excluded and that the meniscus had spread outwards to the edge of the coverslip, before the slide was turned the correct way up. The coverslip was allowed to settle under its own weight.
11. The slide was labelled using bristol board squares before being placed in a drying oven at 40°C for about 6 weeks. Excess balsam which had spread out from beneath the coverslip was scraped off, after it had hardened, with a razor blade.

Remounting slide material

It was sometimes necessary to remount a potentially useful specimen because it was unstained, inadequately cleared or poorly mounted. The glass slide was scored either side of the coverslip with a diamond pen and the slide was broken into three sections. The two outer sections were soaked in water to remove the labels. The central section with the specimen was placed in a large watch-glass containing xylene and covered with a glass square. The actual solvent used depends on the mountant; xylene is appropriate for Canada balsam. This was left for 24 hours or until the mountant had dissolved. Less time was required if the slide had recently been made. The specimen was then transferred with a fine spatula to glacial acetic acid, before being remounted according to the method outlined above. If possible, the original labels were stuck on the new slide as they were often of historical value.

3.2.4 Method of illustration

The method most commonly used to illustrate Coccoidea morphology was originally developed by Ferris (1948). These illustrations are basically maps with symbols to show the distribution and relative abundance of the different pores, ducts and setae. Although these maps are time consuming to produce, they save considerable time for all subsequent workers and can be more informative than lengthy descriptions (Williams, 1985).

In this study, each illustration was prepared from a single, slide-mounted, teneral female specimen with the ventral aspect illustrated on the right side and the dorsal aspect on the left. The specimen was carefully chosen to be representative of the population and to have at least one half of the body intact and to be not noticeably distorted. Enlarged details of important characters were drawn around the main illustration of the body (see Fig. 2.3). They were illustrated using a Zeiss microscope with phase contrast (x 100-400) and a camera lucida. The drawings were initially made on A3 size paper so that a lot of detail could be included, and then reduced to A4 size paper. The magnification of each illustration varied to fit the page size although measurements are given in the morphological descriptions. Scaling was not possible and of limited use because the body shape and size changes so drastically with age in relation to other constant structures, such as appendages, due to allometric growth after maturation.

3.2.5 Selection of morphological characters

Only adult females were studied in the morphological investigations for the reasons outlined in Sections 2.1 and 2.2. Adult female Coccidae possess many morphological characters potentially useful for investigation. Important characters include appendages, setae and wax-producing structures such as pores and ducts. The characters investigated were chosen on the basis of two criteria. Firstly, diagnostic characters recently used in keys (Borchsenius, 1957; Danzig, 1986; Gill, 1988; Kosztarab & Kozár, 1988; Hamon & Williams, 1984). Secondly, characters were included to provide a more balanced view of the structures available. This second category permitted investigation of characters, not traditionally used for diagnosis. Initially, 28 characters were chosen, 13 measured and 15 enumerated. In addition, 2 characters were derived from 6 of the primary characters.

The measurements of slide-mounted aphids, particularly diameters of tubular appendages, can vary according to the mountant used and the thickness of preparation (MacGillivray, 1958). Only a single mountant, Canada balsam, was therefore used in this investigation to avoid introducing an additional source of variation.

Full character set

Generally only teneral females were used because it was not possible to record all the characters from heavily sclerotised, post-reproductive females. Some characters such as the numbers of wax pores and tubular ducts were always more difficult to observe on older specimens. Characters which have recently been used in identifying the nominal species in the complex are numbered below: 1, 6, 7, 8, 9, 22 (Kosztarab & Kozar, 1988; Danzig, In Bei-Bienko, 1987). Examples of characters commonly included in descriptions are numbered below: 2, 3, 4, 5, 10, 12, 13, 14, 15, 16, 17, 28, 30 (Borchsenius, 1957; Gill, 1988; Kosztarab & Kozár, 1988; Danzig, 1986). Finally, characters chosen to provide a more balanced view of the structures avaliable are numbered below: 11, 18, 19, 20, 21, 23, 24, 25, 26, 27, 29. All the characters recorded are shown in Figs. 2.3, 2.4 and 3.1.

Abbreviations which are used in Tables, Figures and Appendices where there are space constraints, are given in parentheses after the name of the character. Several of the measured characters are given as means because most of them occur in pairs due to the bilateral symmetry of the body.

A. Measured characters

Body

1. Maximum length of body (BodyL, Fig. 2.3) *10 μm*

   This was measured along a midline between the extremities of the head and the longest anal flap (anal flaps were, occasionally, considerably unequal in length). Overall body length and width can increase by up to 6 times between the final moult and oviposition but were included in the analysis as body size does appear to be influenced by host-plant species.

2. Width of body (BodyW, Fig. 2.3) *10 μm*

   This was measured between the bases of the posterior spiracular setae and did not take into account convexity of the dorsum.

Dorsal appendages

The appendages, both dorsal and ventral, give a better indication of size than body length and width, as they vary little after the final moult.

3. Mean maximum length of anal operculum (OperL, Fig. 2.4) *μm*

   The anal opercula were prone to distortion during slide-mounting adding another source of variation, particularly according to whether they were closed, or partially or fully open.

4. Mean maximum width of anal operculum (OperW, Fig. 2.4) *μm*

   This was measured perpendicularly from the inner margin to the outer angle. Care was necessary to identify the outer angle when the opercula were open.

Ventral appendages

5. Mean length of antenna (AntL, Fig. 2.3) *μm*

   Care was necessary to record the total straight length as the sum of the segment lengths when antennae were bent or curved.

6. Maximum length of clypeal shield and labium combined (MouthL, Fig. 2.4) *μm*

   The mouthparts were often broken or distorted, making both length and width difficult to record. In some specimens the lengths of the clypeal shield and labium had to be measured separately and then combined.

7. Maximum width of clypeal shield (MouthW, Fig. 2.4) *μm*
8. Mean length of hind femur + trochanter (HFTL, Fig. 2.4) *μm*
9. Mean length of hind tibia, tarsus and claw (HTTL, Fig. 2.4) *μm*

   Care was necessary to measure the straight length of tibia and

tarsus when they were at an angle to each other.

Setae

10. Length of longest dorsal body seta adjacent to preopercular pores (DStL, Fig. 2.3) μm

Seta length varies over the dorsum, the setae near the margin and in two mid-lateral rows bordering the preopercular pores being the longest. The seta measured was situated just anterior to the opercula and bordering the preopercular pores. It had to be as close to horizontal as possible to record the true length and did not include the base.

11. Length of ventral body seta anterior to antennal base (VStL, Fig. 2.3) μm

The seta chosen was situated between an antennal base and the front margin. The precise location varied slightly, but did not include submarginal setae, which were longer.

12. Length of central anterior spiracular seta (CSpStL, Fig. 2.3) μm

The spiracular setae were particularly variable in length, shape and even number. When the two central anterior spiracular setae varied noticeably in length, a mean was taken. The ratio of the lengths of central and lateral spiracular setae have been used in the separation of this group.

13. Length of lateral anterior spiracular seta (LSpStL, Fig. 2.3) μm

The two lateral spiracular setae in each group also sometimes varied considerably in length. In such cases a mean length was also recorded.

B. Enumerated characters

Appendages

14. Mean number of segments per antenna (AntSegN, Fig. 2.3)

Segments which were only partially divided were considered single. They were numbered from the base to the terminal segment. The antennal segments most often fused were 3 + 4 and occasionally 4 + 5.

Setae

15. Numberof setae present in a semi-circle between the antennal bases (InAntSt, Fig. 2.3)

All setae in the semi-circular row were counted, including setae that were no longer than the average ventral body setae.

16. Number of marginal setae present between anterior and posterior spiracular setae in row 1 (MgSt1, Fig. 3.1)

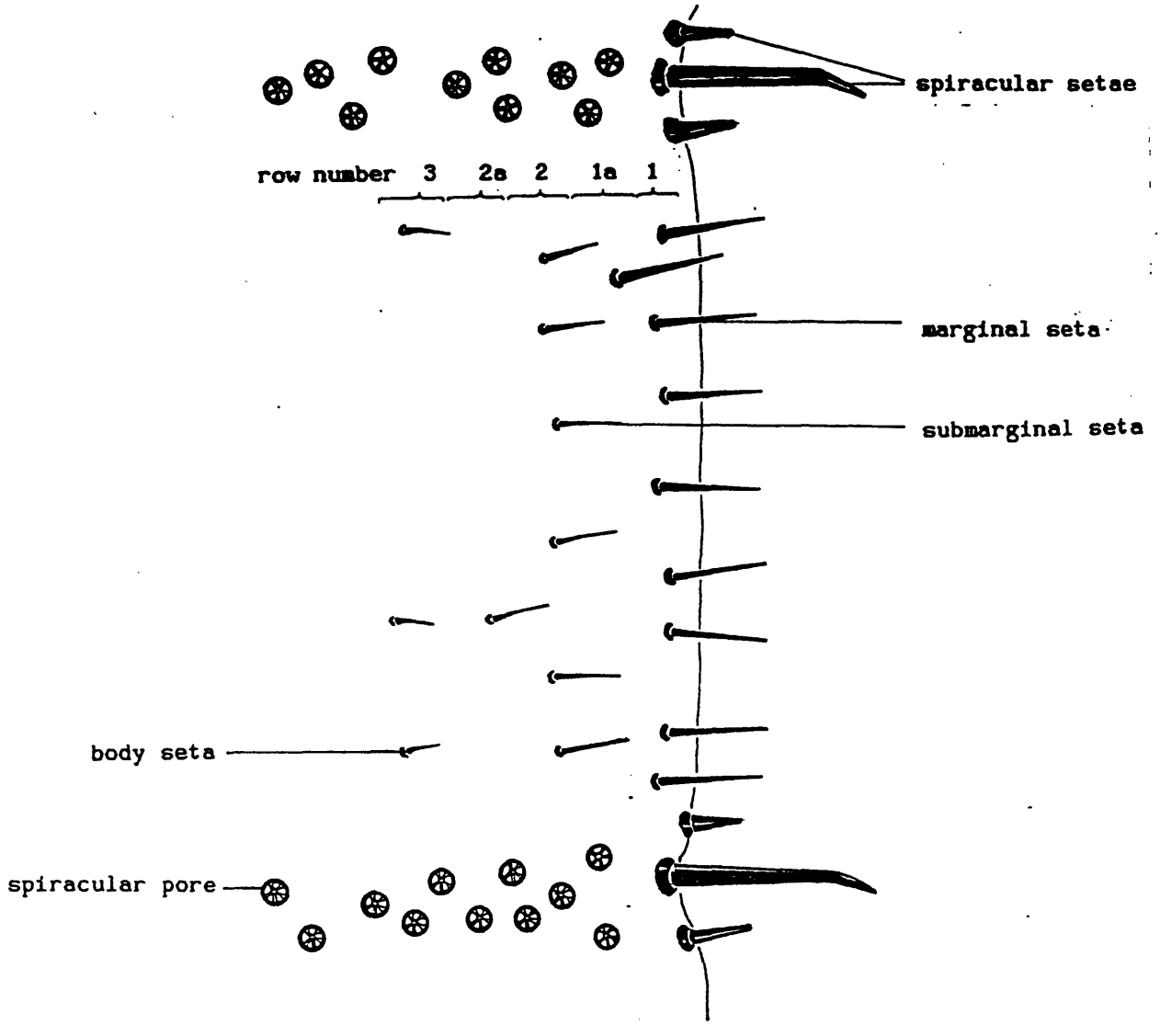


Fig. 3.1 Details of the morphological characters selected for analysis

Number and arrangement of marginal and submarginal setae varied considerably, sometimes even on either side of the same individual. There were usually two main rows, marginal (row 1) and submarginal (row 2), but many setae could not be satisfactorily allocated to one group or the other. The setae were therefore allocated to 5 rows because the arrangement was so irregular. Row 1 consisted of the outer most row of marginal setae.

17. Number of marginal setae present between anterior and posterior spiracular setae in row 1a (MgSt1a, Fig. 3.1)

This consisted of setae located between the main rows of marginal (row 1) and submarginal (row 2) setae. Submarginal setae are shorter than marginal setae but a strict division was not always possible. Row 1a usually consisted only of displaced marginal setae but could include both types.

18. Number of submarginal setae present between anterior and posterior spiracular setae in row 2 (SMgSt2, Fig. 3.1)

This consisted of the main row of submarginal setae.

19. Number of submarginal setae present between anterior and posterior spiracular setae in row 2a (SMgSt2a, Fig. 3.1)

This usually contained only a few setae but on some individuals formed a complete third row.

20. Number of submarginal/body setae present between anterior and posterior spiracular setae in row 3 (SMgSt3, Fig. 3.1)

Submarginal setae are longer than other ventral body setae. Row 3 actually consists of ventral body setae rather than submarginal setae.

Wax-producing pores and ducts

21. Number of preopercular pores (PreoPN, Fig. 2.3)

These were distinguished from other dorsal pores by having thicker rims and were generally much larger. Preopercular pores joined or with more than one loculus were recorded as single. These pores were very difficult to count in older specimens as they become translucent and widely dispersed. They varied considerably in diameter, rim thickness and number.

22. Number of spiracular pore present in an anterior spiracular furrow (SpirPN, Fig. 2.3)

The pores were counted in the spiracular furrow which had the least

disturbance such as folding or overlapping derm. All the pores, including those located around the spiracle and by the front coxa, were counted regardless of number of loculi present in each pore. Most were quinquelocular but some were multilocular near the coxa.

23. Number of multilocular pores present on abdominal segments 1+2 (MPN12, Fig. 2.3)

    Ventral segmentation is obscure but can usually be detected in the mid-thoracic and mid-abdominal regions. The numbering of abdominal segments is controversial but the system used here follows Hamon & Williams (1984). Multilocular pores in the three rows preceeding the prevulvular setae were counted. The rows were usually distinct even if the abdomenal segmentation was unclear. Occasionally, the pores did not take up the stain during slide preparation, particularly in mature, heavily sclerotised or newly moulted individuals and were therefore very difficult to see.

24. Number of multilocular pores present on abdominal segment 3 (MPN3, Fig. 2.3)

25. Number of multilocular pores present on abdominal segment 4 (MPN4, Fig. 2.3)

26. Number of tubular ducts, each with a thick inner filament, present on the head anterior to the antennal bases (TubDucL, Fig. 2.3)

    An imaginary line was drawn transversely across the head, just anterior to the antennal bases, and the ducts counted anterior to this line. Two types of tubular duct with thick inner filaments were counted. One type had the duct and filament almost equal in length, the second type had a longer filament than duct. Care was taken not to score dorsal tubular ducts. The numbers of tubular ducts were difficult or impossible to score in older sclerotised specimens as they were hardly visable.

27. Number of tubular ducts with slender inner filament, present on the head anterior to the antennal bases (TubDucS, Fig. 2.3)

28. Number of submarginal tubercles (SubTubN, Fig. 2.3)

    Submarginal tubercles were sometimes difficult to see on both newly moulted teneral females and on mature, post-reproductive females. They varied considerably, both in size and in distance from the margin. They were smaller and situated closer to the margin on teneral females.

C. Derived characters

29. Structural ratio of hind leg (Ratio, Fig. 2.4)

    Mean length of hind femur and trochanter (character 8) / mean length of hind tibia, tarsus and claw (character 9).

30. Total number of submarginal setae present between anterior and posterior spiracular setae (TSMgSt, Fig. 2.3)

    This is equal to the sum of the setae allocated to rows 1a, 2, 2a and 3 (characters 17+18+19+20).

Reduced character set

The number of characters was reduced to make analysis quicker, and to eliminate unnecessary data. By reducing the number of characters, a greater number of specimens could be included in the analysis.

The initial analyses using the 28 primary or 22 primary and 2 derived characters were intended to give an idea of the relative importance of each of the characters to the analysis. The number of characters was gradually reduced after canonical variates and principal components analyses were conducted and the plots and intergroup distances were examined. The importance of each character on any particular axis was indicated by the latent vector loadings for that axis, which were considerably different when comparing the two methods of analysis. A series of canonical variates analyses was carried out, removing the least significant character each time. The number of characters was thus reduced and the effect on the results observed. The number of primary characters was reduced to 10, with good congruence between plots based on the full and reduced character data sets. Other factors considered when eliminating characters from the investigation were the time taken to record the character, ease of recording, reliability in older specimens and correlation with other characters in the data set. All the characters used for multivariate analysis, theoretically, should be independent of each other to avoid bias in the plots. In practice this is impossible, but characters were chosen that have as little correlation with one another as possible.

The latent vector loadings indicated that the numbers of marginal and submarginal setae present between the anterior and posterior spiracular setae were some of the most significant characters. Unfortunately, these characters are all highly correlated with one another. They would also be

scored differently by different people and could even be scored differently by the same person when repeated at a later date. Therefore, characters 17+18 and 19+20 above, were combined to reduce the number of closely correlated characters and to reduce the risk of adding subjectivity as another source of variation.

The final 8 characters retained after interim analysis which were the most important in the latent vector loadings of the canonical variates analysis were:

1. Length of dorsal body seta present, adjacent to preopercular pores (DStL)
2. Number of marginal setae present between anterior and posterior spiracular setae in rows 1+1a (MgSt)
3. Number of submarginal setae present between anterior and posterior spiracular setae in rows 2+2a (SMgSt)
4. Number of setae present in a semi-circle between the antennal bases (InAntSt)
5. Number of dorsal submarginal tubercles (SubTubN)
6. Number of tubular ducts with thick inner filament situated on the head anterior to the antennal bases (TubDucL)
7. Number of tubular ducts with slender inner filament situated on the head anterior to the antennal bases (TubDucS)
8. Number of multilocular pores present on abdominal segment 4 (MPN4)

Comparison between the full and reduced-character set

There is good congruence between the CVA plots in Figs. 3.4 and 3.5 of the field-collected British specimens arranged into 14 host-plant groups (see Section 3.2.6 below), using the full 28 (n = 238) and reduced 8 (n = 335) character sets, respectively. In both CVA plots, the specimens collected from grapevine separate out from the majority of specimens along canonical variate 1, whereas the specimens collected from birch separate out from the majority of specimens along canonical variate 2. In both analyses, the most important characters in the latent vector loadings of the first five canonical variates are the numbers of marginal, submarginal and interantennal setae. The only significant difference is the amount of variance accounted for by the first two canonical variates. In Fig. 3.4 only 45% of the total variance is accounted for by the first two axes, compared to 71% in Fig. 3.5.

There is, therefore, no significant loss of information in reducing the number of primary characters from 28 to 8. When the number of characters was reduced further, the grouping of the specimens according to host-plant species became less clear, and there were significant differences between the full and reduced character plots. The minimum number of characters found to produce similar results to the full character set was 8.

3.2.6 Statistical analysis

Grouping data for canonical-variates analysis

Specimens of the P. vitis complex were grouped for the canonical variates analysis according to the host-plant species or genera and geographical area from which they were collected. Some specimens were grouped according to the host genus rather than species because of the uncertainty of the identity of the host species. For example, specimens from Ribes nigrum, R. sanguineum, R. uva-crispa and R. sylvestris were treated as separate groups although specimens collected from the genus Betula were grouped together. There were so few specimens collected from some closely related host species that these were also grouped together, such as individuals collected from Sorbus aria and S. aucuparia. Specimens of the P. vitis complex were allocated to a total of 14 host-plant groups listed in Table 3.1. Four geographical areas were recognised, Britain, mainland Europe, North America and New Zealand.

It was initially intended that parasitized specimens should be removed from the analyses to exclude the addition of another source of variation. This was not possible however, as the occurance of parasitism was so high, particularly amongst individuals reared in the host-transfer experiments in Chapter 5. The effects of parasitism on morphology are evaluated in Chapter 4, in order to assess its significance on the results of this chapter. It is important to stress that parasitism was not found to have any significant effect on the morphological variation of the P. vitis complex in this study.

Computers used for analysis

The computers used were Cyber 180/855 and Cyber 180/930 at Imperial College Computer Centre (ICCC) with NOS 2.4 and NOS/VE; Amdahl 5890 model 300 (MVS Service) with Phoenix 3 at University of London Computer Centre (ULCC); and the IBM 6150 with RT Advanvced Interactive Executive (AIX)

Operating System Version 2.2.1, at the Natural History Museum. The following software packages were used for the analyses: Genstat 4 Release 04 and Genstat 5 Release 1.3, Lawes Agricultural Trust (Rothamsted Experimental Station); SPSS-X Release 2.0 from Northwestern University; Minitab developed by the staff of the Statistics department of Pennsylvania State University; Taxpack developed by Ian White of the International Institute of Entomology, an Institute of CAB International.

3.3 Results

3.3.1 Field-collected specimens from Britain

Principal components analysis of British specimens

British field-collected specimens segregate due to size differences and show some grouping according to the host-plant species or genus from which they are collected, using principal components analyses (PCA). Fig. 3.2 shows the first two principal components plotted for the PCA of the British field-collected specimens (n = 238) using the full 28 character set. Separation of individual specimens occurs mostly along the first principal component, which accounts for 70% of the total variation; the second principal component accounts for a further 11% of the variation. Two characters dominate the latent vector loadings of the first principal component: body length and width. The most significant characters in the latent vector loadings of the second principal component are the lengths of the antenna, leg segments and body width. It can be seen from Fig. 3.2 that the specimens collected from grapevine, birch and peach are generally larger than specimens collected from hawthorn, flowering currant and blackcurrant, regardless of the locality from which they are collected. The specimens group according to the host plant and not locality, as the samples from each host-plant species or genus are collected from several different localities.

The first five latent vector loadings of the PCA are dominated by measured rather than enumerate characters which is the reverse of the situation in the canonical variates analyses (see below).

Body length and width dominated the first analysis but these characters are a poor indication of size as they vary considerably with age. The PCA was repeated with body length and width removed; the result is plotted in Fig. 3.3. The resulting PCA plot was similar to the PCA plot in Fig. 3.2, being dominated by the other measured characters. There was a greater

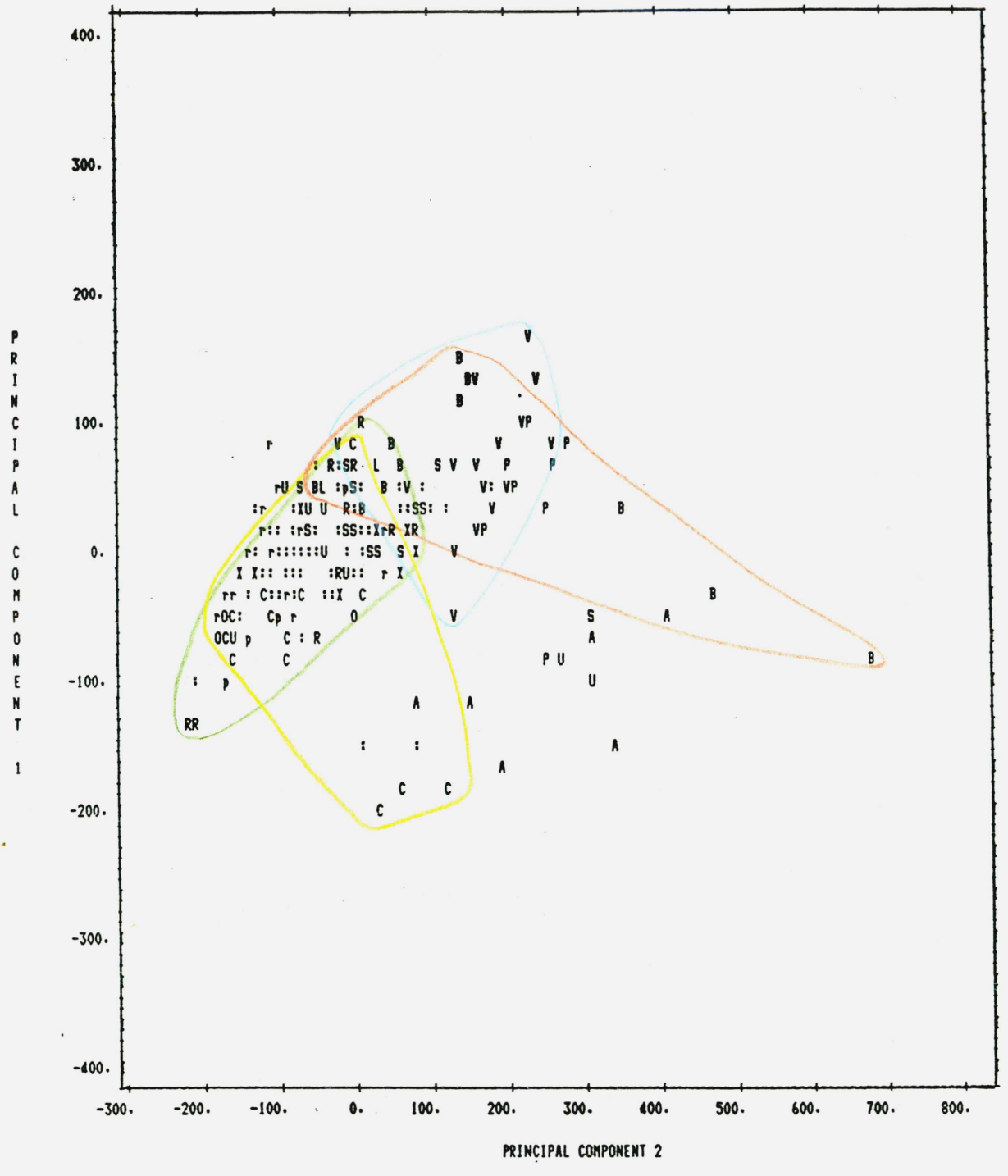


Fig. 3.2 Principal components analysis of British field-collected specimens of the Pulvinaria vitis complex, arranged into 14 host-plant groups, using 28 morphological characters; n = 238

Key for Figs 3.2–3.5
Host-plant groups: A = Alnus glutinosa; B = Betula spp.; C = Crataegus monogyna; E = Euonymus sp.; P = Prunus spp.; p = Pyracantha coccinea; R = Ribes nigrum; r = Ribes sanguineum; L = Ribes sylvestre; U = Ribes uva-crispa; S = Salix spp.; X = Saxifraga sp.; O = Sorbus spp.; V = Vitis vinifera; : = overlapping groups.

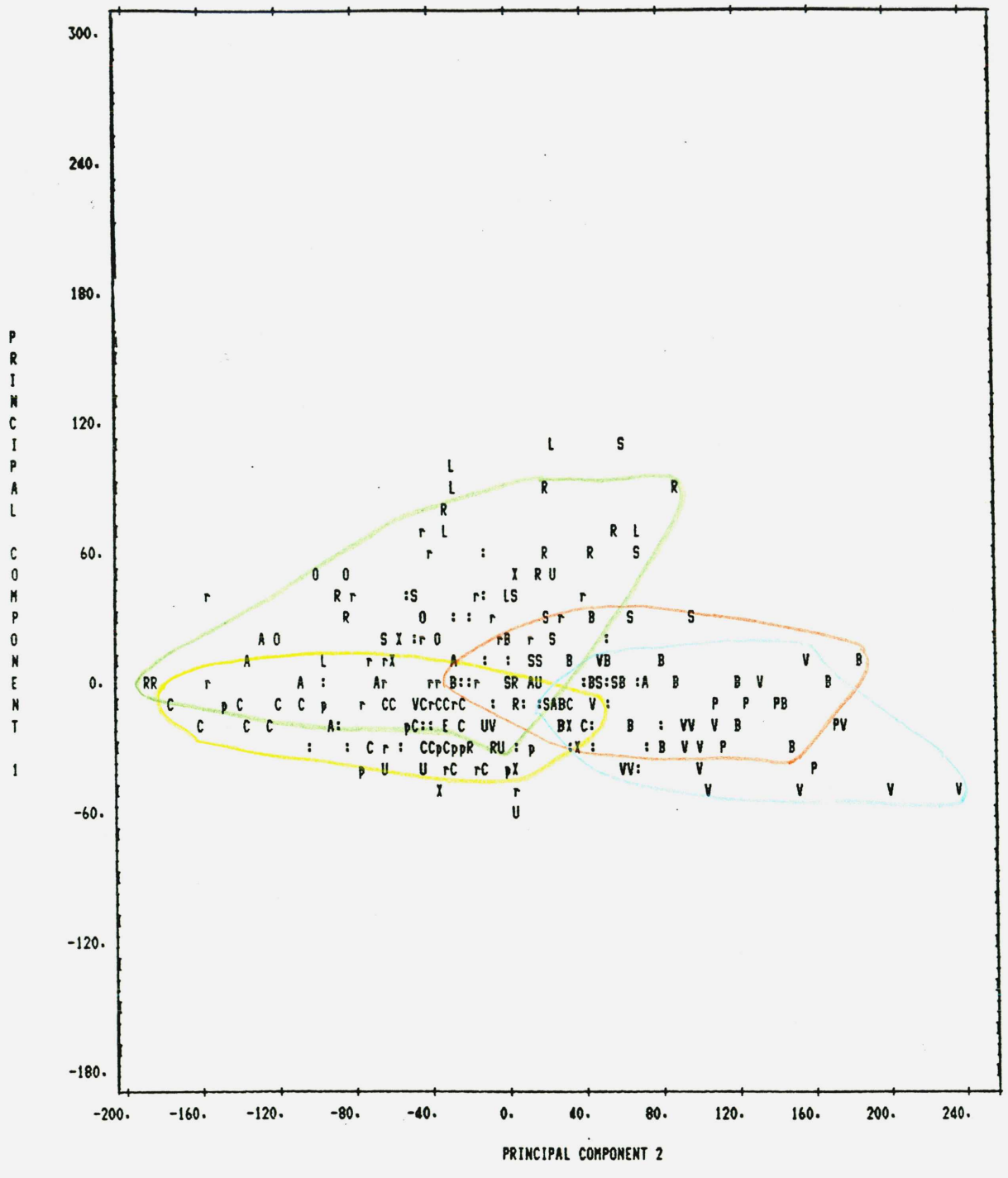


Fig. 3.3 Principal components analysis of British field-collected specimens of the Pulvinaria vitis complex, arranged into 14 host-plant groups, using 26 morphological characters; n = 238
See Fig. 3.2 for key to the host-plant groups.

spread however, along the second principal component. The specimens also grouped according to host plant when the effect of size was removed from the analysis but the grouping was less clear.

To summarise, British specimens group according to the host-plant species or genus from which they were collected using principal component analyses. The most important characters in the latent vector loadings were measured.

Canonical variates analysis of British specimens

British field-collected specimens clearly group according to the host-plant species or genus from which they are collected, using canonical variates analysis (CVA). Figs. 3.4 and 3.5 show the results of the CVA of the field-collected specimens. Only the first two canonical variates are plotted as most of the variation is accounted for in these two axes and these plots show the clearest separation. Fig. 3.5 shows the CVA plot for field-collected specimens from Britain (n = 335) using the reduced number of characters. The first canonical variate accounts for 42% of the total variation and the second for 30%. The specimens collected from grapevine separate out from the majority of specimens along the first axis. The most significant character in the latent vector loadings of canonical variate 1 is the number of interantennal setae. The specimens collected from birch separate out from the majority of specimens along the second axis. The most significant characters in the latent vector loadings of canonical variate 2 are the numbers of marginal and submarginal setae present between the anterior and posterior spiracular setae, and to a lesser extent the number of interantennal setae. The two groups of specimens with their group means furthest apart were those collected from birch and saxifrage. The two groups of specimens with the closest group means were collected from Euonymus spp. and Sorbus spp. The separation of specimens into host-plant groups was more distinct when the number of host-plant groups were reduced.

To summarise, both canonical variates and principal components analyses segregate British field-collected specimens of the P. vitis complex into groups according to the species or genus of host plant from which they are collected. These results using multivariate analyses show that the morphological variation of the P. vitis complex is associated with host-plant species; the results do not show whether this is variation is genetically or environmentally induced. The most important morphological characters in the

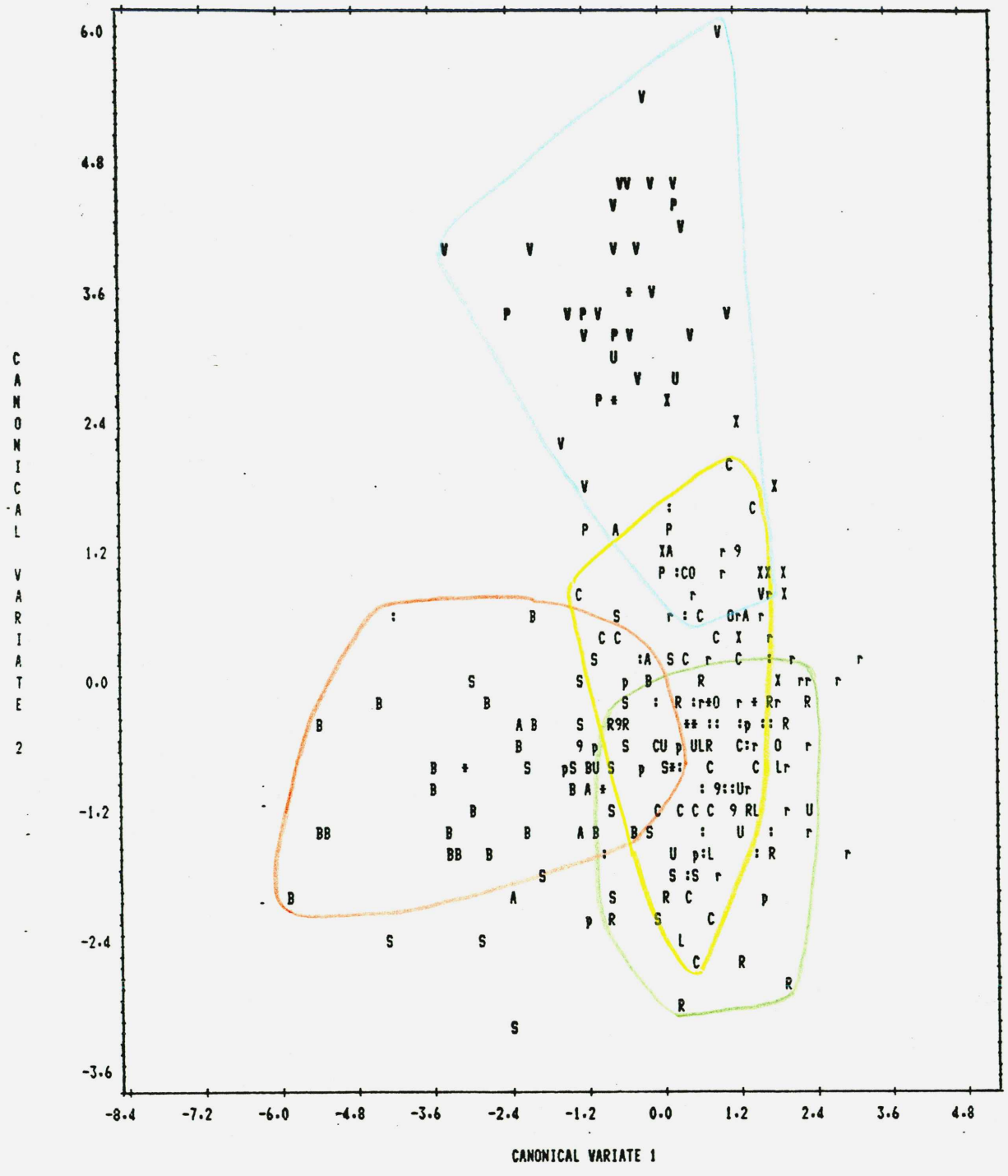


Fig. 3.4 Canonical variates analysis of British field-collected specimens of the Pulvinaria vitis complex, arranged into 14 host-plant groups, using 28 morphological characters; n = 238
See Fig 3.2 for key to the host-plant groups; scores for means (*)

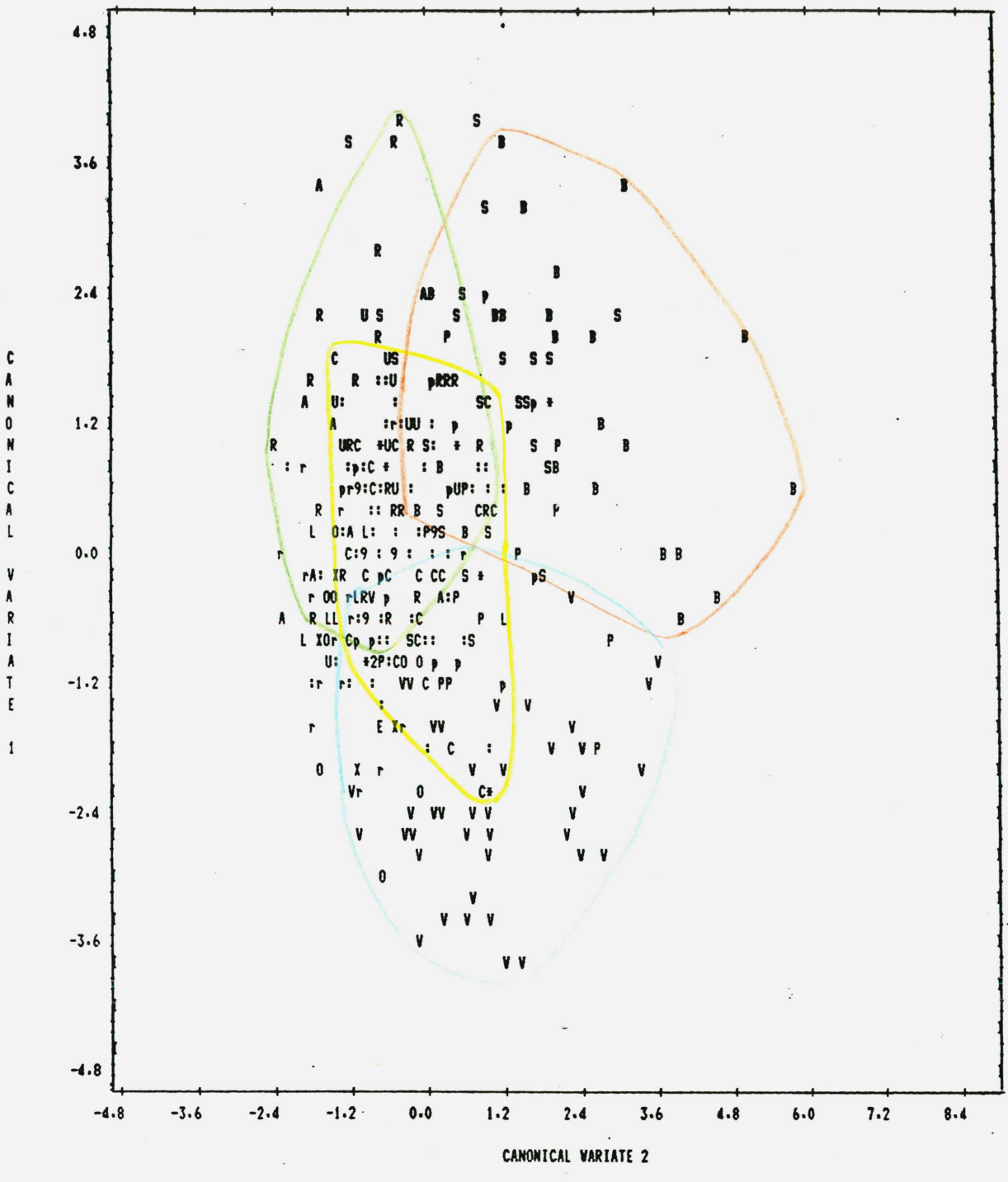


Fig. 3.5 Canonical variates analysis of British field-collected specimens of the Pulvinaria vitis complex, arranged into 14 host-plant groups, using 8 morphological characters; n = 335
See Fig 3.2 for key to the host-plant groups; scores for means (*)

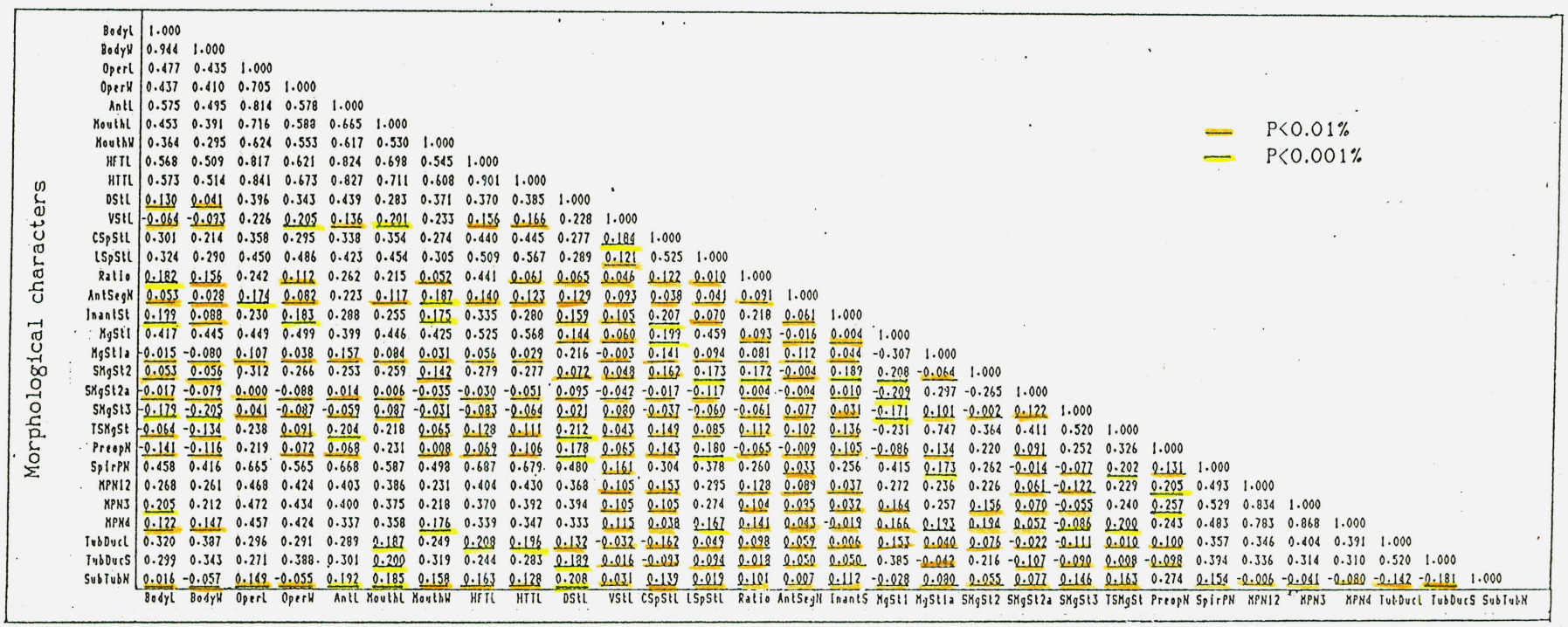
separation of individuals using principal components analyses were measured, in particular body length and width. The most important characters in the separation of groups of specimens using canonical variates analyses were enumerate, in particular the number of interantennal setae, numbers of marginal setae present between the spiracular setae and the dorsal seta length. These enumerate characters are highly correlated with the size of the individual, which is in turn, related to the potential fecundity of the insect.

Correlation between characters

The correlation matrix for the 28 primary and 2 derived morphological characters recorded from 238 field collected specimens, significant at the 0·01% and 0·001% level, is given in Table 3.1. Of the 406 correlation values (derived characters are excluded from the following discussion), only 236 (58%) are significant at the 0·01% level and 206 (51%) at the 0·001% level; 59 (14.5%) of the correlation values between characters are negative but only 2 of the negative values are significant at 0·001%. Most of the smaller values are insignificant at the 0·01% and 0·001% level.

There are a large number of high positive values with 15 values over 0·70 significant at 0·001%. All the measured characters are highly correlated with one another, except for the lengths of setae, suggesting isometric growth. Ventral setae length has almost no significant correlation values with any of the other characters at the 0.001% significance level. The measured characters with the greatest positive correlation with all other characters, significant at the 0·001% level, are: opercular length and width, antennal length, mouth parts length, hind femur and trochanter length, and hind tibia and tarsal length; suggesting some degree of isometric growth. Lengths of appendages have greater correlation with indirectly associated characters than with body length and width, suggesting that they provide a better indication of size. For example, the highest absolute correlation values at 0·001% significance with an indirectly associated character for body length is 0·58 and body width 0·51, compared to 0.84 for opercular length, 0.83 for antennal length and 0.82 for hind femur and trochanter length. There is relatively little allometric growth which was expected as both body length and width vary considerably with maturity.

Three enumerate characters are also highly correlated with the measured



	BodyL	BodyW	OperL	OperW	AntL	MouthL	MouthW	HFTL	HTTL	DStL	VStL	CSpStL	LSpStL	RatIo	AntSegW	InantS	MgSt1	MgSt1a	SMgSt2	SMgSt2a	SMgSt3	TSMgSt	PreopN	SpirPN	MPN12	MPN3	MPN4	TubDucL	TubDucS	SubTubN
BodyL	1.000																													
BodyW	0.944	1.000																												
OperL	0.477	0.435	1.000																											
OperW	0.437	0.410	0.705	1.000																										
AntL	0.575	0.495	0.814	0.578	1.000																									
MouthL	0.453	0.391	0.716	0.588	0.665	1.000																								
MouthW	0.364	0.295	0.624	0.553	0.617	0.530	1.000																							
HFTL	0.568	0.509	0.817	0.621	0.824	0.698	0.545	1.000																						
HTTL	0.573	0.514	0.841	0.673	0.827	0.711	0.608	0.901	1.000																					
DStL	0.130	0.041	0.396	0.343	0.439	0.283	0.371	0.370	0.385	1.000																				
VStL	-0.066	-0.093	0.226	0.205	0.136	0.201	0.233	0.156	0.166	0.228	1.000																			
CSpStL	0.301	0.214	0.358	0.295	0.338	0.354	0.274	0.440	0.445	0.277	0.186	1.000																		
LSpStL	0.324	0.290	0.450	0.486	0.423	0.454	0.305	0.509	0.567	0.289	0.121	0.525	1.000																	
RatIo	0.182	0.156	0.242	0.112	0.262	0.215	0.052	0.441	0.061	0.065	0.046	0.122	0.010	1.000																
AntSegW	0.053	0.028	0.174	0.082	0.223	0.117	0.187	0.140	0.123	0.129	0.093	0.038	0.041	0.091	1.000															
InantSt	0.199	0.088	0.230	0.183	0.288	0.255	0.175	0.335	0.280	0.159	0.105	0.207	0.070	0.218	0.061	1.000														
MgSt1	0.417	0.445	0.449	0.499	0.399	0.446	0.425	0.525	0.568	0.144	0.060	0.199	0.459	0.093	-0.016	0.004	1.000													
MgSt1a	-0.015	-0.080	0.107	0.038	0.157	0.084	0.031	0.056	0.029	0.216	-0.003	0.141	0.094	0.081	0.112	0.044	-0.307	1.000												
SMgSt2	0.053	0.056	0.312	0.266	0.253	0.259	0.142	0.279	0.277	0.072	0.048	0.162	0.173	0.172	-0.004	0.189	0.208	-0.064	1.000											
SMgSt2a	-0.017	-0.079	0.000	-0.088	0.014	0.006	-0.035	-0.030	-0.051	0.095	-0.042	-0.017	-0.117	0.004	-0.004	0.010	-0.209	0.297	-0.265	1.000										
SMgSt3	-0.179	-0.205	0.041	-0.087	-0.059	0.087	-0.031	-0.083	-0.064	0.021	0.080	-0.037	-0.060	-0.061	0.077	0.031	-0.171	0.101	-0.002	0.122	1.000									
TSMgSt	-0.064	-0.134	0.238	0.091	0.204	0.218	0.065	0.128	0.111	0.212	0.043	0.149	0.085	0.112	0.102	0.136	-0.231	0.747	0.364	0.411	0.520	1.000								
PreopN	-0.141	-0.116	0.219	0.072	0.068	0.231	0.008	0.069	0.106	0.178	0.065	0.143	0.180	-0.065	-0.009	0.105	-0.086	0.134	0.220	0.091	0.252	0.326	1.000							
SpirPN	0.458	0.416	0.665	0.565	0.668	0.587	0.498	0.687	0.679	0.480	0.161	0.304	0.378	0.260	0.033	0.256	0.415	0.173	0.262	-0.014	-0.077	0.202	0.131	1.000						
MPN12	0.268	0.261	0.468	0.424	0.403	0.386	0.231	0.404	0.430	0.368	0.105	0.153	0.295	0.128	0.089	0.037	0.272	0.236	0.226	0.061	-0.122	0.229	0.205	0.493	1.000					
MPN3	0.205	0.212	0.472	0.434	0.400	0.375	0.218	0.370	0.392	0.394	0.105	0.105	0.274	0.104	0.095	0.032	0.164	0.257	0.156	0.070	-0.055	0.240	0.257	0.529	0.834	1.000				
MPN4	0.122	0.147	0.457	0.424	0.337	0.358	0.176	0.339	0.347	0.333	0.115	0.038	0.167	0.141	0.043	-0.019	0.166	0.193	0.194	0.057	-0.086	0.200	0.243	0.483	0.783	0.868	1.000			
TubDucL	0.320	0.387	0.296	0.291	0.289	0.187	0.249	0.208	0.196	0.132	-0.032	-0.162	0.049	0.098	0.059	0.006	0.153	0.040	0.076	-0.022	-0.111	0.010	0.100	0.357	0.346	0.404	0.391	1.000		
TubDucS	0.299	0.343	0.271	0.388	0.301	0.200	0.319	0.244	0.283	0.189	0.016	-0.093	0.094	0.018	0.050	0.050	0.385	-0.042	0.216	-0.107	-0.090	0.008	-0.098	0.394	0.336	0.314	0.310	0.520	1.000	
SubTubN	0.016	-0.057	0.149	-0.055	0.192	0.185	0.158	0.163	0.128	0.208	0.031	0.139	0.019	0.101	0.007	0.117	-0.028	0.080	0.055	0.077	0.146	0.163	0.274	0.154	-0.006	-0.041	-0.080	-0.142	-0.181	1.000

Table 3.1 Correlation matrix for 30 morphological characters recorded from British field-collected specimens of the Pulvinaria vitis complex, significant at the 0·01 % and 0·001 % level; n = 238; character abreviations are those listed in Section 3.2.5

characters and therefore with size. These are the number of spiracular pores, marginal setae and to a lesser extent multilocular pores on abdominal segments 1 and 2.

The body increases in width more than in length with age, thus becoming broader with maturity. The operculum also becomes broader with over-all size. The femur and trochanter were found to contribute a greater proportion than the tibia and tarsus to over-all leg length with increasing size.

The numbers of wax-producing multilocular pores, and to a lesser extent spiracular pores are highly correlated with one another. They were also found to be highly correlated with size as indicated by opercular length and width. The number of large and small tubular ducts are highly correlated with one another, and to a lesser extent with the numbers of wax producing pores.

The characters with the least correlation with all the other characters are the numbers of submarginal setae in rows 2a and 3, antennal segment number, interantennal setae number and the lengths of the body, ventral seta and lateral spiracular seta. All the correlation values less than 0.212 are insignificant at the 0·001% level. The following characters have no or very little significant correlation with any other character: number of submarginal tubercles, antennal segments and the number of submarginal setae present in rows 2a, 1a and 3.

The only characters with negative correlation to any other character are the numbers of marginal or submarginal setae in rows 1 and 2a. Only two negative correlations are significant at the 0·001% level. These are easily explained by the subjective method of allocating the setae to groups. Marginal or submarginal setae are allocated to either of two groups so one will increase at the expense of the other, giving rise to a negative correlation between them.

To summarise, all the measured characters are highly correlated with one another, except the lengths of setae, suggesting isometric growth. Numbers of spiracular pores, marginal setae and to a lesser extent multilocular pores are also highly correlated with the measured characters.

Data summary of British specimens

The range and mean for each of the morphological characters in both the

full and reduced character sets, recorded from British field-collected specimens, are given in Tables 3.2 and 3.3. Some care needs to taken when comparing the data from specimens collected from the different host-plant groups due to the unhomogeneous sample sizes.

Each of the 30 characters recorded from the British field-collected specimens show more variation than expected from the present published morphological decriptions of the nominal species within the P. vitis complex. The greatest variation occurred with the enumerate characters rather than the measured. For example, total numbers of preopercular pores 7-170, spiracular pores 25-168, tubular ducts with large filament 0-65 and tubular ducts with slender filament 0-50. The characters which had the least variation were the number of antennal segments 6-8 and the structural ratio of the hind leg 0.59-0.94.

The characters displaying the greatest variability are those associated with wax production such as numbers of pores, ducts and marginal setae. The external morphology of these wax-producing structures, type and function of wax produced and possible reasons for the considerable variation in the numbers are discussed further in Chapter 8.

The mean values for all the characters are consistently higher for specimens collected from certain host plants compared to those collected from others. For example, specimens collected from grapevine, peach, birch and willow have higher mean values for all the characters compared to specimens collected from hawthorn, flowering currant and Sorbus species. The differences however, were not found to be significant for any of the characters when considering the standard deviations between pairs of the P. vitis complex groups. All the characters showed continuous variation and when plotted, all had unimodal distributions. The following characters had skew distributions: Body length and width, numbers of antennal segments, tubular ducts and submarginal setae.

To summarise, all the morphological characters examined show considerable continuous variation which is associated with the host-plant taxa from which they are collected. The enumerate characters show a wider range of variation than the measured.

3.3.2 Field-collected specimens from outside Britain

Field-collected specimens of the P. vitis complex were also examined from

Host	n	BodyL (10 µm) Min	Max	Mean	±	BodyW (10 µm) Min	Max	Mean	±	OperL (µm) Min	Max	Mean	±	OperW (µm) Min	Max	Mean	±	AntL (µm) Min	Max	Mean	±
AG	10	246	577	413.90	113.19	181	466	297.90	102.81	124	187	156.80	21.48	74	100	86.80	9.70	319	435	370.30	35.01
BP	24	194	755	317.21	121.16	119	658	240.75	130.45	168	213	190.33	11.39	84	116	100.75	8.27	368	503	429.96	35.16
CM	33	156	366	236.33	52.05	94	326	166.00	58.67	110	187	160.06	16.96	65	101	81.82	10.10	274	445	367.67	38.85
EU	1	239	239	239.00	*	184	184	184.00	*	175	175	175.00	*	93	93	93.00	*	381	381	381.00	*
PP	8	361	481	437.25	36.70	259	384	314.37	36.54	187	200	192.62	5.18	77	97	90.50	6.87	390	516	472.37	38.18
PC	12	165	255	219.58	25.39	104	172	146.17	21.62	132	179	160.33	12.38	70	93	83.58	6.35	311	443	389.58	39.09
RN	23	143	338	257.52	46.56	74	223	173.78	37.25	129	200	171.65	18.63	55	93	81.65	9.89	257	463	386.35	48.28
RS	37	129	308	196.76	42.86	82	210	130.65	31.50	129	184	161.08	13.00	61	110	79.41	10.51	294	464	375.46	32.86
RL	10	167	275	212.30	30.62	108	201	140.80	24.53	161	200	171.50	13.04	77	90	83.00	5.72	342	413	379.30	23.43
RU	12	167	511	256.33	121.66	95	401	172.75	98.29	155	187	163.83	10.31	70	93	80.83	7.84	334	426	393.75	26.10
SA	27	167	500	268.41	67.94	120	393	200.07	55.96	148	200	177.52	11.36	77	123	92.19	10.58	354	432	396.59	20.93
SO	12	168	355	247.75	62.58	97	248	172.50	60.19	158	189	175.67	10.19	71	90	80.00	5.38	342	450	394.92	37.54
SX	6	157	284	208.33	50.05	88	195	125.50	40.76	135	168	153.17	12.35	71	84	77.17	4.12	310	387	350.50	27.11
VV	23	221	455	379.65	63.80	139	310	238.00	46.43	164	213	188.70	12.22	81	106	94.78	6.80	361	542	455.78	41.17
	238	129	755	271.12	96.40	74	658	187.81	81.07	110	213	171.46	18.14	55	123	86.19	11.28	257	542	396.30	46.33

Host	n	MouthL (µm) Min	Max	Mean	±	MouthW (µm) Min	Max	Mean	±	HFTL (µm) Min	Max	Mean	±	HTTL (µm) Min	Max	Mean	±	DStL (µm) Min	Max	Mean	±
AG	10	241	310	264.40	25.38	160	226	174.10	21.74	179	277	219.20	33.84	233	335	281.10	41.40	5	10	7.50	1.65
BP	24	200	358	298.12	28.56	174	284	212.12	23.76	226	319	268.04	25.62	297	416	340.50	27.51	5	14	9.38	2.30
CM	33	219	303	260.39	21.22	148	239	190.70	24.14	180	277	222.15	25.04	235	348	280.55	31.64	4	11	8.00	1.94
EU	1	280	280	280.00	*	210	210	210.00	*	210	210	210.00	*	280	280	280.00	*	7	7	7.00	*
PP	8	271	329	306.00	20.69	206	252	223.75	13.05	232	323	289.62	28.14	316	377	347.00	18.70	7	13	9.75	2.05
PC	12	218	280	251.25	19.62	179	218	194.00	11.00	179	260	230.00	21.82	226	303	276.08	19.21	6	13	9.33	2.02
RN	23	226	303	271.00	21.78	135	226	189.00	20.62	161	264	234.22	23.95	226	327	287.43	25.76	5	11	9.22	1.83
RS	37	226	297	260.68	17.59	142	248	185.62	20.98	174	324	232.22	23.96	232	319	285.97	21.52	5	11	7.92	1.46
RL	10	239	297	273.40	17.17	174	232	192.80	17.10	194	271	222.40	20.43	258	329	287.00	20.07	7	13	9.90	1.97
RU	12	232	310	267.67	23.37	163	202	186.25	13.38	187	257	230.50	21.63	249	324	285.83	24.82	6	10	7.83	1.19
SA	27	252	335	289.56	19.75	168	206	188.93	12.24	206	297	260.70	25.05	264	368	314.70	23.82	5	13	8.37	1.78
SO	12	239	316	276.00	23.53	174	245	201.33	20.66	219	271	245.67	19.35	258	368	298.92	34.17	5	10	6.58	1.62
SX	6	255	303	280.67	18.51	174	187	184.83	5.31	194	239	216.33	22.53	232	297	263.33	24.30	5	10	8.00	2.10
VV	23	258	355	303.39	21.71	187	255	213.61	19.25	226	381	289.52	34.10	297	406	350.52	29.84	7	15	10.22	1.93
	238	200	358	276.26	27.01	135	284	194.83	22.41	161	381	244.35	34.18	226	416	301.80	37.34	4	15	8.60	2.03

Host	n	VStL (µm) Min	Max	Mean	±	CSpStL (µm) Min	Max	Mean	±	LSpStL (µm) Min	Max	Mean	±	Ratio Min	Max	Mean	±	AntSegN Min	Max	Mean	±
AG	10	5	10	7.40	1.51	80	108	95.60	7.96	27	48	40.50	6.69	697	838	782.80	71.93	60	80	77.00	6.32
BP	24	6	11	8.58	1.53	57	110	89.29	14.64	25	72	45.88	12.65	716	890	786.92	38.51	70	80	79.17	2.41
CM	33	6	11	8.64	1.56	59	113	88.48	11.31	17	46	29.06	6.74	714	857	792.48	35.95	60	80	78.03	4.32
EU	1	10	10	10.00	*	79	79	79.00	*	47	47	47.00	*	755	755	755.00	*	80	80	80.00	*
PP	8	7	13	8.50	1.93	95	133	109.37	14.28	36	54	42.00	6.16	734	873	833.00	43.47	65	80	78.12	5.30
PC	12	5	12	8.42	1.93	68	101	84.67	10.55	20	43	28.33	8.49	765	877	832.00	34.10	75	80	78.33	2.46
RN	23	6	14	8.91	1.95	66	103	91.13	10.00	17	48	30.74	9.18	694	865	814.57	40.95	60	80	78.48	4.63
RS	37	5	15	9.05	2.45	72	106	94.03	6.93	23	48	36.68	6.63	746	867	802.84	30.27	70	80	78.92	2.67
RL	10	8	11	10.00	0.82	82	106	92.00	6.86	28	48	37.50	6.26	735	826	774.40	30.05	75	80	78.50	2.42
RU	12	5	11	8.25	2.05	73	106	83.42	10.56	15	47	29.33	9.39	728	856	806.67	36.89	75	80	79.58	1.44
SA	27	7	11	8.59	1.15	85	118	102.63	7.60	31	64	43.70	8.44	717	912	828.33	49.91	70	80	79.26	2.67
SO	12	7	11	8.58	1.51	79	108	96.17	8.67	25	48	35.08	8.55	736	876	825.33	38.60	70	80	78.75	3.11
SX	6	6	8	7.50	0.84	77	113	91.67	13.76	30	49	35.17	7.08	770	863	821.50	39.94	60	80	75.00	7.75
VV	23	6	15	9.61	2.43	87	116	104.39	6.60	28	56	44.22	8.22	757	938	824.43	40.71	65	80	79.35	3.13
	238	5	15	8.76	1.88	57	133	93.95	11.79	15	72	37.01	10.30	597	938	807.95	43.17	60	80	78.63	3.63

Table 3.2 The mean, standard deviation and range for 30 morphological characters recorded from British field-collected specimens of the Pulvinaria vitis complex, arranged according to host-plant species. Character abreviations are those listed in Section 3.2.5.

Key to the host-plant groups: AG = Alnus glutinosa; BP = Betula spp.; CM = Crataegus spp.; EU = Euonymus sp.; PP = Prunus persicae; PC = Pyracantha coccinea; RN = Ribes nigrum; RS = Ribes sanguineum; RL = Ribes sylvestre; RU = Ribes uva-crispa; SA = Salix spp.; SO = Sorbus spp.; SX = Saxifraga sp.; VV = Vitis vinifera

Host	n	InAntSt Min	Max	Mean	±	MgSt1 Min	Max	Mean	±	MgSt1a Min	Max	Mean	±	SMgSt2 Min	Max	Mean	±	SMgSt2a Min	Max	Mean	±
AG	10	7	11	9.10	1.10	6	13	10.40	2.50	0	5	1.80	2.04	3	8	5.90	1.79	0	3	0.60	1.07
BP	24	7	10	8.79	0.59	9	27	15.58	5.58	0	8	2.71	2.40	4	11	7.71	1.65	0	3	0.63	0.97
CM	33	7	11	8.67	0.89	7	17	11.18	2.54	0	6	1.85	1.66	3	8	5.79	1.14	0	3	0.79	1.14
EU	1	8	8	8.00	*	12	12	12.00	*	4	4	4.00	*	6	6	6.00	*	0	0	0.00	*
PP	8	6	11	9.50	1.69	9	16	12.88	2.53	2	9	5.25	2.38	3	9	5.75	2.25	0	2	1.25	0.71
PC	12	8	10	8.75	0.62	7	12	9.67	1.67	1	7	4.25	2.01	3	10	6.67	1.92	0	3	1.08	0.90
RN	23	7	10	8.83	0.72	7	12	9.30	1.43	0	7	3.17	1.83	5	10	6.65	1.43	0	3	1.26	1.01
RS	37	6	12	8.97	1.14	6	15	10.00	1.63	0	6	3.03	1.80	3	10	6.54	1.77	0	3	0.59	0.86
RL	10	8	10	9.20	0.79	6	14	8.50	2.42	1	6	3.20	1.69	5	9	6.90	1.45	0	2	0.90	0.99
RU	12	8	10	8.92	0.79	7	11	9.50	1.17	0	5	3.33	1.56	4	8	5.75	1.06	0	3	1.00	0.95
SA	27	7	10	8.96	0.81	8	20	12.67	2.97	0	8	3.15	2.07	6	11	7.59	1.22	0	2	0.41	0.57
SO	12	8	11	9.42	1.08	7	15	11.00	2.34	0	6	1.50	1.88	5	9	7.08	1.08	0	2	0.42	0.67
SX	6	7	10	8.83	0.98	8	13	10.67	2.07	1	6	3.33	1.86	4	8	6.50	1.64	0	2	1.17	0.75
VV	23	8	13	10.35	1.34	7	18	12.70	3.17	0	7	3.74	2.65	5	10	7.26	1.10	0	4	0.83	1.27
	238	6	13	9.06	1.06	6	27	11.31	3.35	0	9	2.97	2.13	3	11	6.71	1.59	0	4	0.77	0.97

Host	n	SMgSt3 Min	Max	Mean	±	TSMgSt Min	Max	Mean	±	PreopN Min	Max	Mean	±	SpirPN Min	Max	Mean	±	MPN12 Min	Max	Mean	±
AG	10	0	2	1.00	0.82	3	16	9.30	4.24	34	74	54.50	11.65	32	80	55.20	17.12	12	54	28.80	11.32
BP	24	0	5	1.83	1.27	7	20	12.88	3.29	37	90	66.58	14.33	51	168	83.92	26.26	18	52	35.54	8.37
CM	33	0	5	1.91	1.18	6	16	10.33	2.64	17	62	37.21	12.48	38	115	60.76	16.77	14	36	21.82	5.02
EU	1	3	3	3.00	*	13	13	13.00	*	27	27	27.00	*	57	57	57.00	*	34	34	34.00	*
PP	8	0	2	0.88	0.64	9	18	13.13	3.00	37	64	54.88	11.24	67	129	99.75	19.59	13	39	32.38	8.55
PC	12	0	3	1.42	0.90	8	17	13.42	2.50	17	51	34.25	10.20	34	93	69.83	19.08	16	44	30.25	8.04
RN	23	0	4	1.96	1.43	7	19	13.04	3.42	29	157	81.26	42.67	25	93	74.30	15.93	17	46	31.39	7.80
RS	37	0	7	1.84	1.38	6	17	12.00	2.82	7	128	66.11	28.24	43	77	60.05	8.16	12	43	25.08	7.19
RL	10	1	5	2.80	1.23	11	17	13.80	1.75	64	170	121.10	32.30	46	133	69.40	23.91	18	33	24.70	5.01
RU	12	0	3	1.42	1.31	6	16	11.50	2.94	13	108	38.58	26.61	45	88	62.00	12.91	15	34	26.75	6.28
SA	27	0	6	1.85	1.43	9	21	13.00	2.91	30	169	76.15	27.23	50	114	73.11	14.41	16	58	36.11	9.87
SO	12	0	5	2.17	1.80	7	17	11.17	3.54	21	104	57.25	25.24	28	68	52.75	12.17	8	24	17.83	4.53
SX	6	1	5	3.17	1.33	13	15	14.17	0.75	66	99	85.67	12.63	37	57	46.17	6.59	16	28	21.00	4.69
VV	23	0	7	2.22	1.68	8	21	14.04	3.99	23	90	50.30	17.61	51	124	86.61	15.48	14	37	27.35	5.94
	238	0	7	1.88	1.38	3	21	12.33	3.28	7	170	61.56	31.00	25	168	69.37	20.22	8	58	28.06	9.00

Host	n	MPN3 Min	Max	Mean	±	MPN4 Min	Max	Mean	±	TubDucS Min	Max	Mean	±	TubDucL Min	Max	Mean	±	SubTubN Min	Max	Mean	±
AG	10	23	75	40.30	16.14	25	81	49.50	18.33	0	11	4.30	4.90	0	20	9.10	6.03	0	7	2.90	2.73
BP	24	30	65	48.71	9.57	39	80	60.92	10.65	1	50	16.62	14.36	0	65	12.83	13.26	0	9	1.87	2.15
CM	33	18	54	33.82	8.62	25	68	43.42	10.02	0	19	5.85	4.64	0	11	2.79	2.92	0	12	3.48	2.96
EU	1	48	48	48.00	*	53	53	53.00	*	11	11	11.00	*	0	0	0.00	*	9	9	9.00	*
PP	8	19	54	45.00	11.54	27	75	55.63	14.21	2	23	8.00	7.13	2	13	4.50	3.66	2	10	6.12	2.36
PC	12	19	62	42.58	11.43	30	75	56.42	12.77	0	26	12.08	8.18	0	15	6.17	5.02	0	9	3.67	2.71
RN	23	21	71	43.43	10.67	36	83	55.83	11.01	0	20	4.74	5.58	0	36	10.74	9.01	0	13	5.39	3.22
RS	37	19	47	33.00	6.86	30	61	42.41	7.65	0	13	2.51	3.12	0	20	3.00	4.18	0	13	6.16	3.69
RL	10	30	58	40.60	8.09	41	65	50.40	7.72	1	4	1.60	1.07	2	24	8.00	6.25	2	10	5.80	2.74
RU	12	16	52	39.08	10.55	23	63	48.25	12.53	0	13	4.50	3.99	0	25	11.50	9.28	0	5	2.00	1.81
SA	27	21	69	48.04	10.38	43	84	59.52	10.29	0	18	5.11	4.39	0	24	5.70	5.11	0	11	4.04	2.61
SO	12	12	36	25.67	6.69	18	43	33.50	6.29	0	8	2.17	2.17	1	5	2.33	1.37	0	11	5.42	3.18
SX	6	23	39	30.50	5.99	32	45	36.50	4.59	0	12	3.17	4.58	0	6	2.83	2.23	2	10	4.67	2.88
VV	23	17	51	35.78	9.59	24	53	40.83	7.01	0	31	6.43	7.34	0	31	4.30	7.28	1	12	6.96	3.01
	238	12	75	39.13	11.48	18	84	49.03	12.99	0	50	6.13	7.68	0	65	6.20	7.62	0	13	4.57	3.29

Table 3.2 continued

	Values	DSetL Min	Max	Mean	±	MarSet Min	Max	Mean	±	SMarSet Min	Max	Mean	±	InAntSN Min	Max	Mean	±
AG	11	5	10	7.45	1.57	9	14	12.18	1.47	3	10	6.45	1.81	7	11	9.09	1.04
BP	29	5	14	9.66	2.22	12	31	18.31	4.95	5	13	8.59	1.74	7	10	8.62	0.68
CN	50	4	11	8.38	1.77	9	19	13.16	1.94	4	10	6.84	1.40	7	11	8.62	0.85
EU	2	7	7	7.00	0.00	16	19	17.50	2.12	3	9	6.00	4.24	6	8	7.00	1.41
PP	14	6	13	9.50	1.70	12	23	16.36	3.15	5	10	7.50	1.45	8	11	9.57	1.09
PC	26	7	13	9.15	1.57	10	17	13.42	2.25	5	11	7.62	1.58	8	11	9.12	0.86
RN	41	5	12	9.22	1.70	8	16	12.61	2.06	2	10	7.61	1.80	7	10	8.88	0.71
RS	37	5	11	7.92	1.46	9	18	13.03	2.03	4	11	7.14	1.75	6	12	8.97	1.14
RL	10	7	13	9.90	1.97	7	16	11.70	2.79	6	10	7.80	1.48	8	10	9.20	0.79
RU	14	6	10	7.64	1.22	10	16	13.00	1.80	5	9	6.71	1.14	8	10	8.86	0.77
SA	40	5	13	8.05	1.74	9	22	15.38	2.46	6	12	8.45	1.60	7	10	8.83	0.78
SO	12	5	10	7.17	1.95	9	16	13.00	2.49	6	10	7.83	1.19	7	11	9.25	1.06
SX	6	5	10	6.83	1.83	12	16	13.00	1.55	5	9	7.00	1.41	8	11	9.17	1.17
VV	43	7	15	10.49	1.74	11	22	16.51	2.34	7	13	8.44	1.40	8	13	10.35	1.19
	335	4	15	8.79	1.97	7	31	14.31	3.17	2	13	7.65	1.70	6	13	9.08	1.07

	Values	SubTubN Min	Max	Mean	±	MPN4 Min	Max	Mean	±	TubDucL Min	Max	Mean	±	TubDucS Min	Max	Mean	±
AG	11	0	7	3.09	2.66	25	81	48.00	18.08	0	20	8.36	6.22	0	11	4.09	4.70
BP	29	0	9	2.03	2.11	39	86	63.72	12.09	0	65	11.62	12.41	1	50	15.45	13.88
CN	50	0	12	3.72	2.76	25	68	44.86	9.56	0	27	3.94	5.06	0	28	7.40	6.18
EU	2	8	11	9.50	2.12	27	32	29.50	3.54	2	4	3.00	1.41	3	5	4.00	1.41
PP	14	0	10	4.50	2.59	30	75	54.79	12.89	0	13	3.71	3.20	0	23	6.14	5.99
PC	26	0	9	3.15	2.49	30	75	51.77	12.48	0	15	5.42	4.49	0	26	8.00	7.49
RN	41	0	13	5.27	3.25	36	83	57.66	10.25	0	36	11.15	8.07	0	20	5.22	5.27
RS	37	0	13	6.16	3.69	30	61	42.41	7.65	0	20	3.00	4.18	0	13	2.51	3.12
RL	10	2	10	5.80	2.74	41	65	50.40	7.72	2	24	8.00	6.25	1	4	1.60	1.07
RU	14	0	5	1.79	1.76	23	63	48.07	11.70	0	25	10.14	9.21	0	13	3.93	3.95
SA	40	0	11	3.70	2.45	43	92	60.33	10.99	0	24	5.18	4.88	0	18	4.70	4.37
SO	12	2	11	5.83	2.92	32	45	36.33	4.01	0	6	2.92	1.83	0	12	3.17	3.56
SX	6	0	6	3.83	2.99	18	41	30.83	7.47	1	3	1.67	0.82	0	2	1.17	0.98
VV	43	2	12	7.33	2.53	24	65	41.84	9.54	0	31	3.21	5.57	0	39	8.86	10.27
	335	0	13	4.56	3.21	18	92	49.95	13.38	0	65	5.99	7.16	0	50	6.44	7.84

Table 3.3 The mean, standard deviation and range for 8 morphological characters recorded from British field-collected specimens of the Pulvinaria vitis complex, arranged according to host-plant species. Character abreviations are those listed in Section 3.2.5; Host-plant abreviations are those given in Table 3.2.

North America, mainland Europe and New Zealand. In addition, samples of specimens collected by Drozdovsky reported to have 18 or 16 chromosomes in each somatic cell nucleus (Drozdovsky, 1966; see Sections 1.4.6 and 8.2.1) were analysed.

Canonical variates analysis of non-British specimens

The CVA plots for the field-collected specimens from mainland Europe and North America are shown in Figs. 3.6 and 3.7 respectively. The specimens show some grouping according to their host-plant species using CVA which is similar to the field-collected specimens from Britain.

The first two canonical variates for the specimens from mainland Europe (Fig. 3.6) only account for 63% of the total variation. The specimens collected from grapevine and most of the specimens from poplar separate from the majority of the specimens along the first axis. The most important characters in the latent vector loadings of the first canonical variate are the dorsal seta length and the numbers of slender tubular ducts, marginal setae and submarginal setae. The specimens collected from birch separate from the specimens from blackcurrant, willow and Spiraea along axis 2. The latent vector loadings of the second canonical variate are dominated by the number of dorsal submarginal tubercles and the number of interantennal setae.

The first two canonical variates for the specimens from North America (Fig. 3.7) account for 74% of the total variation. The specimens collected from peach and birch separate from the specimens from hawthorn and pyracantha along axis 1. The character which dominates the latent vector loadings of the first axis are the number of slender tubular ducts. The characters which are the most important in the second canonical variate are the number of slender tubular ducts and the dorsal seta length.

To summarise, specimens collected from other geographical areas outside Britain also segregate into groups according to the host-plant taxa from which they were collected, using canonical variates analysis.

Russian specimens with diploid chromosome number of 16 or 18

Fig. 3.8 shows the CVA plot for the specimens collected from blackcurrant and birch in the U.S.S.R., identified as P. ribesiae and P. betulae, respectively. The specimens collected from blackcurrant were divided into

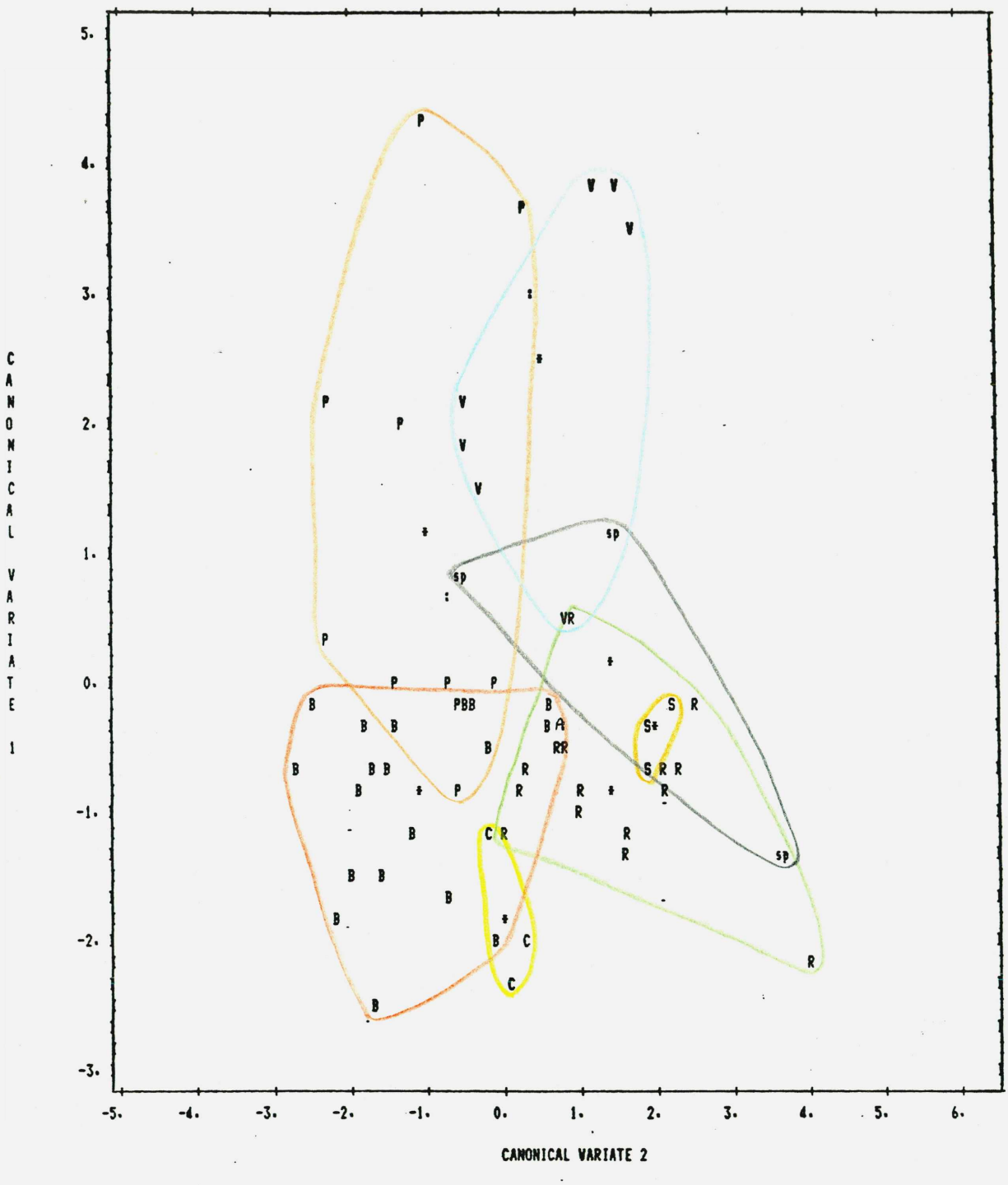


Fig. 3.6 Canonical variates analysis of European field-collected specimens of the Pulvinaria vitis complex, arranged into host-plant groups, using 8 morphological characters; n = 67
Host-plant groups; A = Alnus spp.; B = Betula spp.; C = Cydonia sp.; P = Populus sp.; R = Ribes spp.; S = Salix spp.; sp = Spiraea sp.; V = Vitis vinifera; scores for means (*); : = overlapping groups.

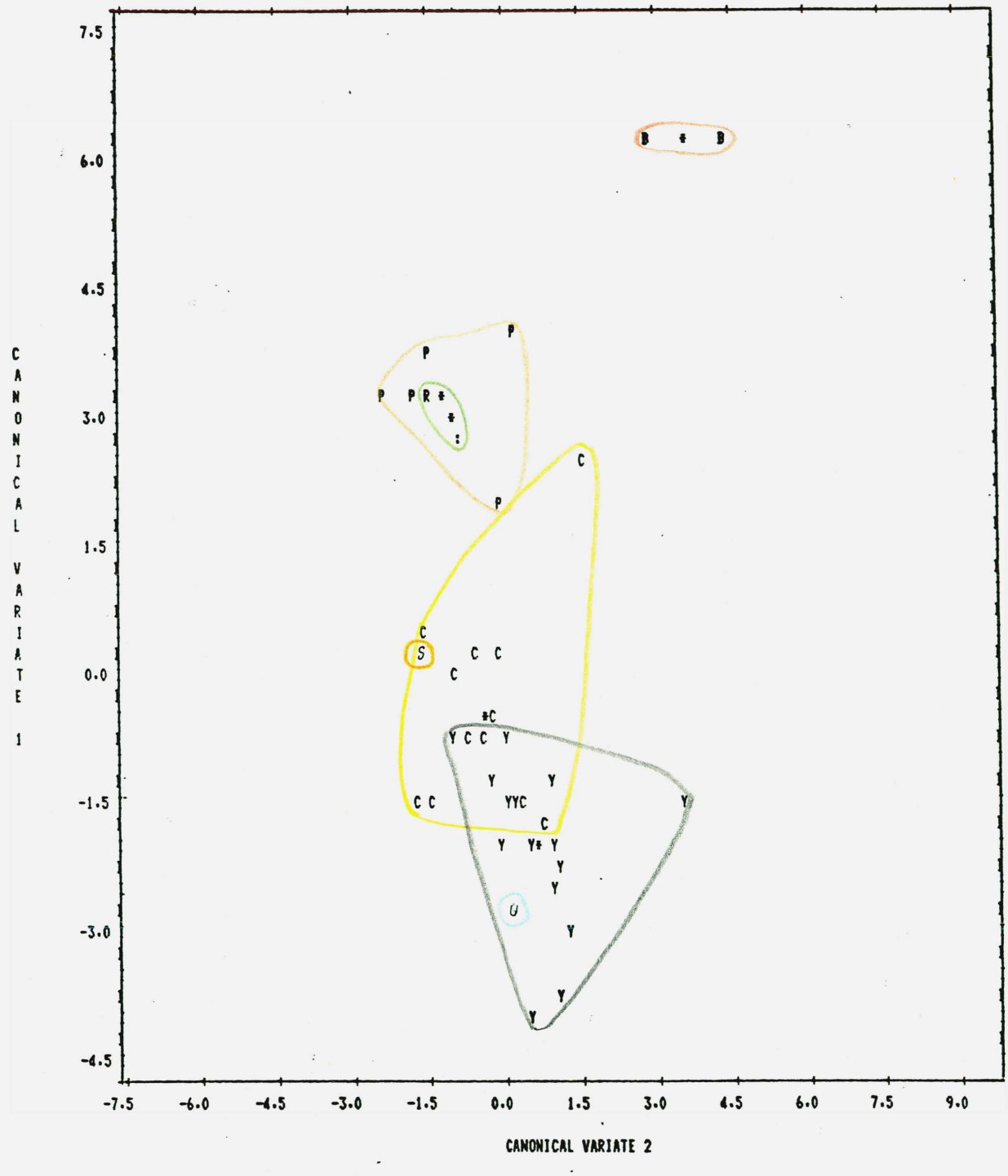


Fig. 3.7 Canonical variates analysis of North American field-collected specimens of the Pulvinaria vitis complex, arranged into host-plant groups, using 8 morphological characters; n = 39
Host-plant groups; (B) = Betula sp.; (C) = Crataegus sp.; (P) = Prunus sp.; (U) = Populus; (Y) = Pyrus sp.; (R) = Ribes sp.; (S) = Salix sp.; scores for means (*)

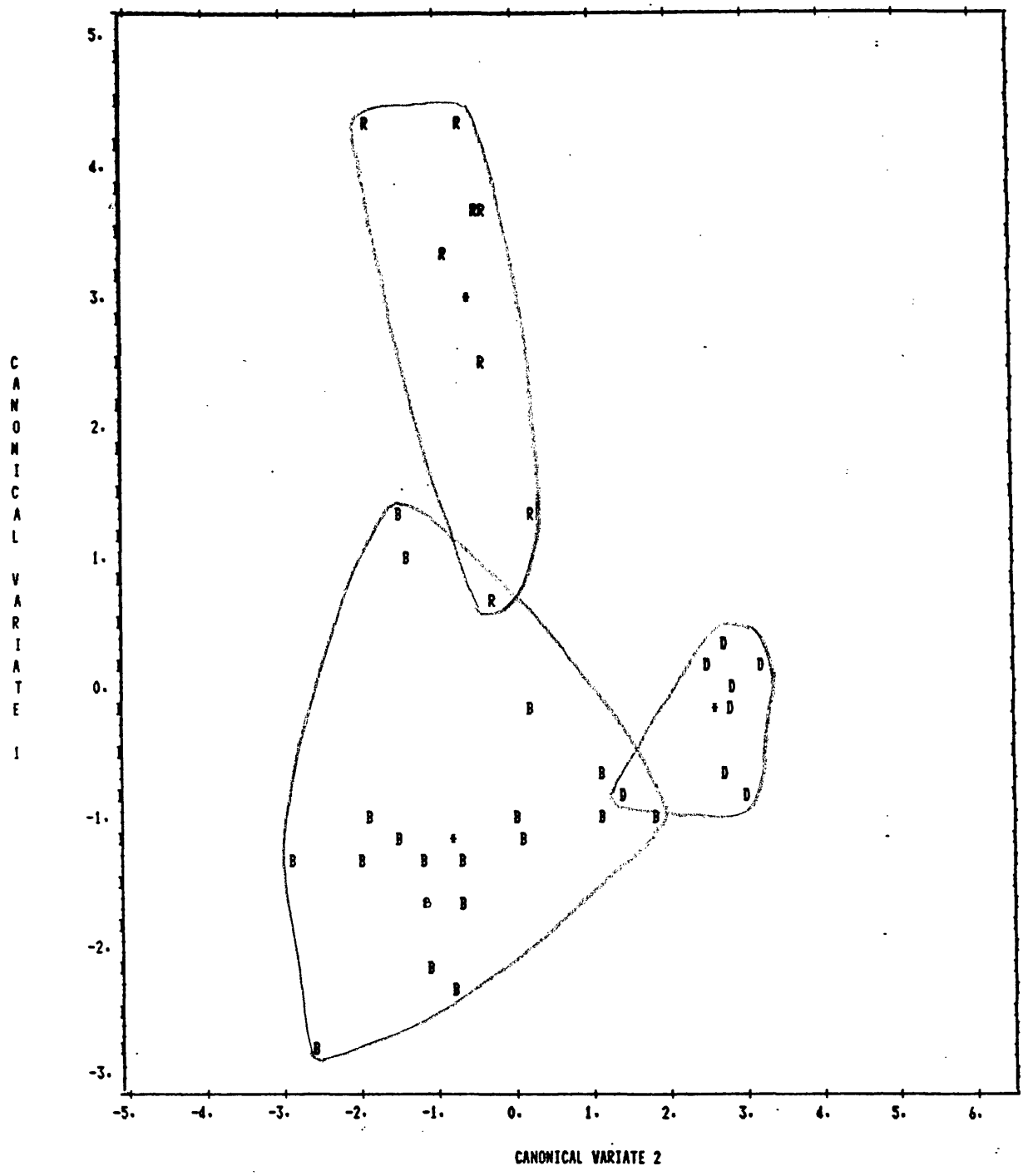


Fig. 3.8 Canonical variates analysis of specimens of the Pulvinaria vitis complex reported to have karyotype of 2n = 18 and 2n = 16, using 8 morphological characters; n = 36
Key: Ⓓ = specimens collected from Ribes sp., karyotype 2n = 18; Ⓡ = specimens collected from Ribes sp., karyotype unknown; Ⓑ = specimens collected from Betula, karyotype 2n = 16 or unknown; scores for means (*)

two groups, those which were reported as having a karyotype of 2n = 18 (Drozdovsky, 1966) and those which had not been karyotyped. There were only 4 specimens collected from birch which were reported to have a karyotype of 2n = 16 (Drozdovsky, 1966) which were suitable for analysis. Therefore, all the specimens collected from birch in the U.S.S.R. were grouped together.

The specimens recorded as having a karytype of 2n = 18 separated from the other specimens along axis 2. The most important characters in the latent vector loadings of the second canonical variate are the numbers of marginal setae present between the anterior and the posterior spiracular setae and the number of dorsal submarginal tubercles. The number of marginal setae is strongly correlated with overall size and the specimens with 18 chromosomes were generally smaller than the other specimens; consequently they had fewer ducts, pores and setae. The number of submarginal tubercles may be artificially lower in this sample because the tubercles were particularly small and difficult to see. Each slide mounted specimen with 2n = 18 had had the dorsal and ventral surfaces separated and mounted side by side which may have resulted in the loss of some of the tubercles during the slide mounting process.

The specimens collected from blackcurrant which had not been karyotyped separated from the specimens from birch along the first axis. The most important characters in the latent vector loadings of the first canonical variate are the number of slender tubular ducts, number of submarginal setae and the number of submarginal tubercles.

When the specimens collected from blackcurrant and birch in the U.S.S.R. were divided into only two groups, according to host plant, the most important characters in the latent vector loadings were the number of slender tubular ducts and the number of submarginal tubercles.

To summarise, the Russian specimens identified as P. ribesiae (karyotype reported to be 2n = 18) show some segregation from specimens identified as P. betulae (karyotype reported to be 2n = 16) when grouped according to host plant, using canonical variates analysis. The segregation is due to size related characters.

Data summary of non-British specimens

The data summaries of the specimens collected from the different geographical regions are not readily comparable as the sample sizes and

principal host-plant species differ. The mean values and ranges of the reduced 8 character set given in Table 3.4, however, are similar for the British, mainland European and North American specimens.

The specimens collected from the European mainland (principally from Hungary and U.S.S.R.) show a particularly wide character variation within and between host-plant groups. For example, the number of multilocular pores on abdominal segment 4 for specimens collected from poplar vary from 23 to 119. The specimens collected from pop lar in the U.S.S.R. had been identified as three different species in the Institute of Zoology, Leningrad, U.S.S.R., P. betulae (L.), P. populi Signoret and P. populeti Borchsenius. These specimens from poplar exhibit a particularly wide degree of variation in size and correspondingly, in setae, pore and duct number. Unfortunately, there were insufficient numbers of slide preparations of teneral specimens available to make conclusive remarks on the relationships between all the samples.

The majority of the specimens identified at the Insitute of Zoology, Leningrad, as the three species above clearly fit into the range of morphological variation found to occur with the P. vitis complex from Britain, although there were a few specimens collected from poplar from a single locality in U.S.S.R. which were exceptionally large with unusually large numbers of pores and setae. These differences in phenotypic plastic morphological characters between populations from different host-plant species and localities may be due to intraspecific genetic variation between demes and not be evidence for different species.

3.3.3 Comparison between British and non-British specimens

Field-collected specimens were examined from Britain, mainland Europe, North America and New Zealand. Unfortunately, only a small proportion of the non-British specimens available could be included in the analyses, thus caution must be taken when comparing the results because the sample sizes and principal host-plant species differ between the geographical areas. The means and ranges for all the characters are very similar for the specimens from each geographical region. The specimens from Britain, North America and mainland Europe separate according to their host-plant species from which they were collected.

There are considerable differences in the most influential characters in the latent vector loadings of the first two canonical variates between the

	Character	Minimum	Mean	Maximum
Britain	DSetL μm	4.000	8.785	15.000
	MarSet	7.00	14.31	31.00
	SMarSet	2.000	7.648	13.000
	InAntSet	6.000	9.081	13.000
	SubTubN	0.000	4.555	13.000
	MPoreN4	18.00	49.95	92.00
	TDuctL	0.000	5.985	65.000
	TDuctS	0.000	6.439	50.000
European mainland	DSetL μm	6.00	11.01	16.00
	MarSet	8.00	17.31	28.00
	SMarSet	3.000	7.104	13.000
	InAntSet	6.000	8.672	13.000
	SubTubN	0.000	4.254	13.000
	MPoreN4	6.00	48.04	119.00
	TDuctL	0.000	5.343	31.000
	TDuctS	0.000	1.851	26.000
North America	DSetL μm	7.00	10.18	13.00
	MarSet	8.00	13.31	19.00
	SMarSet	4.000	8.282	12.000
	InAntSet	7.000	9.590	12.000
	SubTubN	0.000	4.205	11.000
	MPoreN4	20.00	38.28	59.00
	TDuctL	0.000	1.282	9.000
	TDuctS	0.000	9.051	33.000

Table 3.4 The mean and range for 8 morphological characters measured from field-collected specimens of the Pulvinaria vitis complex from Britain (n = 335), European mainland (n = 67) and North America (n = 39). Character abreviations are those listed in Section 3.2.5.

British and non-British samples. The most important characters in the separation of the British specimens according to host-plant group are the numbers of interantennal setae and marginal setae. The most important characters in the separation of the non-British specimens according to host-plant group are the numbers of submarginal tubercles and tubular ducts and dorsal seta length. The majority of non-British samples for each host-plant species are collected from a single locality. Therefore, the CVA plots of the mainland European specimens and the North American specimens show which characters vary according to locality as well as which characters vary with host-plant species. The initial choice of groups for the CVA analysis therefore contains elements of both host and locality.

Some of the most interesting specimens are those collected from poplar and blackcurrant in the U.S.S.R. Some of the specimens from poplar are exceptionally large with correspondingly high numbers of pores, ducts and setae. The specimens from blackcurrant reported to have 2n = 18, are at the other extreme of the morphological variation, being small with low numbers of pores, ducts and setae. These specimens fit at the extremes of the morphological variation found in the P. vitis complex in Britain. More specimens and better slide-mounted preparations are required before a conclusion can be reached on the relationships between these populations. The morphological differences between these samples may only be due to genetic variability between demes of a single species.

3.3.4 The effect of geographical location

Specimens collected from the same host-plant species or genera were grouped according to the geographical location from which they were collected for canonical variates analysis. The resulting CVA plots are shown in Figs. 3.9, 3.10 and 3.11 for specimens collected from birch, currant and grapevine respectively. The specimens do show some grouping according to geographical location. This demonstrates that morphological variation in the P. vitis complex is also associated with geographical locality in addition to host-plant species. The variation associated with geographical location may be environmentally induced due to different climatic conditions and/or genetically induced due to different demes. It is likely that the variation associated with geographical location is both genetically and environmentally determined.

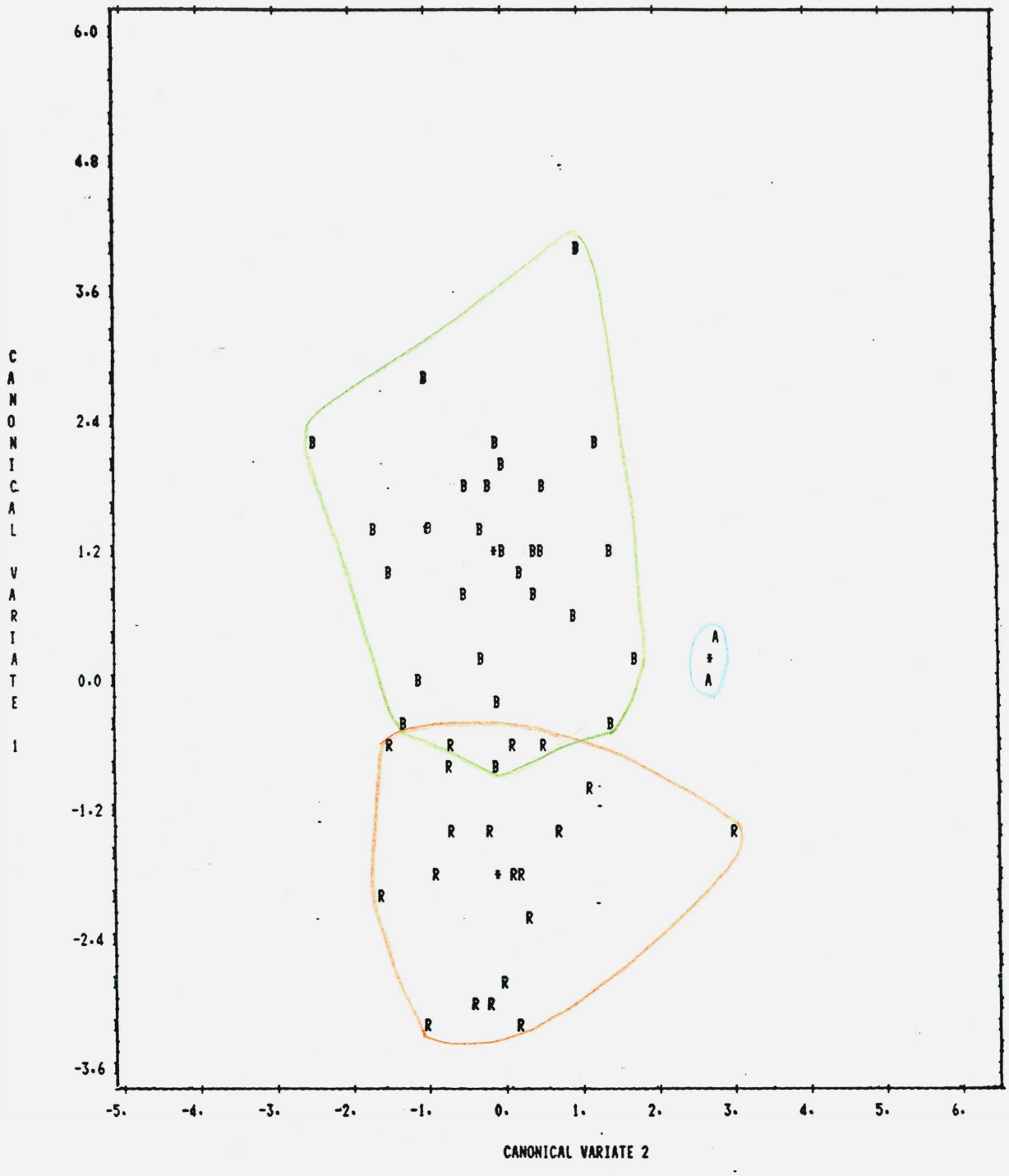


Fig. 3.9 Canonical variates analysis of samples of the Pulvinaria vitis complex, collected from Betula spp., in Britain (B), the European mainland (R) and North America (A); n = 54; scores for means (*)

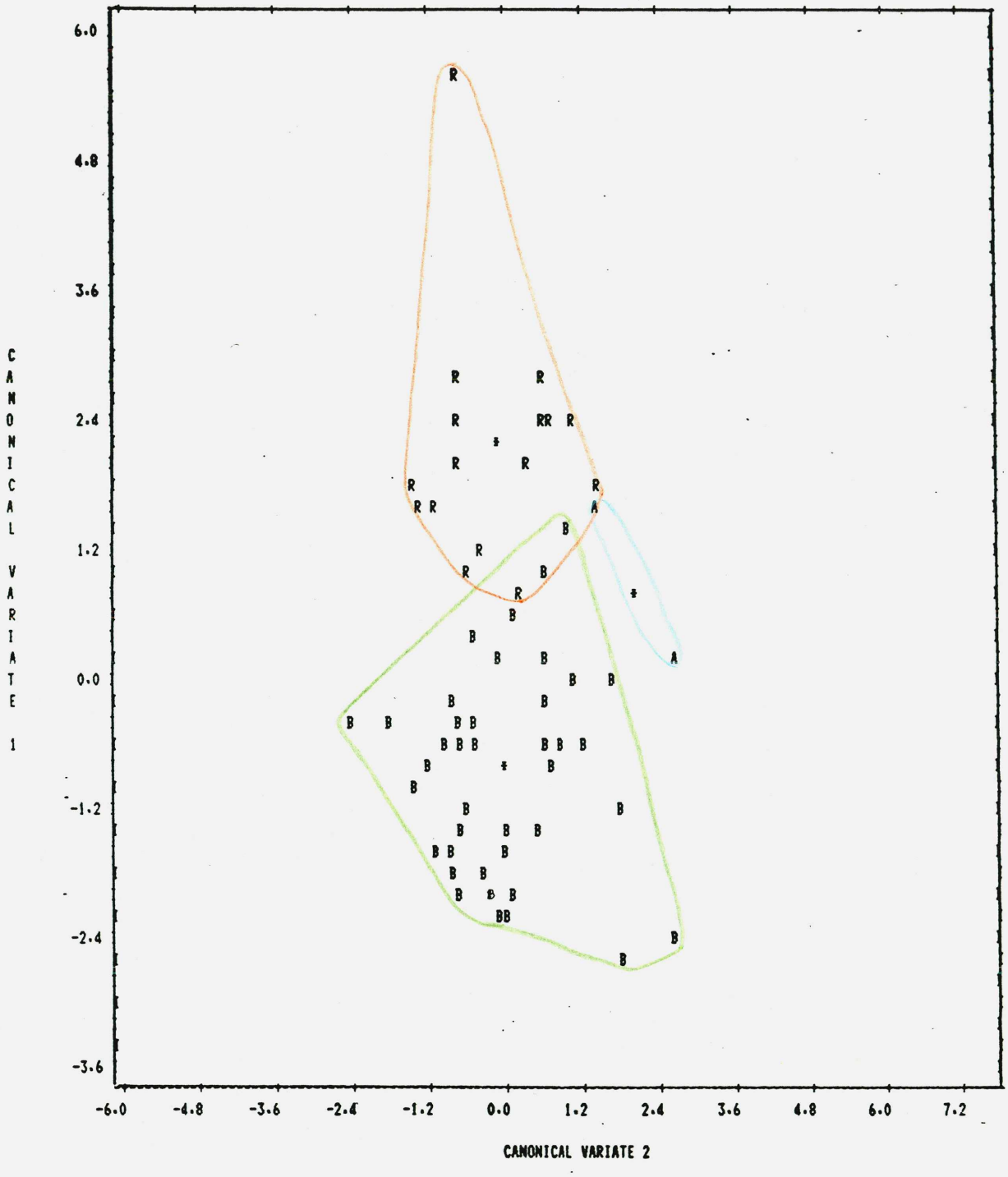


Fig. 3.10 Canonical variates analysis of samples of the Pulvinaria vitis complex, collected from Ribes spp., in Britain (B), the European mainland (R) and North America (A); n = 59; scores for means (*)

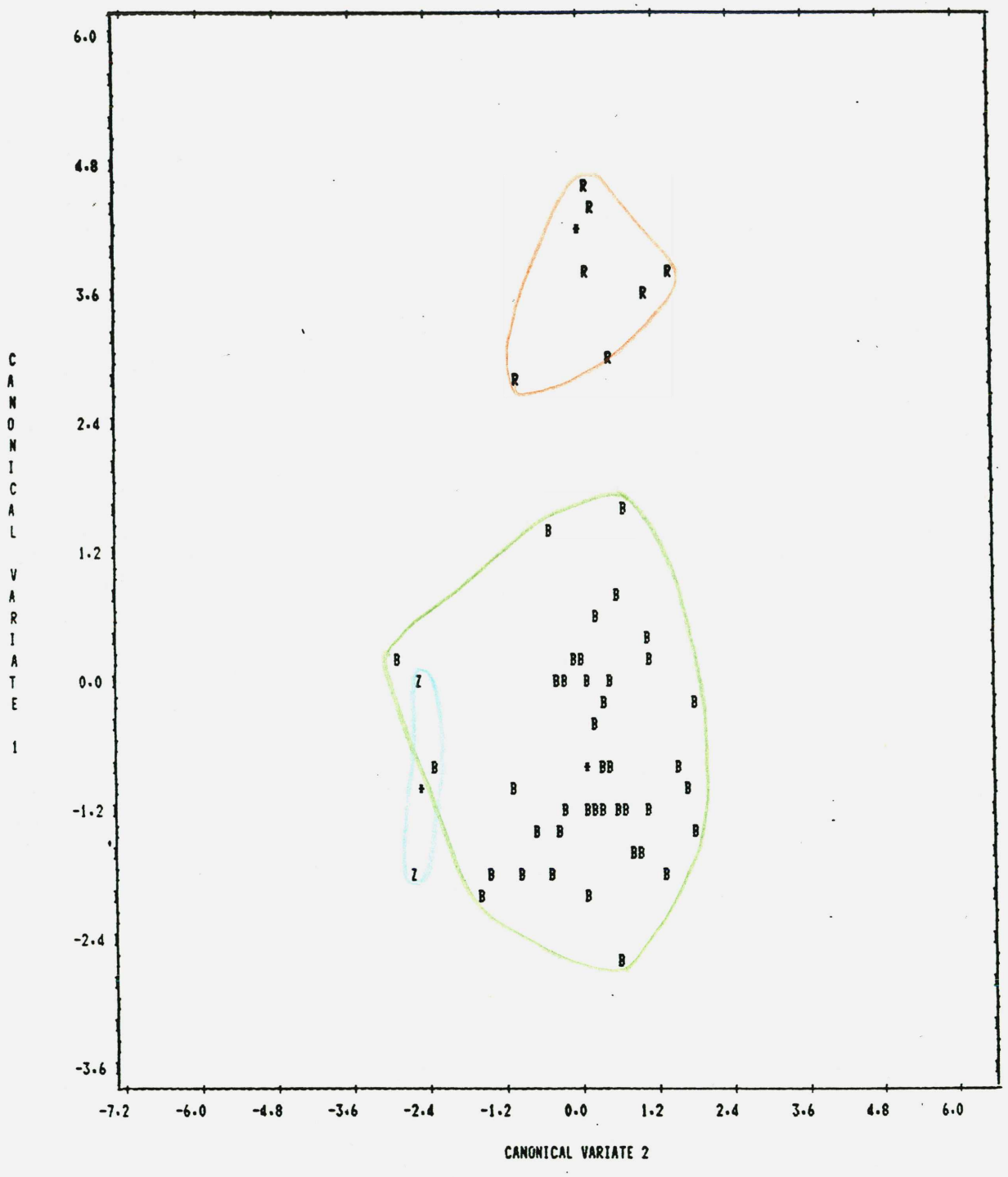


Fig. 3.11 Canonical variates analysis of samples of the Pulvinaria vitis complex collected from Vitis vinifera in Britain (B), the European mainland (R) and New Zealand (Z); n = 54; scores for means (*)

The morphological characters which are the most significant in the latent vector loadings of the first two canonical variates of Figs. 3.9-11, that is, the most important characters in separating specimens according to geographical location, are numbers of submarginal tubercles, marginal and submarginal setae, interantennal setae and dorsal seta length. These are similar to the characters that are the most significant in segregating groups of specimens according to host-plant species; however, there is less consistency in the order of the most important characters in the latent vector loadings of Figs. 3.9-11.

3.4 Discussion

The P. vitis complex in Britain shows considerable morphological variation which has not previously been fully described. The variation is continuous for all the morphological characters investigated in this study; including characters traditionally used as diagnostic for each of the nominal taxonomic species in the complex. It is not surprising, therefore, that the morphological keys to the complex are inadequate and that there are many intermediate forms which do not fit any of the present morphological descriptions of the nominal species. Taxa can not be consistently identified and segregated morphologically, other than on size. The characters with the largest variability are enumerate, in particular, the numbers of pores, ducts and setae associated with wax-production.

The small numbers of specimens examined from mainland Europe, North America and New Zealand fit into the range of morphological variation found in the field-collected specimens from Britain.

The morphological variation is strongly associated with host-plant species and geographical localition. Small groups of specimens of the P. vitis complex from different host-plant species and from different localities can often easily be segregated, but only by size related characters. This explains why different populations on different host-plant taxa have been described as different species. It is interesting to note that the different nominal taxonomic species in the P. vitis complex have been named after the host-plant species on which the extremes of the morphological variation of the scale insect complex are found. A group of specimens collected from hawthorn ("P. oxyacanthae") or currant ("P. ribesiae") can usually easily be segregated morphologically from a group of specimens collected from

grapevine ("P. vitis") or birch ("P. betulae"). The morphological characters which are still used as diagnostic for the nominal species in the complex (see Appendix 1.2), such as numbers of pores and length of the body and appendages, are the characters which vary most with host-plant species. When considerering all the groups of specimens, however, there is continuous variation between the extremes.

Is it possible for a single species of scale insect to exhibit such a wide range of morphological variation? There are many examples of widely-distributed, polyphagous species of scale insect which show considerable morphological variation. Two examples in the Coccidae, Parthenolecanium corni and Coccus hesperidum, have already been mentioned in Section 3.1.2. Pulvinaria floccifera (Westwood) is another cosmopolitan, polyphagous, pest species which shows considerable morphological variation in the number of ventral tubular ducts and number of fringed marginal setae (2-37), (pers. obs.). There are also examples of highly-variable species in all the other larger families of the Coccoidea. The willow scale, Chionaspis salicis (L.) (Diaspididae), has 6 types of different morph which are induced by genetic variation within populations. The different morphs were first described as distinct species (Danzig, 1986). The morphs differ in number and size of dorsal glands on the pygidium. The geographical distribution of the different forms is not homogeneous, that is, C. salicis shows considerable morphological variation in some areas of its range and homogeneity in other areas. This pattern of distribution is typical for polymorphic species. Another example of a polymorphic species of scale insect is the maple mealy bug, Phenacoccus aceris (Signoret) (Pseudococcidae), which varies in the number of circuli it possesses (Danzig, 1970).

The problem for the present study is to determine if the morphological variation found in field-collected specimens of the P. vitis complex, associated with the host plant and locality, is genetically or environmentally induced. There are four aspects of this variation which need consideration. First is the variation induced by the environment within each population, in this case the host-plant species; secondly is the variation due to genetic variability within a population; thirdly, is the variation due to genetic differences between populations; and finally, is the variation due to genetic variability between species? The following 2 chapters aim to resolve this problem.

4 The effect of parasitism on morphological variation

4.1 Introduction

Danzig (1966) reported that parasitism of immature instars dramatically affected the morphology of adult female "P. betulae". Adult females parasitized by Encrytus swederi (Dalman) collected near Partizansk in Primorye, U.S.S.R., showed a marked reduction in the numbers of wax-producing structures, such as tubular ducts and multilocular pores present. These structures are associated with reproduction (see Chapter 6): the tubular ducts produce waxy filaments, forming the ovisac, and the multilocular pores produce short curls of wax to coat the eggs, preventing them from sticking together. Danzig suggested that the reduction in the number of wax-producing pores and ducts associated with reproduction could result in sterility, as the parasitized females would be unable to produce an ovisac. Other morphological characters present in reduced numbers on parasitized adult females were marginal setae, size and number of spiracular setae, and size of the spiracles. The number of parasitized "P. betulae" observed in the study was not given.

A new species of Pulvinaria from Primorye, U.S.S.R., was described in the same paper (Danzig, 1966) that reported the effect of parasitism on morphological variation in "P. betulae". Danzig assumed, by analogy, that the paratype of P. crassispina Danzig was parasitized because it had a much lower number of wax-producing pores and ducts than the holotype.

Danzig (1986) later reported that parasitism does not always affect the morphological variation of adult female Pulvinaria species, and that the morphological differences she observed previously may not have been the result of parasitism. Although there have been many studies on the effect of the host scale insect on parasitoid morphology, there appears to have been no other publications on the effects of parasitism on morphological variation of soft scales.

I have collected a single parasitised adult female Palaeolecanium bituberculatum (Targioni Tozzetti) on blackthorn (Prunus spinosus), England, 1989, which was distinguished from unparasitised specimens on the same host as it did not have the charactersitic dorsal tubercles. The lack of tubercles may have been an effect of parasitism or may have been caused by other factors.

4.2 Methods

The methods used for collecting, rearing and slide mounting scale insects are described in Sections 3.2.2, 5.1.2 and 3.2.3 respectively.

4.2.1 Material examined

All the material examined in the present study came from a single culture, in order to reduce the number of factors influencing morphological variation. Eleven parasitized and 19 unparasitized adult females of the P. vitis complex were obtained from an outdoor culture on blackcurrant (Ribes nigrum) during October. The culture originated from a population on hawthorn (Crataegus monogyna), London, 1987, which was parasitized by Coccophagus lycimnia (Walker) and Metaphycus melanostomatus Timberlake. Both species of parasitoid established themselves in the culture on blackcurrant. C. lycimnia was the commoner of the two parasitoids, and superparasitism (more than a single parasitoid per host) by both species was common. The proportion of scale insects parasitized increased to very high levels in the second year of cultures. Parasitism reached 100% in scale insect cultures on grapevine under glass.

Parasitized scale insects containing mature parasitoid larvae were easily recognised when alive by the strongly convex dorsum, which darkened as the parasitoid larva pupated. At the time of slide preparation, it was noted whether the scale insect was parasitized or not and parasitoid larvae were often mounted on the slide, together with their host. Slide-mounted parasitized scale insects contained the parasitoid egg stalks, which were usually located in the anal flaps.

4.2.2 Rearing adult parasitoids for identification

Parasitized scale insects were removed from the cultures and placed in glass vials with cotton wool plugs to rear the adult parasitoids. When the adult parasitoids had emerged from their hosts, they were killed with ethyl acetate fumes. They were stored in small gelatine capsules containing a small piece of cotton wool to secure them, together with a reference number. The gelatine capsules were stored in the dark as they become yellow and brittle if exposed for long periods to light. The capsules were pinned in a covered collection box.

4.2.3 Morphological characters selected for analysis

Morphological characters of adult female scale insects were selected from the full character set discussed in Section 3.2.5. Thirteen characters were chosen corresponding with those that had been reported to vary with parasitism by Danzig (1966). These characters (6 measured and 7 enumerate) are listed below. Body length and width were not included because they are not a good indication of size in scale insects (see Section 3.3.1).

Measured characters

1. Mean maximum length of anal operculum (OperL, Fig. 2.4) μm
2. Mean maximum width of anal operculum (OperW, Fig. 2.4) μm
3. Mean length of antenna (AntL, Fig. 2.3) μm
4. Mean length of hind femur + trochanter (HTFL, Fig. 2.4) μm
5. Mean length of hind tibia, tarsus and claw (HTTL, Fig. 2.4) μm
6. Length of dorsal body seta present, adjacent to preopercular pores (DsetL, Fig. 2.3) μm

Enumerated characters

7. Number of marginal setae present between anterior and posterior spiracular setae (MarSet, Fig. 3.1)
8. Number of submarginal setae present between anterior and posterior spiracular setae (SMSet, Fig. 3.1)
9. Number of setae present in a semi-circle between the antennal bases (InAntS, Fig. 2.3)
10. Number of submarginal tubercles (Stub, Fig. 2.3)
11. Number of multilocular pores present on abdominal segment 4 (MPN4, Fig. 2.3)
12. Number of tubular ducts with thick inner filament situated on the head anterior to the antennal bases (TDLF, Fig. 2.3)
13. Number of tubular ducts with slender inner filament situated on the head anterior to the antennal bases (TDSF, Fig. 2.3)

4.2.4 Statistical analysis

Methods of multivariate analysis are discussed in Sections 3.1.3 and 3.2.6. The morphological data of the parasitized and unparasitized scale insects were analysed using principal components analysis. This method is used to

investigate the interrelationships between variables and to see the dependence structure occuring within the multivariate matrix.

4.3 Results

4.3.1 Principal components analysis.

Fig. 4.1 shows the principal components analysis plot for the parasitized and unparasitized specimens of the P. vitis complex reared on blackcurrant. The first two principal components are plotted, accounting for 91% of the total variation. There was no significant separation of the parasitized and unparasitized specimens, and plots of combinations of the other principal components did not show any clearer separation. The small degree of separation which did occur was along the first principal component. The most important character in the latent vector loadings of the first two components was antennal length. This character, however, is highly variable and gives a poor indication of size, as it has little correlation with other appendage lengths. Also important in the latent vector loadings of the first two components were the leg segment lengths, operculum length and number of multilocular pores. The lengths of the leg segments and operculum give a good indication of size.

Morphological Character	n = 11 Parasitized sample			n = 19 Unparasitized sample		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
OperL μm	140.0	162.5	193.0	156.0	169.9	192.0
OperW μm	76.00	85.45	98.00	78.00	88.68	104.00
AntL μm	339.0	379.2	450.0	333.0	401.3	485.0
HTFL μm	202.0	231.4	267.0	222.0	243.9	287.0
HTTL μm	261.0	285.6	319.0	274.0	302.7	352.0
Dsetl μm	8.000	9.273	13.000	8.00	10.16	12.00
MarSet	8.00	13.18	19.00	7.00	11.79	17.00
SMSet	4.000	7.091	12.000	4.000	6.895	9.000
InAntS	5.000	7.818	9.000	8.000	8.947	11.000
Stub	0.000	4.273	9.000	2.000	5.421	8.000
MPN4	29.00	47.55	66.00	6.00	46.16	79.00
TDLF	5.00	11.64	27.00	0.00	12.47	26.00
TDSF	4.00	10.09	17.00	4.00	17.53	38.00

Table 4.1 The mean and range of morphological variation of parasitized and unparasitized Pulvinaria vitis complex reared on Ribes nigrum

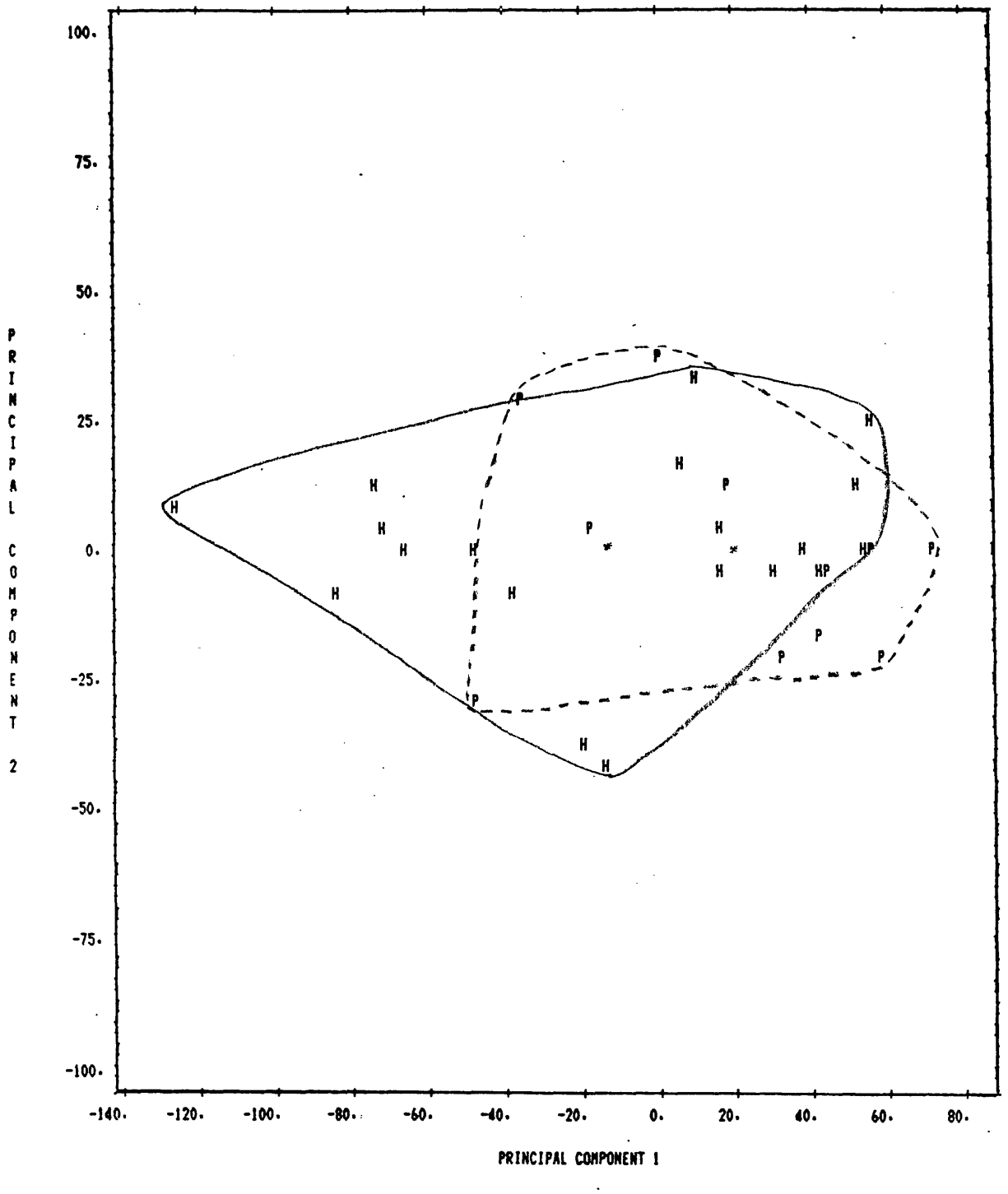


Fig. 4.1 Principal components analysis of parasitized (P) and unparasitized (H) samples of the Pulvinaria vitis complex reared on Ribes nigrum; n = 30; scores for means (*)

Data summary

Morphological data from parasitized and unparasitized scale insects is given in Table 4.1 below. The number of interantennal setae was found to be significantly larger in the unparasitized sample than in the parasitized sample, using the t test, P = 0.01. This character, however, was not important in the vector loadings of the principal components analysis.

4.3.2 The effect of parasitism on reproduction

Parasitized scale insects were still capable of producing viable offspring although the ovisac was smaller and fecundity was reduced. None of the scale insects appeared to have been sterilised by parasitism.

4.4 Discussion

Parasitism did not have a pronounced effect on morphological variation or to cause sterility in adult females of the Pulvinaria vitis (L.) complex in the present study. This result conflicts with the observations of Danzig (1966), who reported that parasitism caused a reduction in the numbers of wax-producing pores and ducts associated with reproduction in adult females of the P. vitis complex. Danzig, however, was reporting the effects of parasitism on scale insect morphology by a different parasitoid species, Encyrtus swederi (Dalman). The most common parasitoid in the present investigation was Coccophagus lycimnia (Walker).

It may be assumed that the effect of parasitism on the host will depend upon the size and number of parasitoid larvae in relation to the size of the host. Adult E. swederi are slightly larger than adult C. lycimnia, however, the number of parasitoid larvae per host is greater for the smaller C. lycimnia. The difference in the effect on morphological variation is unlikely, therefore, to be due to the difference in size of the parasitoid.

If it is assumed that parasitism will have a greater effect on the morphology of a smaller host, then the differences in observations may be due to differences in host size. The scale insects used in this study were reared on blackcurrant, and the mean body length of scale insects on this host-plant species is below the average size for the P. vitis complex (Table 3.2). Parasitism, therefore, would be expected to have an effect on morphology: since it did not, the difference in observations of the effect of parasitism on morphology is unlikely to have been due to differences in mean body size of the host scale insects.

The number of parasitized specimens in this study was small and there may be more subtle differences in morphological variation between parasitized and unparasitized specimens that would only be detected in a larger sample. Parasitism certainly reduces fecundity in the P. vitis complex and probably reduces longevity. Parasitized scale insects are likely to be susceptible to fungal attack at the site of the ovipostion puncture. Adult parasitoids also feed on body fluids of scale insects, often at the site of oviposition (see Section 7.2.3).

Scale insects normally only feed for relatively short periods between long non-feeding periods while they moult. Parasitized scale insects may increase the rate or length of time spent feeding to make up for the loss of tissue reserves used by the parasitoid. Scale insects also have an important internal defence mechanism, mediated by haemocytes, which results in the encapsulation of foreign bodies in the haemocoel. Encapsulated parasitoid eggs and first instar larvae were occasionally observed inside specimens of the P. vitis complex. It is likely therefore that the P. vitis complex has an effective immune system, mediated by haemocytes.

The body margin of specimens of the P. vitis complex, which contained encapsulated parasitoid eggs or larvae, were occasionally deformed at the site of parasitoid oviposition. Encapsulation of the eggs of Habrolepis rouxi Compere (Hymenoptera; Encyrtidae) has been reported to cause deformation of the body margin of adult female Californian red scale, Aonidiella aurantii (Maskell)(Diaspididae)(Blumberg, 1990).

The characters that Danzig (1966) reported to be influenced by parasitism, such as numbers of pores, ducts and setae, are characters that were found in Chapter 3 to vary considerably within a series of specimens from a single population. In the present study, parasitized specimens with unusually low numbers of pores, ducts and setae did occur; however, unparasitized specimens were also found in this condition.

5 The effect of host-plant species on morphological variation

5.1 Introduction

The results of Chapter 3 demonstrated that British field-collected specimens of the Pulvinaria vitis (L.) complex show a large degree of morphological variation, which is associated with both the host-plant species and locality. The morphological characters which separated the specimens according to host plant, using canonical variates analysis, were enumerate; in particular the numbers of pores, ducts and setae. These secondary morphological characters have been traditionally used for diagnosis of the nominal taxonomic species within the P. vitis complex. This chapter investigates whether the morphological variation associated with the host-plant taxa is determined genetically or environmentally.

5.1.1 Genetic and environmentally-induced variation

Only genetic variation may be used for species diagnosis but this variation is often obscured by that induced by environmental conditions (discussed in Section 3.1.1). Genetic variation, therefore, needs to be distinguished from the enviromental variation, in order to determine how many species there are in the P. vitis complex. There are two ways to separate genetic variation from variation induced by the host plant, both of which were used in this study. First, genotypically similar individuals were reared on different host-plant species under similar environmental conditions. Resulting variation is, theoretically, due only to differences between host-plant species. It would have been preferable to have used genotypically identical individuals but it is very rare to have genotypically identical individuals within a sexually reproducing species. The second approach was to rear individuals with different genotypes on the same host-plant species under identical environmental conditions. Resulting variation is, theoretically, due to genetic differences between individuals.

To obtain individuals that are genetically similar, cultures ideally should be started from a single female. This would also ensure that a single species of scale insect from each locality was employed. Unfortunately, starting the cultures with a single female of a univoltine insect was not practical as a minimum of a year would be required to rear enough genetically similar females to initiate the experiment. In the following

study, it has been assumed that individuals of the P. vitis complex from the same population have similar genotypes. This is a reasonable assumption, as populations of the P. vitis complex are genetically isolated due to their immobility and facultative parthenogeneticity (discussed in Section 8.3.3). The sexual function of adult males is also uncertain (see Section 7.1.3). The assumption that individuals from the same population have similar genotypes was tested by comparing intra- and inter-population variation, on the same and different host-plant species. The results of Chapter 3 clearly show that there is less variation between individuals from the same population than between individuals from different populations.

Individual scale insects with similar genotypes will appear morphologically similar, assuming that the individuals reared on the same host-plant species have experienced similar environmental conditions to one another. The cultures in this study were reared under the same conditions to each other, although the microweather would have differed between host plants. Consistent morphological differences between cultures of scale insect originating from different localities would denote different genotypes and suggest separate species, particularly if biological differences were also found between the populations.

The percentage of parasitism in the cultures of the P. vitis complex was high and both parasitized and unparasitized specimens were included in the results. The effect of parasitism on morphological variation was investigated in Chapter 4 in order, to assess its significance on the results of the host transfer experiments. It is important to stress that parasitism was not found to significantly affect the morphological variation of the P. vitis complex in this study and can be ignored as a cause of variation.

Experimentally-induced variation can be compared statistically with variation found in natural populations, and such information may then be used in the identification of field-collected material. Therefore, although this method initially requires the rearing of living material under different environmental conditions, once the limits of variation are defined, preserved material can more easily be identified. This technique is, however, only appropriate for the identification of variable, cosmopolitan, polyphagous pest species because of the time, effort and cost involved.

Correlations of environmentally induced variation between characters can also be usefully studied. An understanding of such variation can then be

applied to other species complexes, allowing a better interpretation of variation observed in field-collected material. Selection, reliability and relative importance of characters used in keys and descriptions of new taxa can also then be assessed.

5.1.2 Past morphological and host-transfer studies

Host-transfer experiments with the P. vitis complex have been conducted in the past to determine the range of host-plant species, and to see if groups could be segregated according to host preference. The first published experiments involved the transfer of a large number of ovisacs from currants (Ribes spp.) to a wide range of plant species (Newstead, 1903). Many of the plants involved were not known host species of the complex. These transfers were completely unsuccessful, leading to the conclusion by Newstead that the woolly scale insects on currant were distinct from "P. vitis".

Successful host-transfer experiments were conducted in the U.S.A. using a scale insect identified as P. vitis, in order to determine synonymies (Sanders, 1909). First instars were successfully transferred from American plane (Platanus occidentalis) and American lime (Tilia americana) to boxwood (Buxus sempervirens). They were subsequently transferred successfully from boxwood to 18 other plant species, some of which are only hosts of the P. vitis complex, some are hosts of both the P. vitis complex and Neopulvinaria innumerabilis (Rathvon), but many are only hosts of N. innumerabilis. As a result, N. innumerabilis, was incorrectly considered a synonym of P. vitis.

More recently, a detailed investigation was conducted to examine the host-plant range of "P. vitis" in Canada (Phillips, 1963). It was succesfully transferred from peach (Prunus persica) to willow (Salix sp.), poplar (Populus sp.), hawthorn (Crataegus sp.), cherry (Prunus cerasus), peach, plum (Prunus domestica), blackcurrant (Ribes nigrum), redcurrant (Ribes slyvestris), gooseberry (Ribes uva-crispa) and grapevine (Vitis vinifera). Ovisacs were only produced in the following year on willow, poplar, peach, plum, blackcurrant and grapevine. Willow was found to be readily colonised in the laboratory but not in the field. It was also reported to be difficult to establish scale insects on hosts other than the one on which the eggs were laid. However, this did not mean the scale insect could not develop on that host. Similarly, success in rearing immatures through the summer and autumn

on a particular host did not mean that they could complete their life cycle on it. For example, "P. vitis" was found, by Phillips (1963), to survive the summer on black medick (Medicago lupulina) growing with infested willows in an insectary, but it could not survive the winter on that host. No attempt was made to investigate the effect of host-plant species on morphology or to elucidate and separate genetic from environmentally induced variation.

In the present study, a wide range of host-plant species were initially included in the transfer experiment in 1988 in order to determine the host plant range and to investigate if groups could be segregated according to survival on a particular host. The host transfers were repeated in the following year, after interim analyses, using fewer host species and a greater number of replicates. A single ovisac was transferred from a population of the P. vitis complex on birch and hawthorn to 10 plants of hawthorn, willow, birch and blackcurrant, making a total of 80 transfers. Birch and hawthorn were chosen as the main hosts in this experiment because scale insects from these host-plant species appeared to be at the extremes of the morphological variation found in field-collected specimens of the P. vitis complex (see Section 3.3.1). These two host species were also readily avaliable.

The species concept used in the present study and the selection of analytical methods is discussed in Sections 3.1.2 and 3.1.3.

5.2 Methods

Collection of material in the field to start the cultures, slide preparation, statistical analysis and selection of morphological characters for study are all discussed in the methods of Chapter 3.

5.2.1 Material studied

The study material consisted of 7 non-clonal populations derived from field-collected samples. The adult females and ovisacs used to start the cultures were collected from a single host-plant species at each locality. The collection data for the source material is given in Appendix 3.2.

It was not possible to examine the effect of the experimental host-plant species on the morphology of a second generation of scale as almost all the cultures were completely destroyed by parasitoids in the second year.

5.2.2 Selection of host-plant species for the cultures

Seven experimental host-plant species were selected. The factors determining the selection of the host-plant species were the number of past collection records, economic importance of the plant, host systematic relationships and availability. The P. vitis complex in Britain is most often found on the following host species arranged in order of the number of reports: hawthorn, flowering currant, birch, blackcurrant, pyracantha and grapevine (see Appendix 3.2). Blackcurrant and grapevine, and to a lesser extent, peach and redcurrant, were also selected because of their economic importance. The different nominal species of the P. vitis complex were described from five of the selected host plants: "P. betulae" on birch, "P. oxyacanthae" on hawthorn, "P. persicae" on peach, "P. ribesiae" on blackcurrant, "P. salicis" on willow and "P. vitis" on grapevine. The plant species chosen are from the five host-plant families that contain the majority of P. vitis complex host-plant species: alder and birch from Betulaceae; willow from Salicaceae; hawthorn, pyracantha and peach from the Rosaceae; blackcurrant, redcurrant and flowering currant from the Grossulariaceae; and grapevine from the Vitaceae.

Although there were 7 source host-plant species and 7 experimental host-plant species, a total of 10 host species were involved (listed below) as the lists of natural and experimental host species were not identical. The host transfers made are summarised in Table 5.1. The following abbreviations for host-plant species are used in the tables, figures and appendices where there is insufficient space to print the names in full. Capital letters are used to indicate natural host plants and lower-case letters for experimental host plants.

AG,	ag	= Alnus glutinosa (L.) Gaertner	alder
BP,	bp	= Betula pendula Roth.	silver birch
CM,	cm	= Crataegus monogyna Jacq.	common hawthorn
PP,	pp	= Prunus persicae L. 'Peregrine'	peach
PC,	pc	= Pyracantha coccinea M. J. Roem.	firethorn, pyracantha
RN,	rn	= Ribes nigrum L. var. Ben Lomond	blackcurrant
RS,	rs	= Ribes sanguineum Pursh.	flowering currant
RSL,	rsl	= Ribes slyvestris Syme.	red or whitecurrant
SA,	sa	= Salix alba L.	white willow
VV,	vv	= Vitis vinifera L. var. black Hamburg	grapevine

5.2.3 Rearing

For rearing in the laboratory, mature females with developing ovisacs

were collected during May and June, in both 1988 and 1989. This stage was selected as ovisacs were easily transferred between host plants and eggs were less likely to be parasitized than adult scales in Britain. In Hungary, however, ovisacs were often found to contain predacious lepidopteran and dipteran larvae.

Each individual ovisac, still attached to a small piece of the original host, was transferred to a new host plant with forceps. The piece of original host was pinned or tied with wire twist ties to the base of the main stem of the new host (see Fig. 5.1). The ovisac faced downwards and the tie or pin was fixed below. During the time of egg hatch, the experimental plants with ovisacs from the same original locality were grouped together, and isolated from other groups of plants with ovisacs from different localities. This was in order to prevent cross contamination. None of the individual plants in each group had direct contact with each other to prevent crawlers walking between plants.

Two ovisacs were transferred to each plant in the initial experiment because a high percentage of scales were parasitized. Parasitized adults still produced ovisacs but their fecundity was lower (See Section 4.3.1). The plants were grown in small pots on the roof of the Natural History Museum, London. They were grown in the open, except for the grapevines, which were grown in an unheated greenhouse. Temperature and light fluctuated with the season. The plants were watered every other day during the summer and care was necessary to avoid soaking the ovisacs when using a hose-pipe.

5.3 Results

The morphological variation of the reared cultures of P. vitis is induced by two sources; the experimental host-plant species (environmental variation) and the original parental deme (genetic variation).

5.3.1 The effect of the experimental host plant

A relatively constant ordering of size of morphological characters was observed among the P. vitis specimens, according to the experimental host-plant species. This ordering of size was regardless of the original host and deme. Tables 5.2-5.9 show the means, standard deviations and ranges for the 8 morphological characters investigated. The influence of the experimental host plant on morphological variation of P. vitis is indicated by the mean

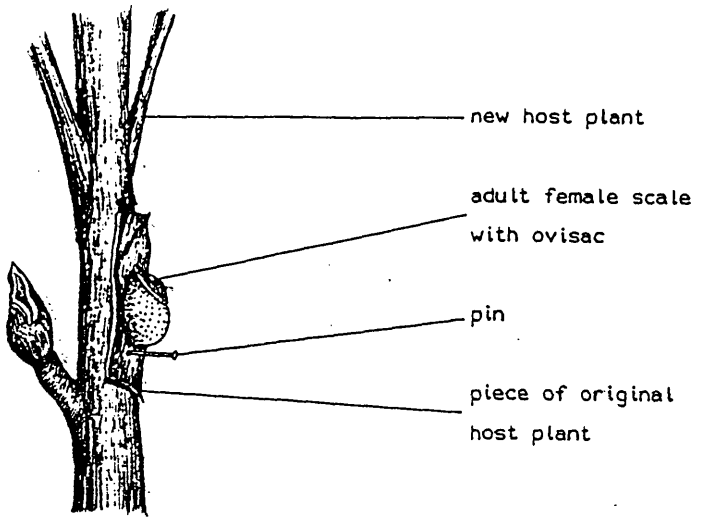


Fig. 5.1 Attachment of the transferred ovisac of the Pulvinaria vitis complex to the experimental host plant, Ribes nigrum

Transfer host plants		1988		1989	
Natural	Experimental	Number of replicates	Adult ♀♀ number	Number of replicates	Adult ♀♀ number
birch	birch	2	5	10	1 ♂♂
birch	hawthorn	1	10	10	0 ♂♂
birch	blackcurrant	2	15 ♂♂	10	0
birch	willow	1	3	10	20 ♂♂
birch	grapevine	2	10		
hawthorn	birch	1	0	10	0
hawthorn	hawthorn	1	0	10	0 ♂♂
hawthorn	blackcurrant	2	21	10	0
hawthorn	redcurrant	1	5		
hawthorn	willow	1	1	10	0
hawthorn	grapevine	1	0		
peach	alder	1	7		
peach	birch	2	15		
peach	hawthorn	1	12 ♂♂		
peach	peach	1	9		
peach	blackcurrant	2	12 ♂♂		
peach	willow	2	15		
peach	grapevine	1	15		
pyracantha	birch	1	0		
pyracantha	hawthorn	1	14		
blackcurrant	birch	1	0 ♂♂		
flowering currant	hawthorn	1	6 ♂♂		
flowering currant	blackcurrant	2	10		
flowering currant	willow	1	10		
flowering currant	grapevine	1	1		
willow	redcurrant	1	2		
grapevine	birch	1	10		
grapevine	hawthorn	1	12		
grapevine	blackcurrant	1	3		
grapevine	willow	2	10		
grapevine	grapevine	2	18		

Table 5.1 Summary of the host transfer experiments
Adult ♀♀ number = indicates the total number of slide-mounted adult females in all the replicates of each transfer; ♂♂ = indicates the presence of males in one or more replicates

Table 5.2
Table of means, standard deviations and ranges for the dorsal setae length (um) of Pulvinaria vitis

NAT. HOSTS.	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		10.0 2.00 7-14	8.73 1.01 7-10			9.75 1.39 8-12		11.0 1.66 8-15	12.9 1.58 11-15	10.4 2.01 7-15	9.66 2.22 5-14
CM		9.00	8.33 1.58 6-10			9.83 1.44 8-13		11.0 1.00 10-12		9.58 1.58 6-13	8.38 1.77 4-11
PP	9.00 0.58 8-10	9.21 1.05 8-11	9.42 1.16 8-12	11.0 0.83 10-12		10.0 1.38 7-12		9.65 1.06 8-12	10.9 1.16 9-15	10.0 1.43 7-15	9.50 1.70 6-13
PC			7.00 1.52 5-10							7.00 1.52 5-10	9.15 1.57 7-13
RN											
RS			8.94 1.06 8-12			8.78 1.24 7-11		9.15 0.88 8-11	10.0	8.97 1.07 7-12	7.92 1.46 5-11
SA						8.00				8.00	8.05 1.74 5-13
VV		10.3 1.20 9-13	8.50 0.67 7-9			8.33 0.58 8-9		9.90 1.37 7-12	10.8 2.28 8-15	9.91 1.65 7-15	10.4 1.74 7-15
Exp. Pop.	9.00 0.58 8-10	9.90 1.53 7-14	8.47 1.40 5-12	11.2 0.85 10-12		9.47 1.43 7-13		9.96 1.43 7-12	11.3 1.96 8-15		
Nat. Pop.	7.45 1.57 5-10	9.66 2.22 5-14	8.38 1.77 4-11	9.50 1.70 6-13		9.22 1.70 5-12		8.05 1.74 5-13	10.4 1.74 7-15		

Table 5.3
Table of means, standard deviations and ranges of the number of marginal setae between the spiracular setae of Pulvinaria vitis

NAT. HOSTS.	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		18.8 4.07 13-26	14.0 3.56 10-23			20.7 2.44 17-25		18.6 2.83 14-23	28.7 2.15 25-32	19.9 5.20 10-32	18.3 4.95 12-31
CM		16.0	14.3 1.58 12-17			12.3 2.67 7-19		12.3 2.89 9-14		12.8 2.59 7-19	13.1 1.94 9-19
PP	14.2 2.21 11-17	15.0 2.75 11-19	14.0 1.60 10-16	14.3 2.65 9-17		14.8 2.68 10-19		14.8 2.23 9-18	16.4 2.19 12-21	15.0 2.42 9-21	16.3 3.15 12-23
PC			13.2 2.29 9-16							13.2 2.29 9-16	13.4 2.25 10-17
RN											
RS			13.0 1.44 11-15			14.0 1.73 9-14		16.1 1.70 11-17	14.0	12.7 1.59 9-17	13.0 2.03 9-18
SA						13.0				13.0	15.3 2.46 9-22
VV		14.0 2.41 10-19	11.3 1.87 9-15			14.0 1.73 12-15		16.8 1.70 13-19	15.5 2.62 11-20	14.5 2.86 9-20	16.5 2.34 11-2
Exp. Pop	14.2 2.21 11-17	15.98 3.72 10-26	13.2 2.29 9-23	14.3 2.65 9-17		14.1 3.99 7-25		15.5 2.89 9-23	18.8 5.78 11-32		
Nat. Pop.	12.1 1.47 9-14	18.3 4.95 12-31	13.1 1.94 9-19	16.3 3.15 12-23		12.6 2.06 8-16		15.3 2.46 9-22	16.5 2.34 11-22		

Table 5.4
Table of means, standard deviations and ranges of the number of submarginal setae between the spiracular setae of Pulvinaria vitis

NAT. HOSTS.	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		9.29 1.99 6-13	8.36 1.57 6-11			9.94 2.11 4-13		9.67 2.00 6-13	9.45 2.11 4-12	9.41 1.99 4-13	8.59 1.74 5-13
CM		6.00	7.00 1.22 6-9			6.97 1.65 4-12		7.33 0.58 7-8		6.98 1.49 4-12	6.84 1.40 4-10
PP	8.29 1.98 5-11	7.79 1.37 5-10	8.75 1.82 5-11	8.67 1.32 7-11		6.82 1.72 4-9		8.00 1.62 4-10	9.17 1.34 6-11	8.31 1.68 4-11	7.50 1.45 5-10
PC			8.21 1.12 6-10							8.21 1.12 6-10	7.62 1.58 5-11
RN											
RS			10.1 1.59 7-13			8.09 1.44 5-11		8.35 1.18 6-10	9.00	8.73 1.61 5-13	7.14 1.75 4-11
SA						6.50 0.71 6-7				6.50 0.71 6-7	8.45 1.60 6-12
VV		7.78 1.48 4-10	7.50 0.90 6-9			6.33 1.53 5-8		8.76 1.37 6-11	9.43 1.28 7-12	8.31 1.53 4-12	8.44 1.40 7-13
Exp. Pop.	8.29 1.98 5-11	8.26 1.78 4-13	8.21 1.12 6-10	8.67 1.32 7-11		7.78 2.02 4-13		8.65 1.63 4-13	9.30 1.49 4-12		
Nat. Pop.	6.45 1.81 3-10	8.59 1.74 5-13	6.84 1.40 4-10	7.50 1.45 5-10		7.61 1.80 2-10		8.45 1.60 6-12	8.44 1.40 7-13		

Table 5.5
Table of means, standard deviations and ranges of the number of setae between antennal bases of Pulvinaria vitis

NAT. HOSTS	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		9.71 0.85 8-11	8.73 0.79 8-10			8.94 0.77 7-10		9.22 0.81 8-11	9.27 1.19 7-11	9.21 0.91 7-11	8.62 0.68 7-10
CM		7.00	8.33 0.87 7-9			8.53 1.07 5-11		9.33 0.58 9-19		8.51 1.03 5-11	8.62 0.85 7-11
PP	9.14 0.90 8-10	9.21 0.97 8-11	9.50 0.52 9-10	9.22 0.97 8-11		9.00 0.63 8-10		9.29 0.77 8-11	10.3 1.13 8-12	9.53 1.01 8-12	9.57 1.09 8-11
PC			10.1 1.10 8-12							10.1 1.10 8-12	9.12 0.86 8-11
RN											
RS			8.50 0.52 8-9			8.39 0.58 7-9		8.65 0.93 7-10	8.00	8.50 0.70 7-10	8.97 1.14 6-12
SA						9.00 0.00 9-9				9.00 0.00 9-9	8.83 0.78 7-10
VV		9.39 0.70 8-11	8.58 1.00 6-10			9.00 0.00 9-9		9.76 0.89 9-12	10.5 1.55 9-13	9.59 1.19 6-13	10.3 1.19 8-13
Exp. Pop.	9.14 0.90 8-10	9.40 0.90 7-11	9.00 1.03 6-12	9.22 0.97 8-11		8.66 0.84 5-11		9.24 0.92 7-12	10.1 1.37 7-13		
Nat. Pop.	9.09 1.04 7-11	8.62 0.68 7-10	8.62 0.85 7-11	9.57 1.09 8-11		8.88 0.71 7-10		8.83 0.78 7-10	10.3 1.19 8-13		

Table 5.6
Table of means, standard deviations and ranges of the number of dorsal submarginal tubercles of Pulvinaria vitis

NAT. HOSTS	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		5.47 2.24 2-9	7.73 2.69 1-11			4.06 1.53 2-7		6.56 2.50 3-10	5.00 2.72 1-9	5.70 2.57 1-11	2.03 2.11 0-9
CM		0.00	4.89 2.98 1-11			5.00 2.26 0-9		6.67 2.08 5-9		4.98 2.49 0-11	3.72 2.76 0-12
PP	9.43 1.51 8-12	6.00 2.22 1-10	9.58 1.44 6-12	6.44 2.88 2-10		5.73 2.41 2-10		7.00 2.50 4-12	9.08 2.39 3-14	7.69 2.69 1-14	4.50 2.59 0-10
PC			5.14 2.28 1-8							5.14 2.28 1-8	3.15 2.49 0-9
RN											
RS			6.88 1.96 4-11			6.00 1.83 2-9		6.90 1.97 4-11	8.00	6.57 1.93 2-11	6.16 3.69 0-13
SA						0.50 0.71 0-1				0.50 0.71 0-1	3.70 2.45 0-11
VV		5.89 2.65 2-10	6.42 2.75 4-12			4.67 2.31 2-6		6.43 2.68 1-10	8.64 1.98 4-12	6.66 2.70 1-12	7.33 2.53 2-12
Exp. Pop.	9.43 1.51 8-12	5.66 2.47 0-10	6.80 2.74 1-12	6.44 2.88 2-10		5.07 2.21 0-10		6.71 2.37 1-12	8.04 2.82 1-14		
Nat. Pop.	3.09 2.66 0-7	2.03 2.11 0-9	3.72 2.76 0-12	4.50 2.59 0-10		5.27 3.25 0-13		3.70 2.45 0-11	7.33 2.53 2-12		

Table 5.7
Table of means, standard deviations and ranges of the number of multilocular pores on abdomenal segment 4 of Pulvinaria vitis

NAT. HOSTS.	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		59.3 8.87 48-83	53.2 10.04 38-75			60.3 11.96 32-82		69.0 13.83 45-93	65.6 12.48 37-78	62.9 12.37 32-93	63.7 12.09 39-86
CM		48.0	39.8 6.55 28-47			64.6 13.65 6-79		51.0 7.81 42-56		5 3.5 10.63 6-79	44.8 9.56 25-68
PP	47.1 5.76 39-55	45.3 3.37 40-54	50.0 6.18 40-64	63.0 8.69 51-78		50.1 9.85 36-65		57.7 11.28 41-75	47.2 7.46 35-60	51.8 9.96 35-78	54.7 12.89 30-75
PC			36.3 8.45 20-48							36.3 8.45 20-48	51.7 12.48 30-75
RN											
RS			43.9 6.34 35-59			45.1 5.68 33-56		52.2 13.93 9-77	54.0	46.1 9.97 9-77	42.4 7.65 30-61
SA						40.5 4.95 37-44				40.5 4.95 37-44	60.3 10.99 43-92
VV		45.4 4.84 38-56	20.3 5.28 16-32			57.0 12.77 46-71		62.1 10.18 50-80	56.5 9.69 41-79	48.1 15.71 16-80	41.8 9.54 24-65
Exp. Pop.	47.1 5.76 39-55	50.2 9.04 38-83	40.5 12.71 16-75	63.0 8.69 51-78		49.5 12.19 6-82		59.8 13.54 9-93	54.0 11.71 35-79		
Nat. Pop.	48.0 18.08 25-81	63.7 12.09 39-86	44.8 9.56 25-68	54.7 12.89 30-75		57.6 10.25 36-83		60.3 10.99 43-92	41.8 9.54 24-65		

Table 5.8
Table of means, standard deviations and ranges of the number of thick filamented tubular ducts on the head region of Pulvinaria vitis

NAT. HOSTS.	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		8.12 6.28 0-23	3.36 7.16 0-24			3.81 3.56 0-11		10.2 9.07 3-36	8.64 5.28 2-20	7.25 6.81 0-36	11.6 12.41 0-65
CM		7.00	2.33 2.29 0-7			12.1 7.01 0-27		20.3 4.51 16-25		8.74 7.50 0-27	3.94 5.06 0-27
PP	3.00 2.31 1-7	2.57 2.21 0-8	0.58 0.90 0-3	8.33 3.67 3-14		3.64 7.54 0-25		9.24 5.98 0-18	6.83 3.53 2-13	5.16 5.01 0-25	3.71 3.20 0-13
PC			0.07 0.27 0-1							0.07 0.27 0-1	5.42 4.49 0-15
RN											
RS			0.44 0.63 0-2			3.43 2.41 0-10		4.70 2.92 1-14	4.00	2.97 2.65 0-14	3.00 4.18 0-20
SA						23.5 20.51 9-38				23.5 20.51 9-38	5.18 4.88 0-24
VV		4.00 2.70 0-10	0.42 0.79 0-2			12.6 9.50 3-22		4.19 3.44 0-11	3.79 2.94 0-9	3.58 3.58 0-22	3.21 5.57 0-31
Exp. Pop.	3.00 2.31 1-7	5.06 4.71 0-23	1.05 3.05 0-24	8.33 3.67 3-14		7.41 7.65 0-38		7.39 6.68 0-36	6.32 4.14 0-20		
Nat. Pop.	8.36 6.22 0-20	11.6 12.41 0-65	3.94 5.06 0-27	3.71 3.20 0-13		11.1 8.07 0-36		5.18 4.88 0-24	3.21 5.57 0-31		

Table 5.9
Table of means, standard deviations and ranges of the number of slender filamented tubular ducts on the head region of Pulvinaria vitis

NAT. HOSTS.	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		8.29 7.41 1-29	2.82 3.06 0-11			7.69 7.56 0-28		15.0 16.85 1-60	29.6 17.65 11-14	12.2 13.82 0-64	15.4 13.88 1-50
CM		5.00	4.89 3.76 0-11			14.8 9.05 4-38		16.3 6.11 11-23		11.3 8.40 0-38	7.40 6.18 0-28
PP	11.1 7.80 2-22	2.64 2.37 0-7	0.50 1.45 0-5	5.22 3.27 2-10		5.82 7.61 1-27		10.6 5.69 2-22	11.0 6.83 0-24	7.04 6.58 0-27	6.14 5.99 0-23
PC			0.57 0.65 0-2							0.57 0.65 0-2	8.00 7.49 0-26
RN											
RS			3.44 4.75 0-14			7.43 6.47 0-22		8.95 8.37 1-36	5.00	6.33 6.63 0-36	2.51 3.12 0-13
SA						24.0 16.97 12-36				24.0 16.97 12-36	4.70 4.37 0-18
VV		3.11 2.74 0-9	1.00 1.21 0-4			16.6 9.07 10-27		5.86 6.17 0-26	4.64 3.48 0-11	7.32 8.40 0-39	8.86 10.27 0-39
Exp. Pop.		4.78 5.35 0-29	2.11 3.25 0-14			10.5 9.00 0-38		10.1 10.42 0-60	13.2 13.18 0-64		
Nat. Pop.	4.09 4.70 0-11	15.4 13.88 1-50	7.40 6.18 0-28	6.14 5.99 0-23		5.22 5.27 0-20		4.70 4.37 0-18	8.86 10.27 0-39		

Table 5.10
Table showing the numbers of individuals of Pulvinaria vitis analysed from the host transfer experiments

NAT. HOSTS	EXPERIMENTAL HOST PLANTS										
	ag	bp	cm	pp	pc	rn	rs	sa	vv	Exp. Pop.	Nat. Pop.
AG											
BP		17	11			16		18	11	73	29
CM		1	9			30		3		43	50
PP	9	14	12	9		11		17	24	94	14
PC			14							14	26
RN											
RS			16			23		20	1	60	37
SA						2				2	40
VV		18	12			3		21	14	68	43
Exp. Pop.	9	50	74	9		85		79	50	354	
Nat. Pop.	11	29	50	14		41		40	43		

values along the rows. Theorectically, the data in the rows shows the variation of individuals with similar genotypes reared under different environmental conditions.

The mean values for the morphological characters of specimens reared on grapevine, birch and peach are larger than the mean values of specimens reared on hawthorn, blackcurrant and alder, regardless of the original parental host-plant species. However, when the mean values of each character are plotted, the confidence limits show considerable overlap between the samples from each host plant. When the data is combined each character shows a continuous gradation and all produce modal distributions.

To summarise, the morphological variation of the P. vitis complex in Britain is not only associated with the different host-plant species, as demonstrated in Chapter 3, but is in part, induced by the host-plant species.

Canonical variates analysis with data grouped according to experimental host-plant species

Specimens with similar genotypes segregate into different morphological groups according to the host-plant species on which they were reared, using canonical variates analysis (CVA). The results of the CVA are plotted in Figs. 5.2-5.11. Only the first two canonical variates are plotted as almost all the variation was accounted for by the first two axes and segregation into groups was no clearer when other combinations of canonical variates were plotted.

Figs. 5.2-5.6 show the CVA plots for the transfers from birch, hawthorn, peach, flowering currant and grapevine to the experimental host-plants respectively. All the CVA plots for the host-transfer specimens show some degree of grouping according to experimental host-plant species. For example, Fig. 5.2 shows the CVA plot of the transfer from birch to 5 experimental host species: birch, hawthorn, blackcurrant, willow and grapevine. The first two axes account for almost 90% of the total variation. Clear grouping of the specimens according to experimental host-plant species can be seen. The specimens reared on grapevine and hawthorn are separated from the majority of the specimens along canonical variate 1. The most

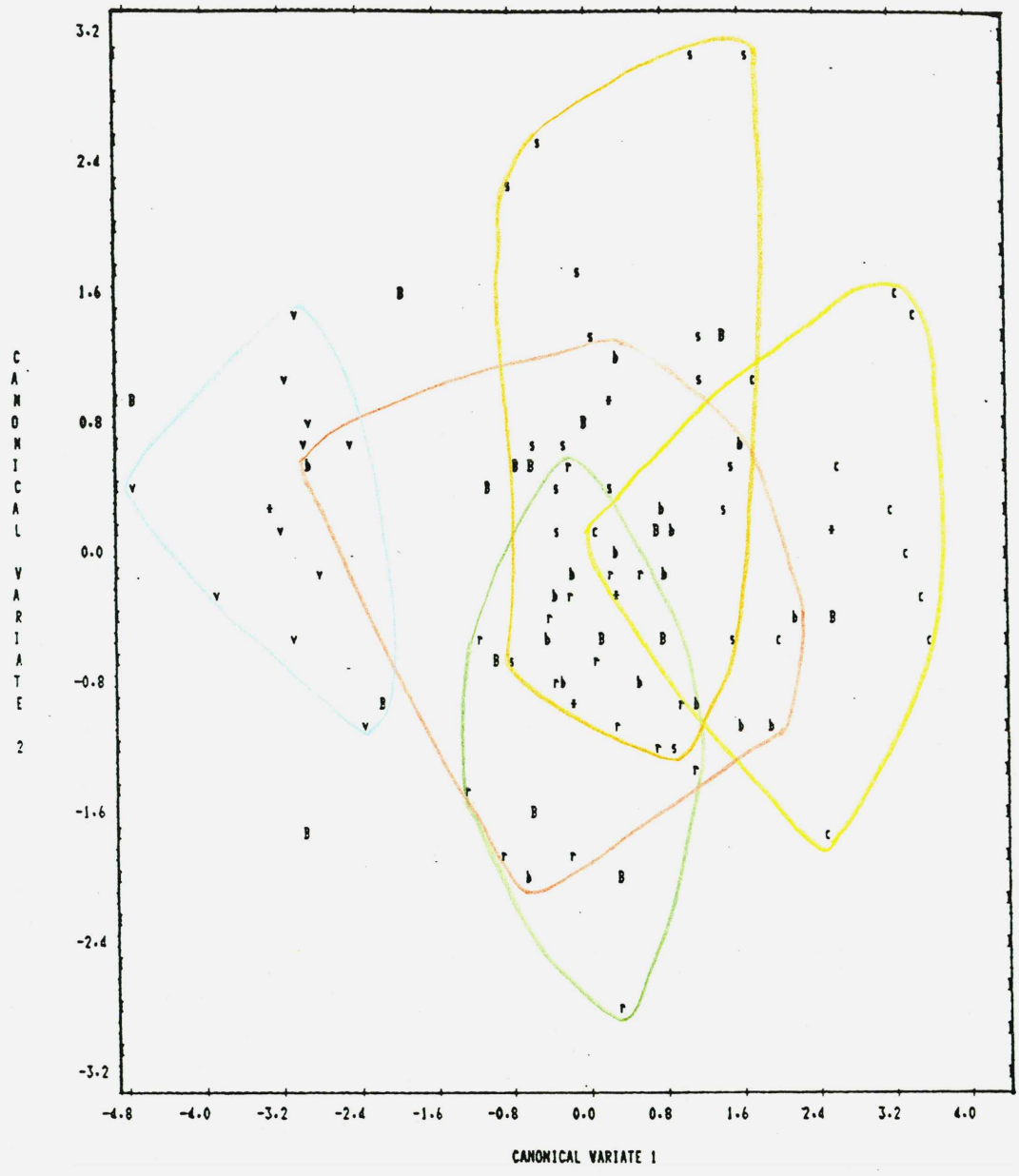


Fig. 5.2 Canonical variates analysis of samples of the Pulvinaria vitis complex transferred from birch (B) and reared on: birch (b); hawthorn (c); willow (s); blackcurrant (r) and grapevine (v). Scores for means (*)

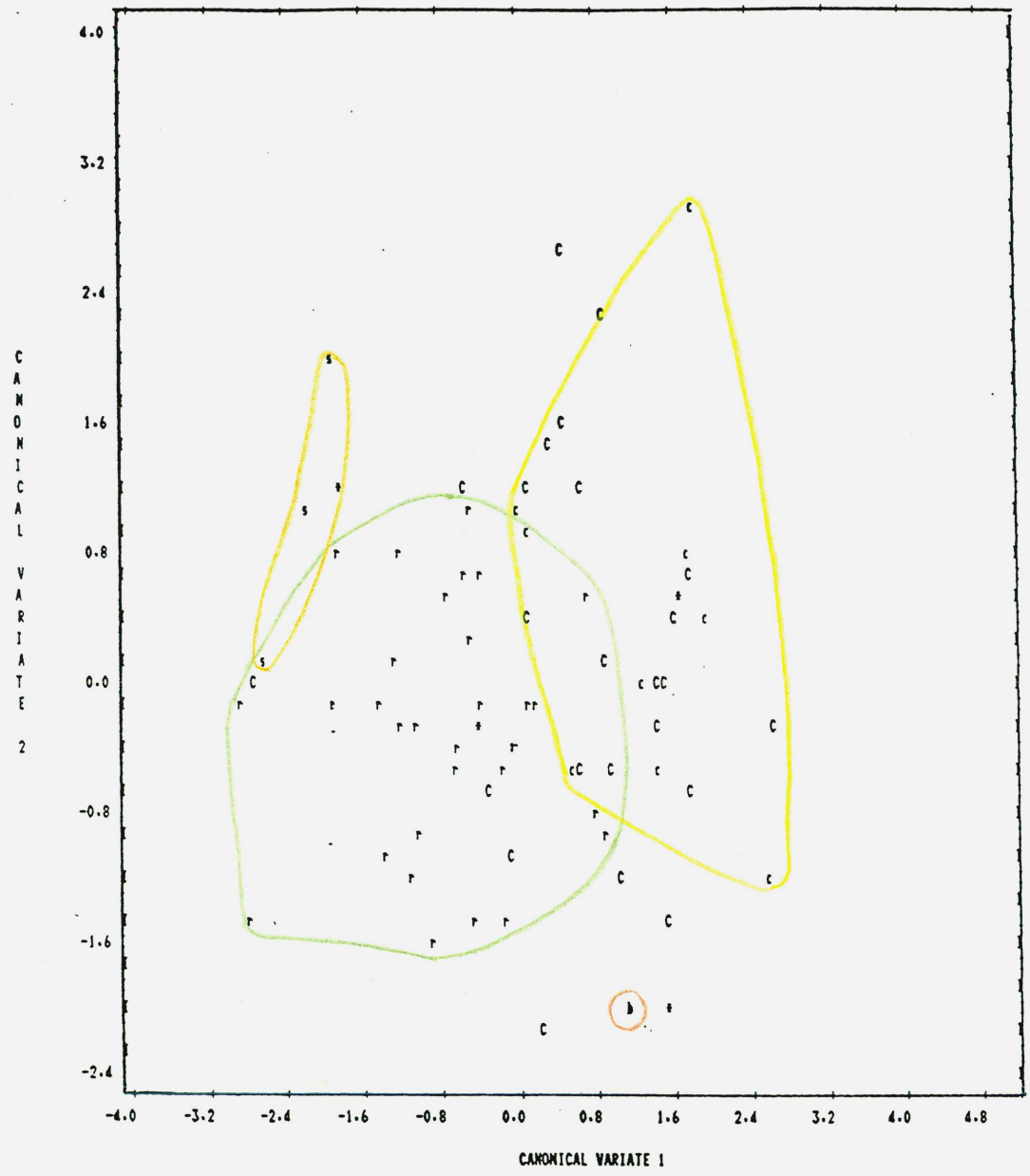


Fig. 5.3 Canonical variates analysis of samples of the Pulvinaria vitis complex transferred from hawthorn (C) and reared on: birch (b); hawthorn (c); willow (s) and blackcurrant (r). Scores for means (*)

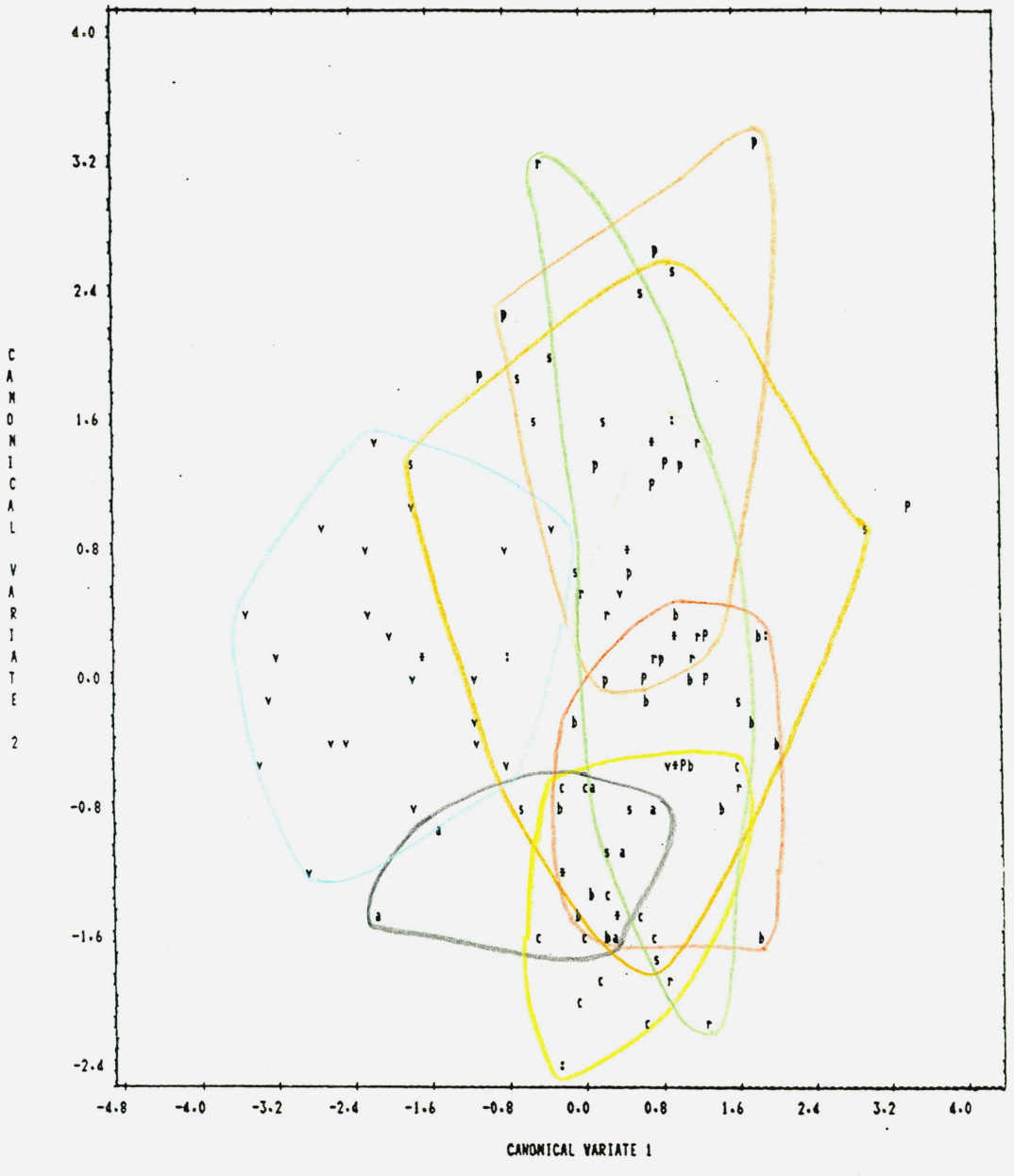


Fig. 5.4 Canonical variates analysis of samples of the Pulvinaria vitis complex transferred from peach (P) and reared on: alder (a); birch (b); hawthorn (c); peach (p); willow (s); blackcurrant (r) and grapevine (v). Scores for means (*). : = overlapping samples.

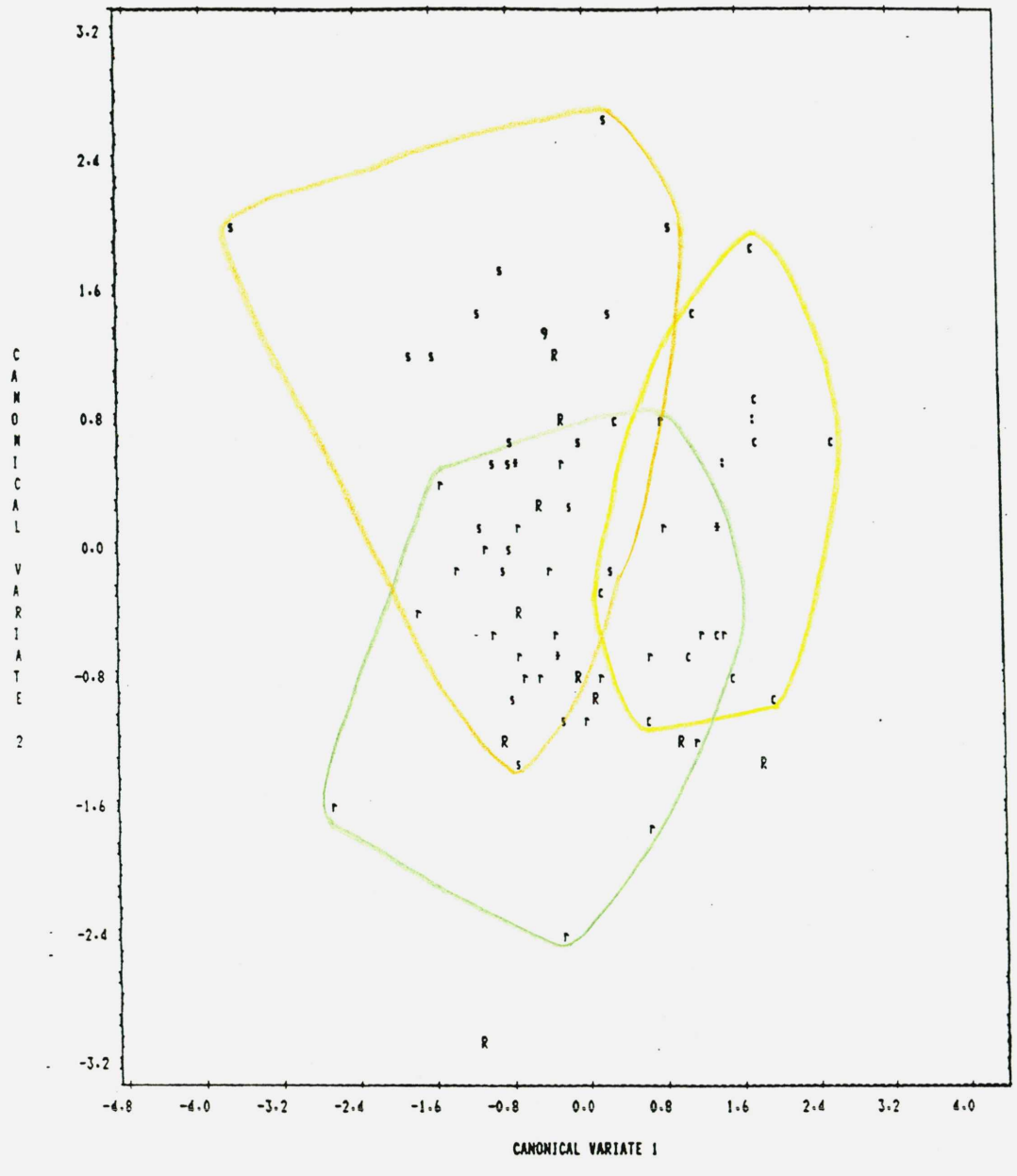


Fig. 5.5 Canonical variates analysis of samples of the Pulvinaria vitis complex transferred from flowering currant (R) and reared on: hawthorn (c), willow (s) and blackcurrant (r). Scores for means (*). : = overlapping samples.

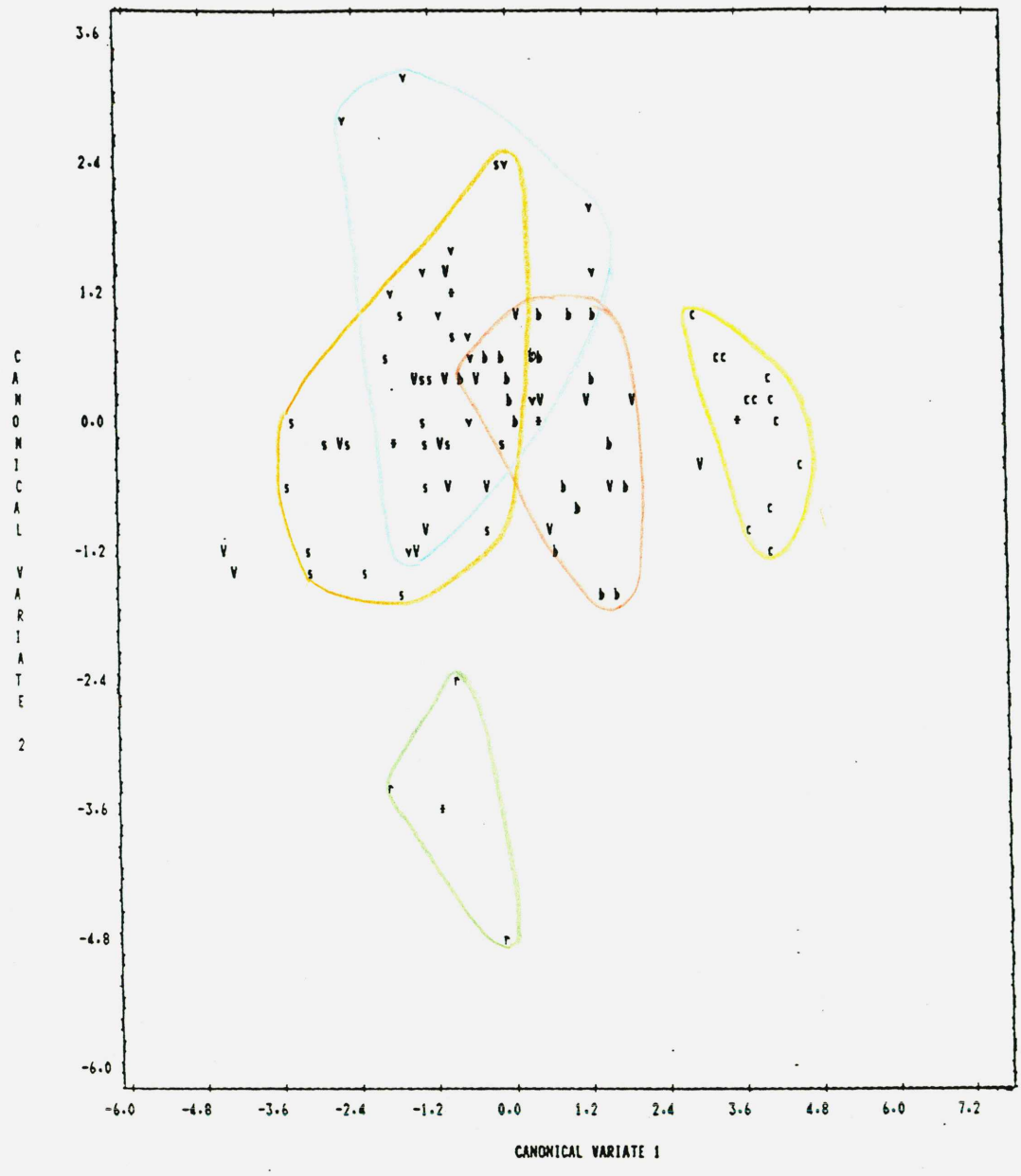


Fig. 5.6 Canonical variates analysis of samples of the Pulvinaria vitis complex transferred from grapevine (V) and reared on: birch (b) hawthorn (c); blackcurrant (r); willow (s) and grapevine (v). Scores for means (*)

important morphological characters in the latent vector loadings of the first canonical variate are the numbers of interantennal setae and the numbers of marginal setae present between the spiracular setae. The specimens reared on blackcurrant and willow are separated from each other along canonical variate 2. The most important characters along axis 2 are the dorsal seta length, number of interantennal setae and the number of dorsal submarginal tubercles. The specimens which were reared on birch (control transfer) appear more or less in the centre of the plot. Specimens collected from birch from the original locality are superimposed on to the plot but have not been included in the analysis. The field-collected specimens are widely spread over the plot and overlap all the other groups of specimens.

Specimens reared on grapevine also separate out from the majority of specimens in Figs. 5.3 and 5.4 in a similar way to Fig. 5.2. The most important character for the separation of specimens from grapevine in each plot are the number of interantennal setae. Specimens reared on hawthorn, blackcurrant and willow are separated in Fig. 5.5 largely by the dorsal seta length, numbers of interantennal setae and numbers of marginal setae present between the spiracular setae.

To summarise, British specimens of the P. vitis complex show host-induced morphological variation. Specimens with similar genotypes were found to segregate with canonical variates analysis into different morphological groups according to the host-plant species on which they were reared. The characters which show the greatest host-induced variation are the numbers of interantennal setae, marginal setae, submarginal tubercles and dorsal seta length. These characters are all highly correlated with body size.

5.3.2 The effect of the parental deme

It has been demonstrated that the morphological variation of the P. vitis complex is, in part, induced by the host-plant species. However, the morphological variation of P. vitis, associated with the host plant is also, in part, genetically determined. The mean values of the morphological characters of each population, show a relatively constant ordering in size, according to the parental deme from which they were derived. For example, the populations derived from the deme on birch were consistently larger than the populations derived from the deme on hawthorn, regardless of the experimental host plant.

The influence of the original parental deme, that is, the genetically determined variation, is indicated by the mean values in the columns of Tables 5.2-5.9. The data in the columns shows the variation of individuals with different genotypes reared under similar environmental conditions (the same experimental host-plant species).

Two-way regression analyses was used to test if the mean values of the host transfer scale populations were more similar to the total mean values of field-collected specimens from the parental or experimental host-plant species. The mean values of the host transfer populations were found to be slightly closer to the mean values of field-collected specimens from the parental host-plant species. Although there was no significant differences in the regression values, the results suggest that the morphological variation of the scales in the host transfer experiments was determined more by the parental deme than by the experimental host plant on which they were reared. The difference in regression values, however, may be due in part to the data along the rows being more complete than the data in the columns.

To summarise, the morphological variation of the P. vitis complex in Britain is, in part, genetically determined.

Canonical variates analysis with data grouped according to parental deme

Specimens reared on the same host-plant species segregate into groups according to their original parental deme, using canonical variates analysis. In other words, individuals reared under similar environmental conditions segregate into morphological groups according to their genotype.

CVA plots for the transfers of the P. vitis complex from the different demes and reared on birch, hawthorn, blackcurrant, willow and grapevine, are shown in Figs. 5.7-5.11, respectively. If the morphological variation of the scales was determined solely by the host-plant species, all the specimens would appear randomly in the CVA plots; however, there was some grouping according to the original parental deme. Morphological variation of the P. vitis complex is therefore, also due in part to genotypic differences between demes.

Unfortunately, most of the morphological characters which show genetically-determined variation, such as numbers of setae, also show a large degree of environmentally-induced variation. There are some differences, however, between the most important morphological characters in the latent

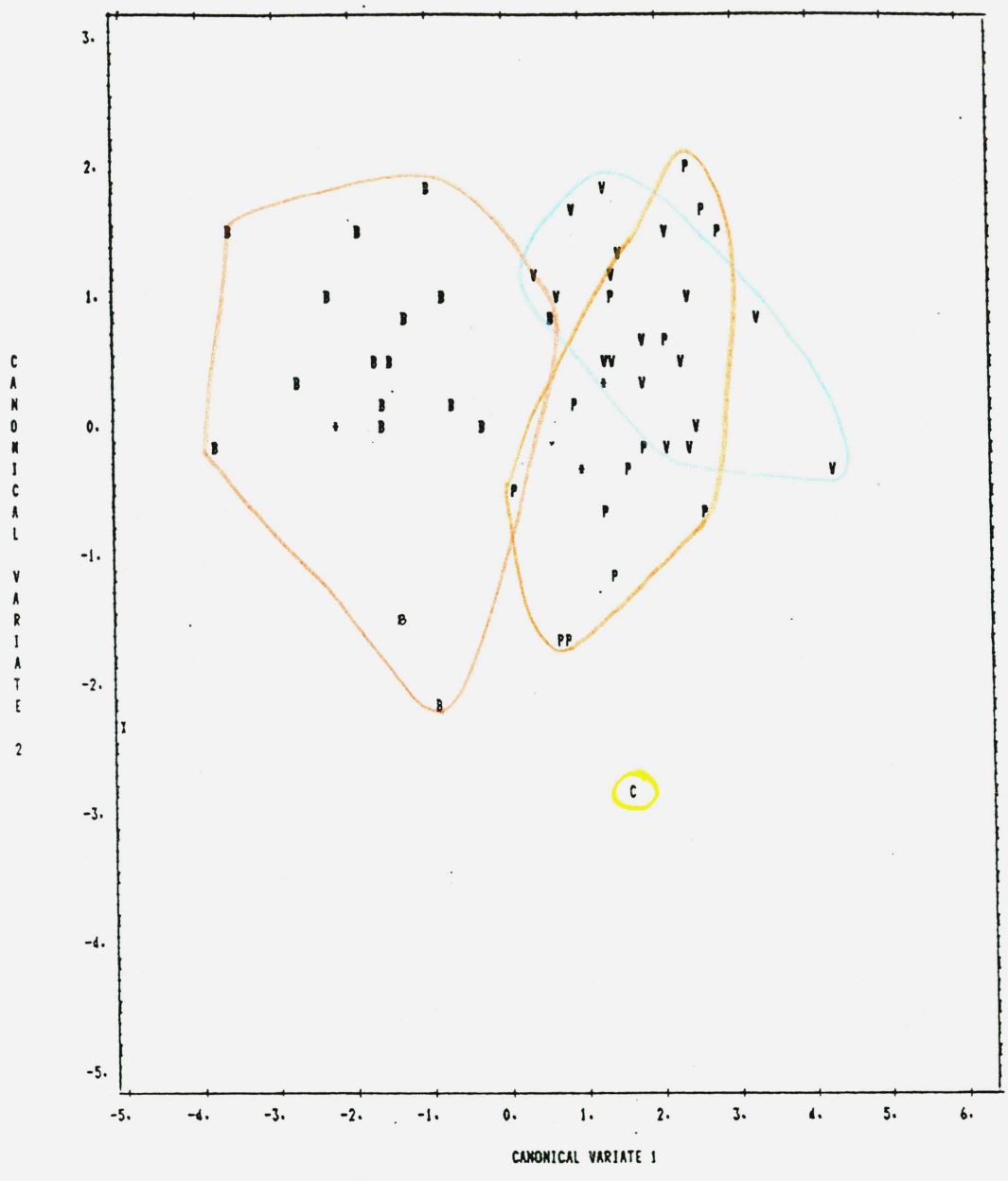


Fig. 5.7 Canonical variates analysis of samples of the Pulvinaria vitis complex reared on birch, derived from populations on: birch (B); hawthorn (C); peach (P) and grapevine (V). Scores for means (*)

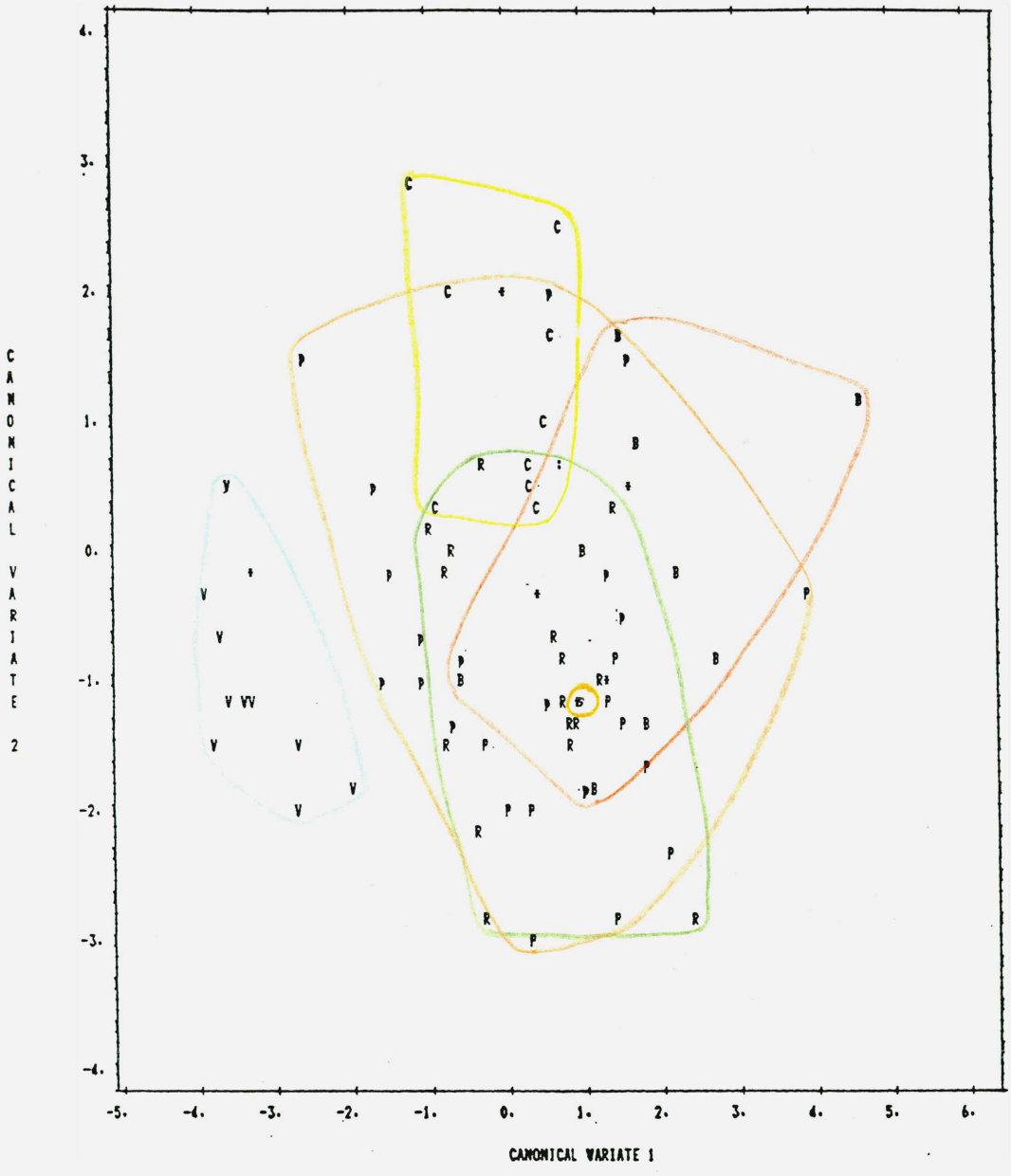


Fig. 5.8 Canonical variates analysis of samples of the Pulvinaria vitis complex reared on hawthorn, derived from populations on: birch (B); hawthorn (C); peach (P); blackcurrant (R); willow (S) and grapevine (V). Scores for means (*)

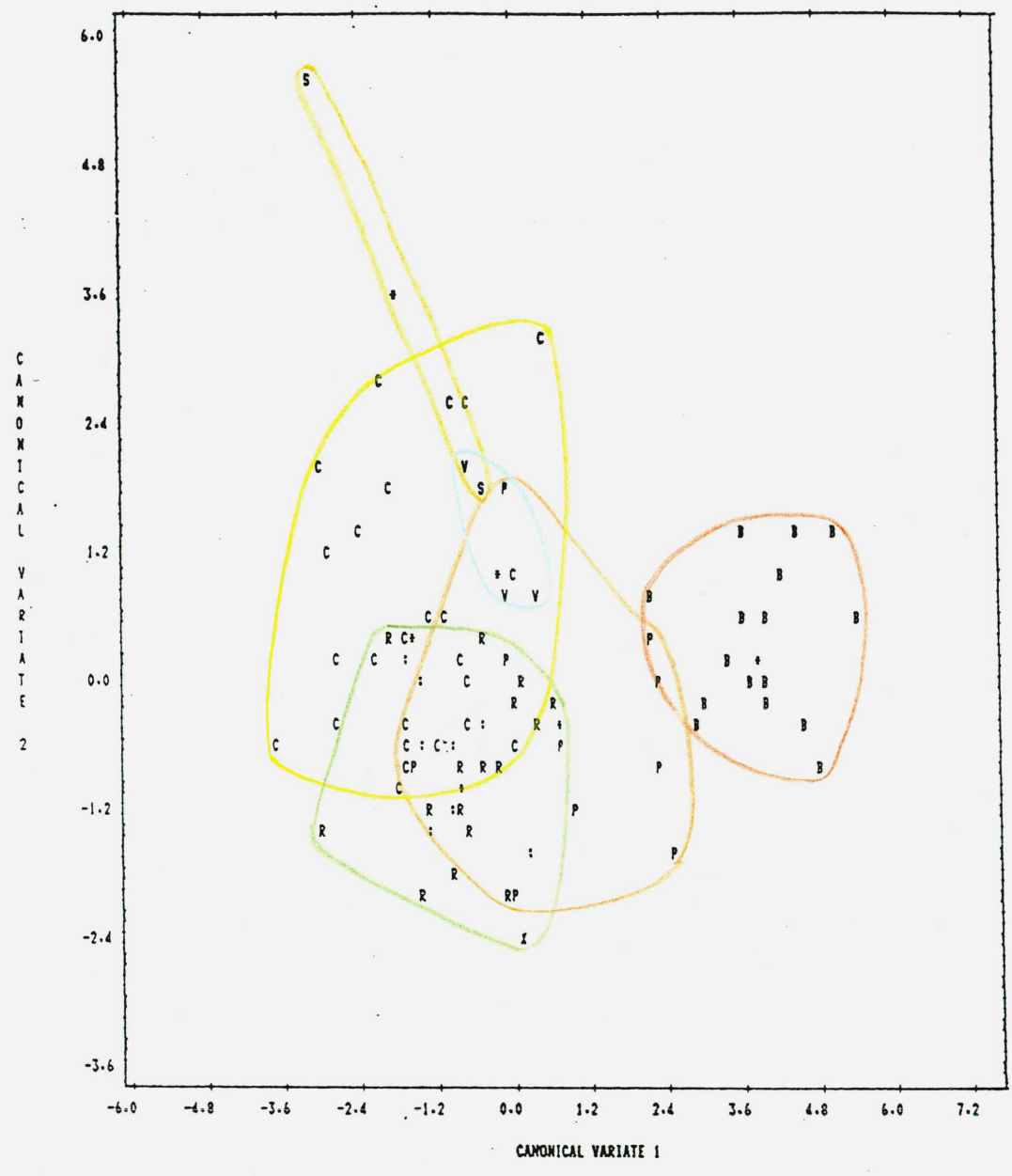


Fig. 5.9 Canonical variates analysis of samples of the Pulvinaria vitis complex reared on blackcurrant, derived from populations on: birch (B); hawthorn (C); peach (P); blackcurrant (R); willow (S) and grapevine (V). Scores for means (*). : = overlapping samples.

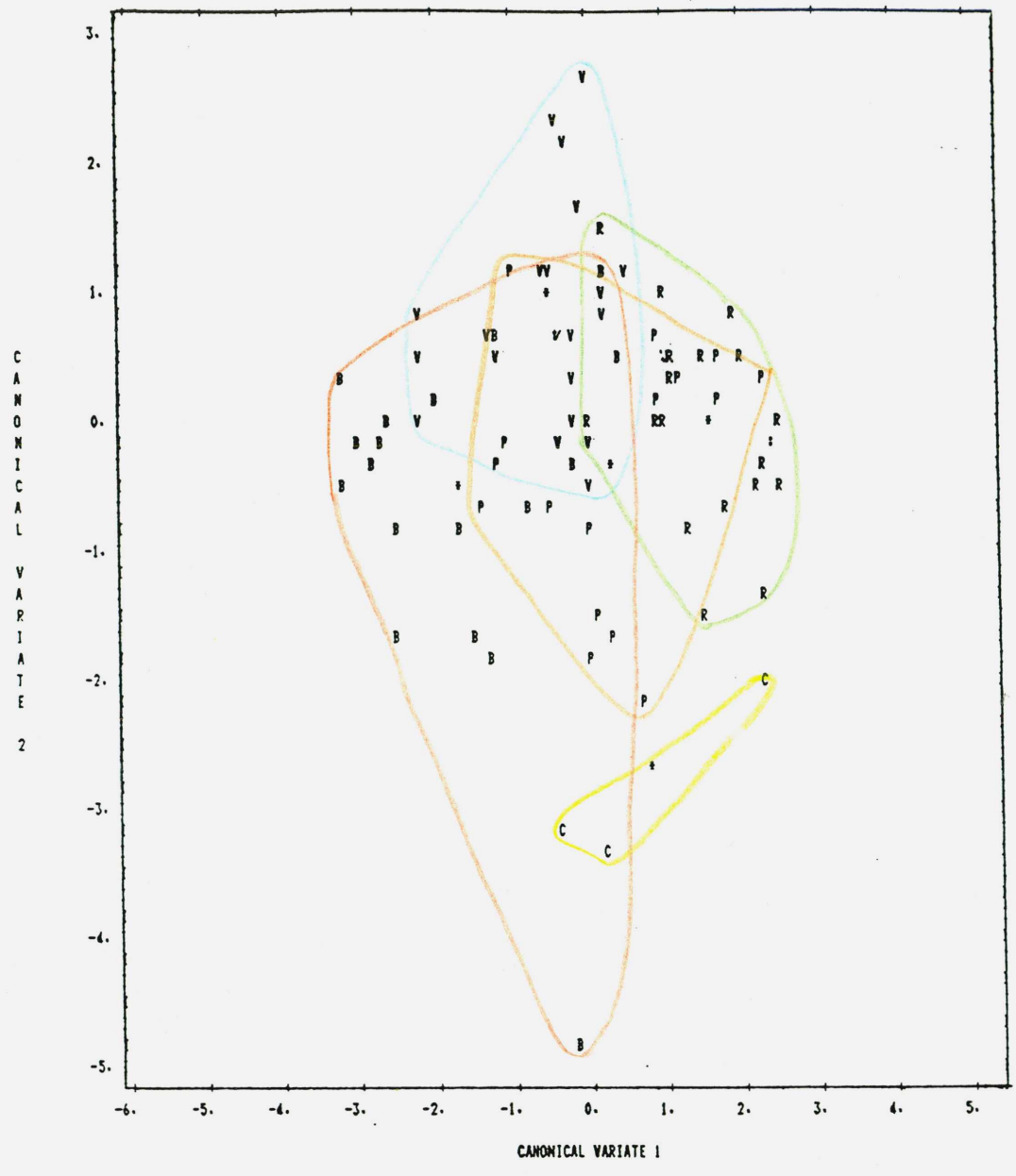


Fig. 5.10 Canonical variates analysis of samples of the Pulvinaria vitis complex reared on willow, derived from populations on: birch (B); hawthorn (C); peach (P); blackcurrant (R) and grapevine (V). Scores for means (*)

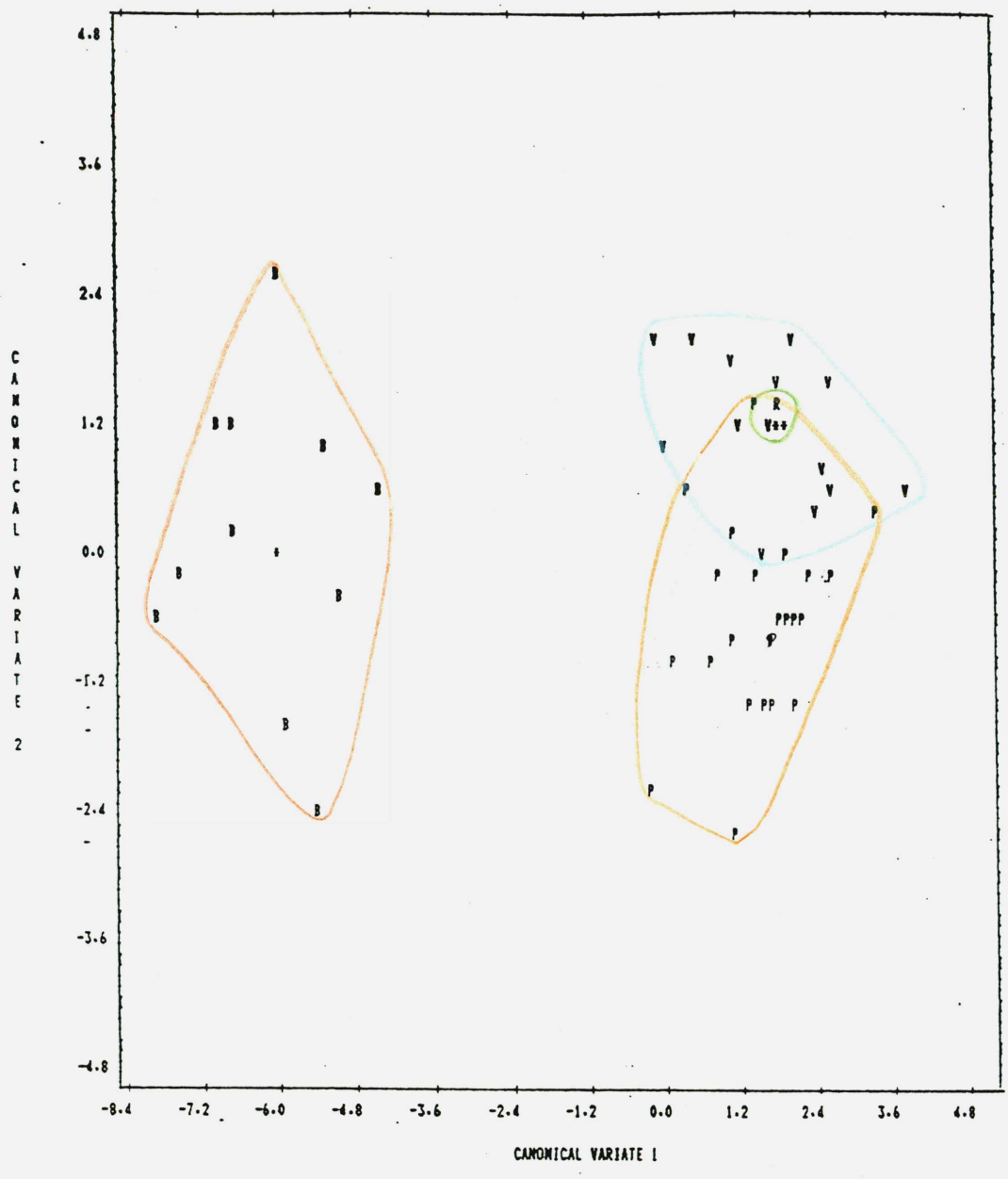


Fig. 5.11 Canonical variates analysis of samples of the Pulvinaria vitis complex reared on grapevine, derived from populations on: birch (B); peach (P); blackcurrant (R) and grapevine (V). Scores for means (*)

vector loadings in the CVA plots 5.7-5.11 and 5.2-5.6. For example, Fig. 5.8 shows the CVA plot for specimens reared on hawthorn derived from 6 different parental demes. The first two axes account for 84% of the variation. The specimens which were originally derived from grapevine are segregated from the majority of specimens along the second axis. The characters most significant to the latent vector loadings of canonical variate 2 are the numbers of multilocular pores and the numbers of tubular ducts with slender inner filaments. Specimens which were originally derived from hawthorn are segregated from those derived from peach and flowering currant along the second axis. The most important characters in the latent vector loadings of the second canonical variate are the numbers of submarginal setae between spiracular setae, dorsal seta length, and interantennal setae number.

To summarise, the morphological variation of the P. vitis complex in Britain was found to be related to parental deme, that is, genetically determined, as well as induced by the host-plant species. The characters which show the most genetic variation are related to size.

5.3.3 Data summaries and correlation between characters

The range and mean for 8 morphological characters for all the experimentally reared specimens, field-collected specimens and combined data are given in Table 5.11. There is good congruence between the total mean values of the experimental and natural population data although all the mean values are slightly higher for the experimentally reared specimens. A difference is expected due to differences in sample sizes and host-plant species between the natural and experimental groups. The range of variation found in the experimental and natural populations was also similar.

The correlation matrix for the experimental morphological data is shown in Table 5.12. None of the pairs of characters are highly correlated with each other except the numbers of tubular ducts with thick inner filaments and those with slender inner filaments.

The numbers of individuals of adult female P. vitis complex analysed from each host-transfer are shown in Table 5.10 and the data summaries and standard deviations for 8 morphological characters are given in Tables 5.2-5.9. Care needs to taken when comparing the data between different host transfers due to the different sample sizes.

Character	Minimum	Mean	Maximum
Experimentally reared specimens			
DSetL μm	5.00	9.73	15.00
MarSet	7.00	15.23	32.00
SMarSet	4.00	8.43	13.00
InAntSet	5.00	9.20	13.00
STubN	0.00	6.42	14.00
MPoreN4	6.00	50.98	93.00
TDuctL	0.00	5.53	38.00
TDuctS	0.00	8.15	64.00
Field-collected specimens			
DSetL μm	4.00	8.79	15.00
MarSet	7.00	14.31	31.00
SMarSet	2.00	7.65	13.00
InAntSet	6.00	9.08	13.00
STubN	0.00	4.56	13.00
MPoreN4	18.00	46.00	92.00
TDuctL	0.00	5.99	65.00
TDuctS	0.00	6.44	50.00
Combined data			
DSetL μm	4.00	9.27	15.00
MarSet	7.00	14.78	32.00
SMarSet	2.00	8.05	13.00
InAntSet	5.00	9.14	13.00
STubN	0.00	5.51	14.00
MPoreN4	6.00	50.48	93.00
TDuctL	0.00	5.75	65.00
TDuctS	0.00	7.32	64.00

Table 5.11 The range and mean for 8 morphological characters recorded from experimentally reared specimens (n = 354), British field-collected specimens (n = 335) and the combined data (n = 689)

DSetL	1.000							
MarSet	0.389	1.000						
SMarSet	0.174	0.361	1.000					
InAntSet	0.140	0.136	0.171	1.000				
STubN	0.060	-0.017	0.168	0.222	1.000			
MPoreN4	0.348	0.438	0.257	0.099	0.026	1.000		
TDuctL	0.209	0.127	-0.066	0.067	-0.064	0.309	1.000	
TDuctS	0.239	0.326	0.055	0.107	-0.012	0.261	0.628	1.000
	DSetL	MarSet	SMarSet	InAntSet	STubN	MPoreN4	TDuctL	TDuctS

Table 5.12 Correlation matrix for 8 morphological characters recorded from experimentally reared specimens (n = 254)

Abbreviations used in the tables for the morphological characters are those given in Section 3.2.5

5.3.4 Success of host transfers

First instars from the transferred ovisacs successfully established themselves on all the experimental host-plant species with varying degrees of success. It was relatively easy to establish scales on host-plant species other than the plant species on which the eggs were laid. However, only a small number of scale insects reached maturity in most of the transfers. The transfers of scale insect from peach and birch to the other host-plant species were considerably more successful than from hawthorn or flowering currant. This was probably due in part to the adult females from peach and birch being larger and having a higher fecundity. There was no evidence that taxa could be separated on host preference alone in this experiment as had been reported previously (Newstead, 1903). The transfers were considerably more successful in 1988 than 1989 (discussed in Section 7.1.2); see Table 5.1.

5.4 Discussion

The Pulvinaria vitis complex in Britain shows considerable morphological variation. This variation results from genetic variability within and between populations/demes; and environmental conditions. However, since the characters most effected are all related to size and correlated with each other, the genetic variation expressed by the different characters might be expected to be redundant.

A significant part of the morphological variation has been shown in the present study to be induced by the host-plant species. Host-induced variation in morphology and/or biology has been suggested in many polyphagous species of Coccoidea, but demonstrated experimentally in only a few species (see Section 3.1.2). Host-induced variation has been studied in detail in many Hymenopterous parasitoids. For example, Trichogramma semblidis (Aurivillius) (Trichogrammatidae: Hymenoptera) shows extremely different morphs according to the host upon which it is reared (Salt, 1937). This is not surprising as the whole life cycle of each individual scale insect or parasitoid is normally passed on a single host. Cox (1983) did not find host-plant species to affect morphological variation of a number of species of mealybug (Pseudococcidae), although the number of host-plant species investigated was small.

'Host forms' of a single species of scale insect may develop genetically

or environmentally. Different species of host plant will have different nutritional values for scale insects. The 'host forms' of the P. vitis complex differ from one another by size related characters, which suggests they may result in part from differences in host nutritional value. 'Host forms' of scale insects may also result from differences in selection pressure produced by the host. Scale insects survive by adapting to the phenotypic characteristics of the host plant, and must overcome host phenoimmunity and genetic-immunity. Scale insects adapt to these defences by being able to succesfully reproduce in populations with low male frequencies and by forming demes on individual hosts (Cooper & Oetting, 1986). Selection pressure, over many years, results in scale populations which are increasingly adapted to the defensive character of the individual host plant. Such scale populations may show genetic and morphological variability related to the host-plant species. Polyphagous scale insect species have host preferences, shown by fecundity, survivorship or sex ratio (Cooper & Oetting, 1986). These host preferences vary within the geographical range of the scale insect, which may be mediated by the climate.
Genetically based morphological variation has been demonstrated in many scale insect species, for example Chionaspis salicis (Signoret) discussed in Section 3.4.

Other environmental factors, such as temperature, can also affect morphological variation of scale insects. The rate of development of species of mealybug increased at higher temperatures resulting in smaller teneral adults with correspondingly smaller appendages and enumerated characters (Cox, 1983). It is interesting to note that the reverse was found with the P. vitis complex reared on grapevine under glass at higher temperatures. The rate of development was increased but the teneral adults were larger than specimens reared outdoors, at lower temperatures, on different host species. The nutritional value of the host may, therefore, be more important than temperature over a narrow range of temperatures. Host and temperature have also been shown to significantly affect morphological variation of minor anatomical characters frequently used in Trichogramma taxonomy (Pinto et al., 1989).

Much of the morphological variation of the P. vitis complex in Britain results from genetic variability both within populations and between demes. Unfortunately for the taxonomist, the characters which are genetically

variable also show the greatest degree of host-induced variation. The morphological characters which are most affected by the host plant are enumerate and have been considered important in the taxonomy of the P. vitis complex (see Section 3.4). An understanding of the potential magnitude of intraspecific variation in the secondary characters is neccesary before they are used for identification. Unfortunately, species are typically based on a few indviduals from a limited geographical area and intraspecific variation is often not taken into account. To ignore such plasticity would eventually fill the literature with species names that can be associated with little more than the type material on which they are based. Species within the P. vitis complex should only be described from limited samples if major anatomical differences are found. The problem is that the broadly based collections necessary for the study of variation are often not available. There are advantages of tentatively treating minor variants informally as a 'form' or 'race' until this data is accumulated.

How many species are there in the P. vitis complex in Britain? Differences in morphology, life cycle (studied in Chapter 7), host preference and reproductive method (studied in Chapter 8) between different populations of the P. vitis complex have been used as evidence for the existence of more than one species within the complex. This study has clearly shown that the morphological characters that are frequently used as diagnostic show considerable plasticity, varying significantly with the host-plant species. It is not surprising then, that taxa have been described as distinct species on different host-plant species. Small samples of scale insects collected from different host-plant species or different localities can be morphologically distinguished, however, when larger samples are examined the variation is found to be continuous for all characters.

Species are traditionally recognised by unique, genetically-determined morphological traits. Although much of the morphological variation of the P. vitis complex is genetically determined, no reliable consistent morphological differences have been found between populations of the P. vitis complex. Consequently, there is no evidence for more than one taxa in Britain based on morphological data alone.

In conclusion, there is a single morphological species in Britain. Its morphological variation is determined both by genetic variability within and between populations and by environmental conditions. P. vitis is a highly

plastic species, morphologically and probably also genetically (see Chapter 9). It is broadly polyphagous, has a wide geographical distribution, is highly adaptable and appears to be able to readily colonise new geographical areas after introduction by man; for example, North America, New Zealand and Brazil.

6 External morphology and function of wax-producing structures

6.1 Introduction

A characteristic of scale insects is the production of visible wax. The types and functions of the wax produced have been discussed by several authors (Cox & Pearce, 1983; Morales, 1990). Scale insects produce a wide variety of integumentary secretions and the majority of species within each of the families produce a type of wax which is characteristic for the family. Eriococcidae produce a felted sac; Pseudococcidae, a fine powdery wax; and the Asterolecanidae, fine wax fringes. Most Diaspididae produce a hard covering shield composed of wax coated proteinaceous filaments and larval exuviae cemented together with anal secretions (Foldi, 1990). Kerridae (formerly Lacciferidae) produce a variety of waxes, pigments and resins (Varshney, 1976).

The types of wax produced can also vary considerably between species within a single family. The wax produced by the Coccidae varies from thin waxy coverings of the genus Coccus L.; sculptured, glassy cases of the genus Inglisia Maskell; and voluminous, watery-wax covers of the genus Ceroplastes Gray; to fine, delicate, star-shaped, semi-transparent wax produced by the genus Vinsonia Signoret. In addition, almost all scale insects produce wax in association with parturition. Most species of the soft scale genus Pulvinaria produce extensive woolly ovisacs (see Section 2.3) into which the eggs are laid. The chemical composition of the waxes produced varies greatly between families and genera, and the variety of components in the secretions depends on the host-plant species (Brown, 1975).

Species of Coccidae produce a structureless liquid wax from subepidermal glands that forms a relatively featureless film over the dorsum (Foldi, 1978; Tamaki et al. 1969); however, the majority of the wax is produced via distinct sclerotised pores or ducts (Cox & Pearce, 1983). The lumen of each pore or loculus acts as a template through which the wax is exuded to produce distinctive shapes and patterns on different parts of the body. The shape and surface texture of the wax produced is determined by the shape of the duct lumina and the underlying dermal structures (Foldi & Cassier, 1985). The internal and external morphology, number and location on the body of the wax-producing structures are of great taxonomic importance in scale insects (see Sections 2.2 and 3.2.5). The ultrastructure of the wax-producing

glands of scale insects has been reviewed by Foldi & Cassier (1985).

The functions of the wax produced by scale insects are summarised below: prevention of desiccation (Gullan, 1979; Pollister, 1937; Tamaki et al. 1969); attachment to the substrate (Cox & Pearce, 1983); elimination of honeydew to prevent self contamination (Cox & Pearce, 1983; Foldi & Pearce, 1985; Gullen, 1979; Pollister, 1937; Williams & Williams, 1980); prevention of eggs sticking together and assistance of first instar emergence (Hamon et al. 1975; Hashimoto & Ueda, 1985; Phillips, 1963); ventilation of spiracular furrows (Foldi, 1978, 1981); sensory (Cox & Pearce, 1983); protection against natural enemies, fungal infections and pollution (Cox & Pearce, 1983; Foldi, 1981; Foldi & Pearce, 1985), and an aid to balance during adult male flight (Cox & Pearce, 1983; Jakubski, 1965). The usefulness to man of the waxes and other secretions, such as resins and pigments, produced by scale insects has been reviewed by Miller & Kosztarab (1979); for example, the wax of Ericerus pela (Chavannes) (Coccidae) is used to make candles in China.

It has been demonstrated in Chapters 3 and 5 that the number of wax-producing pores, ducts and setal bases of Pulvinaria vitis vary according to host-plant species and locality. The external structure and function of some of these morphological characters was investigated below, using a scanning electron microscope in order to explain how these characters may vary in number due to the reasons above. Observations were also made on secretion of the ovisac.

6.2 Methods

6.2.1 Material examined

Teneral adult female P. vitis were collected from hawthorn (Crataegus monogyna) and flowering currant (Ribes sanguineum), Queens Club Gardens, London. A single adult male P. vitis from the same population was also examined. Third instar female P. regalis Canard were collected from lime (Tilia sp.), Cromwell Road, London and examined for comparison with P. vitis. Third instar female P. regalis are unusual in possessing multilocular pores, which are usually only present in the adult female stage in most Coccidae, as they are associated with reproduction.

6.2.2 Preparation of material for scanning electron microscopy

The first method used was to prepare specimens for examination of the

external morphology of the wax-producing structures. Specimens were first dewaxed and dehydrated by soaking in xylene, followed by chloroform for 24 hours, then were rinsed with absolute alcohol and cleaned using ultrasound.

The second method used was to prepare specimens to investigate the wax produced by the various morphological structures. Specimens were freeze-dried and lightly brushed to remove excessive wax. All specimens were then critical-point dried and coated with gold before examination. The scanning electron microscopes used were a Cambridge 180 series and a Hitachi 800.

6.2.3 Ovisac formation

Mature, convex, adult female P. vitis were carefully removed from the host plant at the end of May. Each scale was placed upside down on the centre of a glass cavity slide. The slides were then placed on damp blotting paper in a plastic petri dish that was covered when observations were not being made. Ovisac formation and oviposition were observed under a stereoscopic microscope under low magnification (x 12.5-35.0).

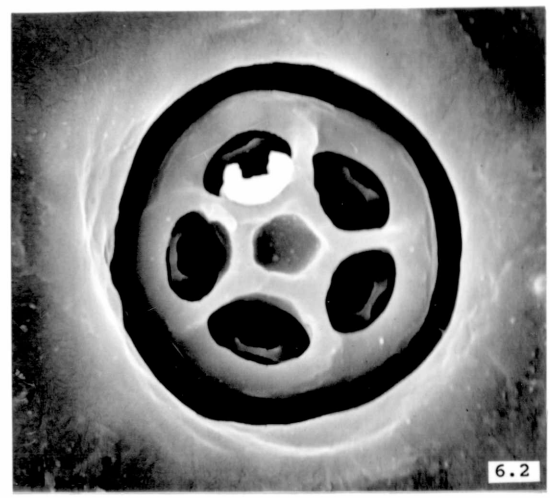
6.3 Results and discussion

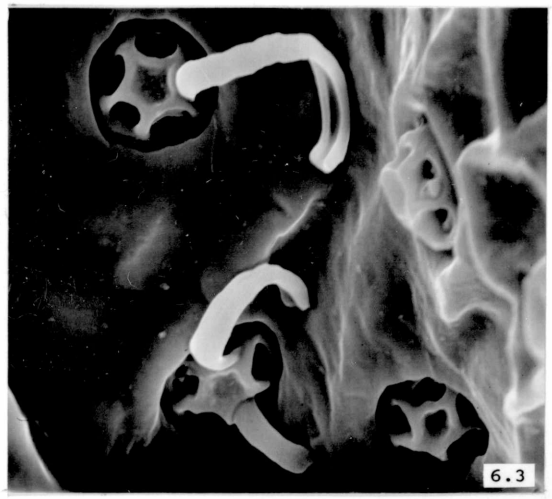
The morphology of the wax-producing structures, seen under a light microscope, and their location on the body of an adult teneral female are shown in Fig. 2.3 ; and are discussed in Sections 2.2 and 3.2.5. The detailed morphology of the wax-producing ducts, pores and setal bases were found to be very similar between P. vitis and P. regalis. The following results and discussion apply principally to P. vitis, but reference is made to P. regalis where it differs.

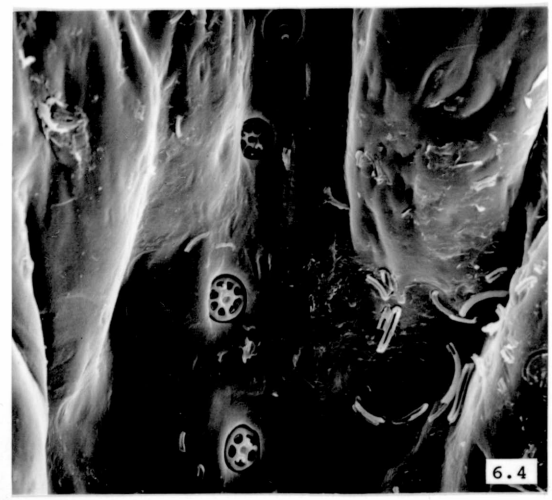
6.3.1 Pores

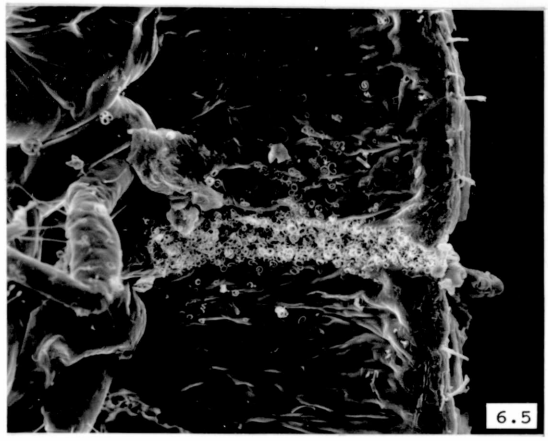
Spiracular pores (Figs. 6.1, 6.2, 6.3, 6.4 and 6.7)

Spiracular pores are located in four spiracular furrows, each furrow lying between a spiracle and the corresponding group of spiracular setae (Figs. 6.5, 6.22, 6.23 and 6.24). Some spiracular pores are also present around the spiracles. Spiracular pores are present in all instars except the adult male. The number of spiracular pores present vary between instars. In P. vitis there are 3-4 pores per furrow in the first instar, 9-12 in the female second instar, 18-26 in the female third instar and 25-168 in the adult female. In P. regalis, there are 90-165 pores per furrow in the adult female









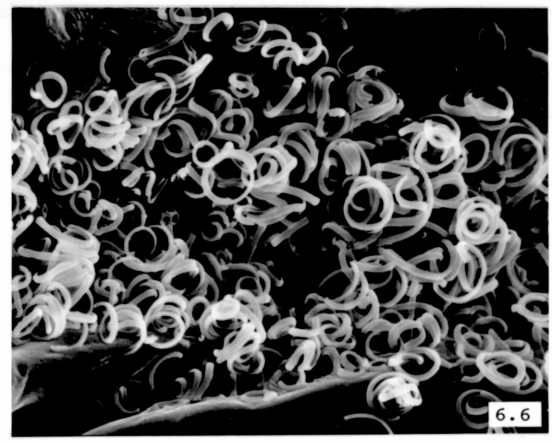


Fig. 6.1 Spiracular pore of Pulvinaria vitis with four loculi (x 17,000)

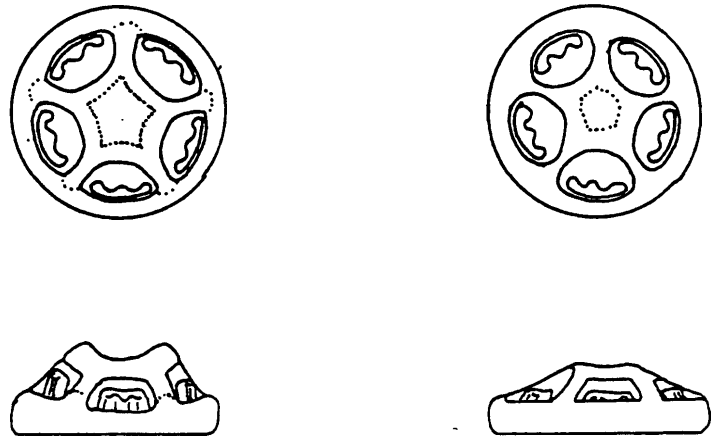
Fig. 6.2 Spiracular pore of Pulvinaria regalis. with five loculi (x 17,000)

Fig. 6.3 Spiracular pores of Pulvinaria vitis exuding short curls of wax (x 7,000)

Fig. 6.4 Spiracular ores of Pulvinaria regalis with five, seven and eight loculi (x 2,200)

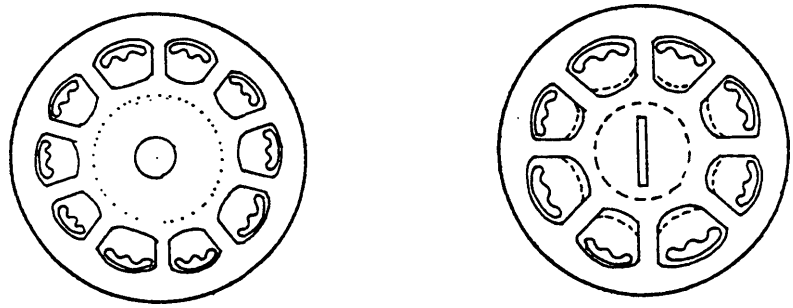
Fig. 6.5 Anterior spiracular furrow of teneral adult female Pulvinaria vitis (x 450)

Fig. 6.6 Short curls of wax in the spiracular furrow of Pulvinaria vitis) (x 1,950)



Pulvinaria vitis Pulvinaria regalis

Fig. 6.7 Diagram of a spiracular pore of Pulvinaria vitis and P. regalis (top and side view)



Pulvinaria vitis Pulvinaria regalis

Fig. 6.8 Diagram of a multilocular pore of Pulvinaria vitis and P. regalis (top view)

(Canard, 1968)

Spiracular pores each consist of a circular disc, diameter 3.1-3.5um, usually located in a circular depression, with 4-8 elipsoid peripheral loculi (each 1.0 x 0.64um), 5 loculi being the most common number present (Figs. 6.2, 6.3 and 6.4). The spiracular pores of P. vitis had a slightly higher profile than those of P. regalis, which is shown diagrammatically in Fig. 6.7.

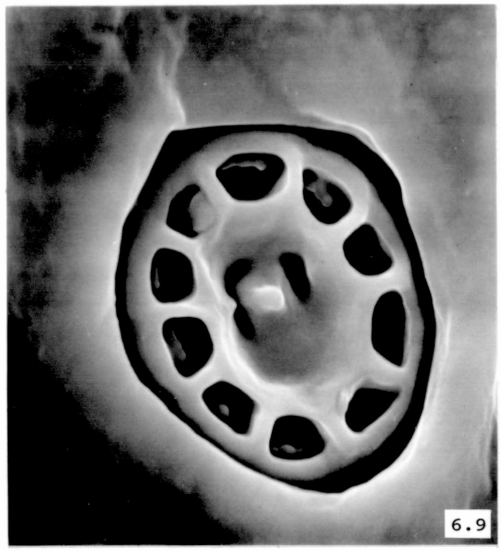
Each loculus produces a curl of hydrophobic wax 0.73-0.82um wide, whose length varies considerably. An internal, bifurcated template at the mouth of each loculus gives the wax a C-shaped cross section causing it to curl into a spiral as it is produced (Figs. 6.2 and 6.3). The wax fills the spiracular furrows to allow ventilation of the spiracles preventing them filling with water. The wax can also act as a barrier to fungal and bacteria pathogens, mites and air pollutants (Figs. 6.5 and 6.6).

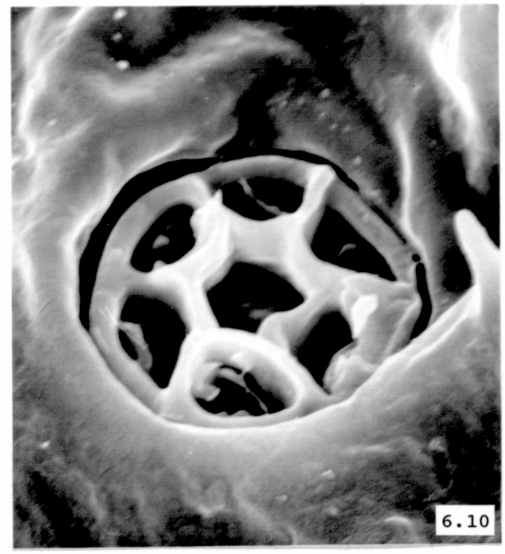
Multilocular pores (Figs. 6.8, 6.9, 6.10, 6.11, 6.12 and 6.18)

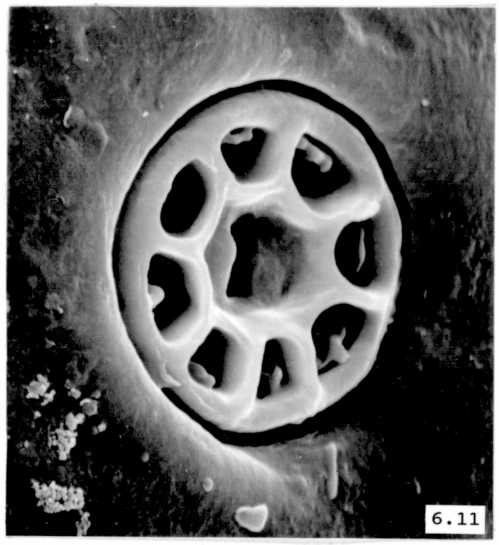
Multilocular pores are usually found in adult females only; however, they are often present also in female third instars of P. regalis. They are distributed in bands across the abdominal segments and are particularly dense around the genital opening (Fig. 6.18).

The external structure of multilocular pores is similar to spiracular pores. In P. vitis, each multilocular pore has a diameter of 4.5-4.9um and contains 7-13 peripheral quadrilateral loculi (each 1.0 x 0.77um), 10 loculi being the most common number present (Fig. 6.9). The central depression of each multilocular pore usually contains a small protuberance. In P. regalis, each multilocular pore has a diameter of 4.4-4.8um and contains 6-10 peripheral quadrilateral loculi (each 1.1 x 0.69um), 8 being the most common number present (Fig. 6.11). The multilocular pores of P. regalis differ from those of P. vitis by containing a slit which is usually rectangular, containing 2 non-wax-secreting loculi, in a central depression. The difference in morphology of the multilocular pores between P. vitis and P. regalis is shown diagrammatically in Fig. 6.8. Only two other species have been illustrated with multilocular pores with a central slit similar to P. regalis; these species are P. hydrangeae Steinweden and P. dodonaeae Maskell.

Each multilocular pore loculus contains an internal bifurcated template, similar to the spiracular pores. Multilocular pores produce short curls of wax which coat the eggs, preventing them from sticking together. The wax







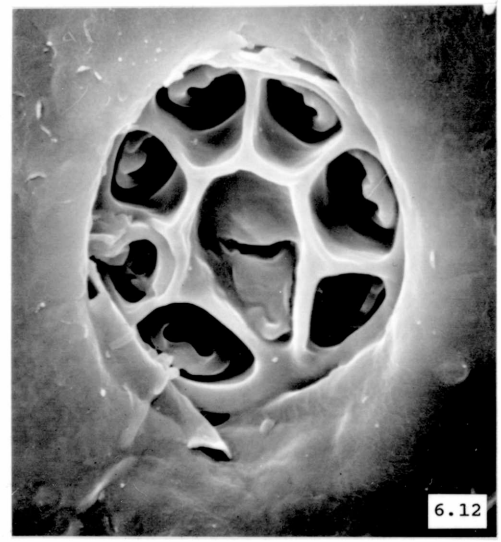


Fig. 6.9 Multilocular pores of Pulvinaria vitis with ten loculi (x 1,300)

Fig. 6.10 Multilocular pore with six loculi (x 17,000)

Fig. 6.11 Multilocular pore with eight loculi (x 13,000)

Fig. 6.12 Multilocular pore with seven loclui (x 15,000)

particles are required for successful egg hatch (Hamon et al., 1975; Hashimoto & Ueda, 1985; Phillips, 1963).

The diameter of individual multilocular pores, the number of loculi present and the size of each loculus varies. The number of loculi present increases with the diameter of the pore, and the size of each loculus decreases very slightly with increasing loculi number. Multilocular pores appear to be large spiracular pores with more loculi. The terminology is, therefore, misleading as multilocular and spiracular pores are morphologically very similar. It would be clearer to use the terms spiracular disc pores and vulvular disc pores.

6.3.2 Ducts

Microducts (Figs. 6.13 and 6.14)

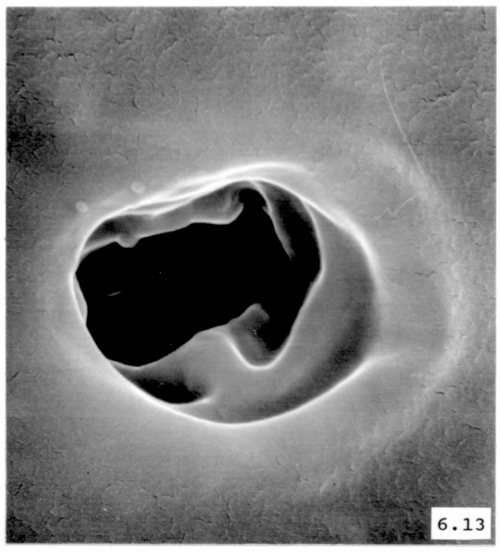
Microducts are found in all instars except the adult male. They are distributed mainly in a narrow ventral marginal band. Each consists of an oval pore 0.68 x 0.45um, surrounded by a pair of sclerotised lips, 1.08 x 0.6um, situated at the base of a relatively deep convoluted chamber, diameter 1.6-1.8um (Figs. 6.13 and 6.14). The type of wax produced and its function is unclear but it is possibly associated with adhesion of the scale to the substrate.

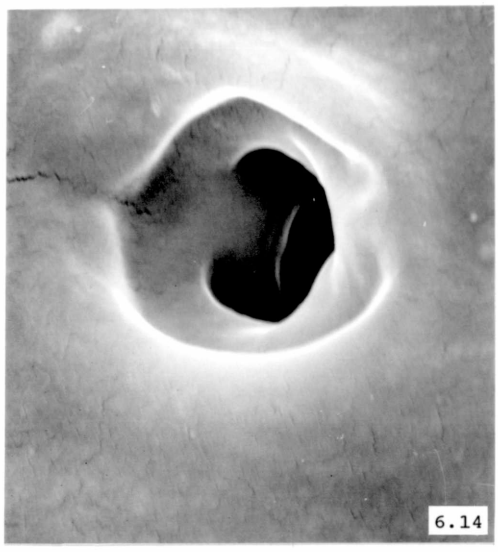
Filamentous ducts (Fig. 6.15)

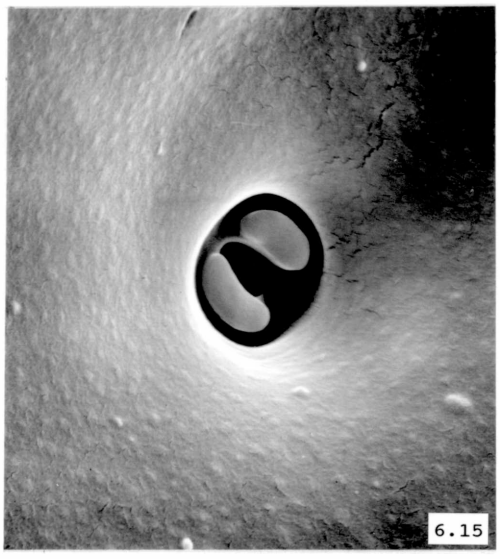
Filamentous ducts are minute ducts evenly distributed over the dorsum of all instars except the adult male. Each consists of an oval pore, 0.55 x 0.45um, surrounded by a pair of sclerotised lips, 0.8 x 0.6um, at the base of a shallow circular depression, diameter 1.35um. Their external morphology appears to be very similar to the ventral microducts except they are smaller. The type of wax they produce and their function is uncertain, but possibly they contribute to the layered flakes of wax covering the dorsum (Figs. 6.25 and 6.26).

Tubular ducts (Figs. 6.16 and 6.17)

Tubular ducts are found in the adult female and second instar males only. There are 2 main types; one with a slender filament and one with a thick filament. Each tubular duct appears to have a simple circular loculus, diameter 0.33um, which is often raised slightly above the ventral surface







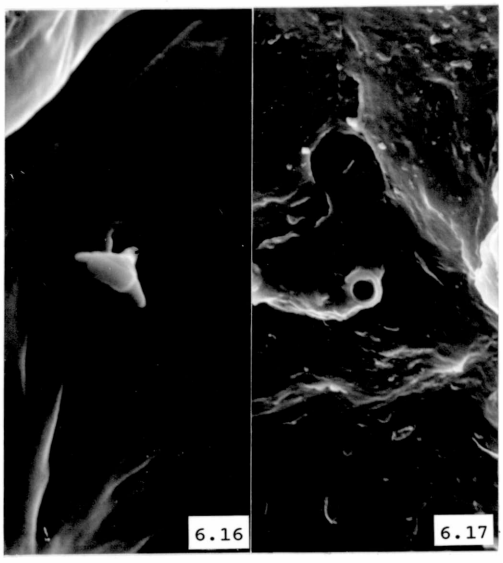


Fig. 6.13 Ventral microduct of teneral adult female Pulvinaria vitis (x 25,000)

Fig. 6.14 Ventral microduct of third instar female Pulvinaria regalis (x 25,000)

Fig. 6.15 Dorsal filamentous duct of teneral adult female Pulvinaria regalis (x 20,000)

Fig. 6.16 Tubular duct of teneral adult female Pulvinaria vitis (x 17,000)

Fig. 6.17 Tubular duct of teneral adult female Pulvinaria vitis (x 19,000)

(Fig. 6.17). The tubular ducts of P. regalis have a hexagonal loculus with a slightly raised rim. Slide-mounted, teneral adult female P. vitis, seen under the light microscope, have large numbers of tubular ducts, densely packed in a submarginal row. The number of tubular duct openings observed on a newly-moulted, dewaxed, adult female P. vitis under the scanning electron microscope was considerably less than expected from examination of slide-mounted specimens. This may have been an artifact of the preparation, or it may show that the majority of tubular ducts are blocked in some way when the adult is newly moulted. The tubular ducts are not required by adult females of P. vitis for or at least another 6 months, when oviposition occurs.

The tubular ducts with slender filaments are distributed in a wide submarginal band from the antennal bases to the anal cleft in the adult female. They produce fine, white, tightly curled and slightly sticky filaments which form the felt-like ovisac wall and fix the ovisac firmly to the substrate.

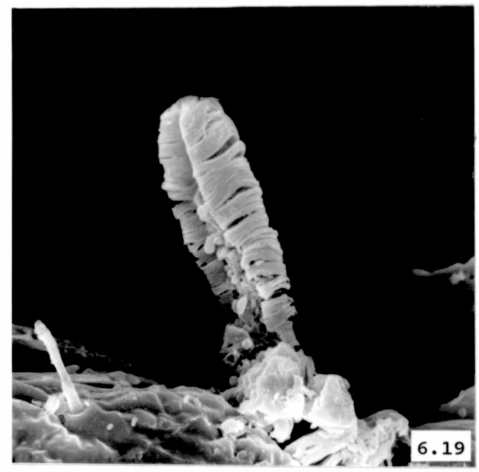
The tubular ducts with thick inner filaments are distributed with the slender tubular ducts in a submarginal band, and more sparsely over the remainder of the venter, except in an area anterior to the antennal bases in the adult female. They are also found around the entire margin of the second instar male. They produce long, straight or loosely coiled, non-sticky filaments which occur within the felt-like ovisac wall and the egg mass, holding it together.

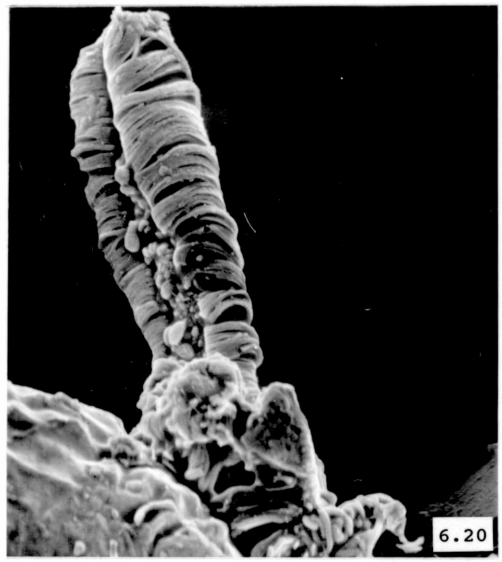
Phillips (1963) reported that the wax filaments produced by the tubular ducts of P. vitis with slender filaments were soluable in ethyl ether whereas those produced by tubular ducts with thick filaments were insoluble in ethyl ether but soluable in potassium hydroxide.

In P. regalis, the tubular ducts produce hollow hexagonal wax filaments that are thickened at each corner, providing strength (Foldi & Pearce, 1985). Tubular ducts in species of Pseudococcidae were found to produce hollow wax filaments with 8 longitudinal ridges on the external surface (Cox & Pearce, 1983).

6.3.3 Setal bases

The majority of setal bases on adult female scales (Figs. 6.18) and adult males (Figs. 6.29 and 6.30) are not involved in wax production; however, the





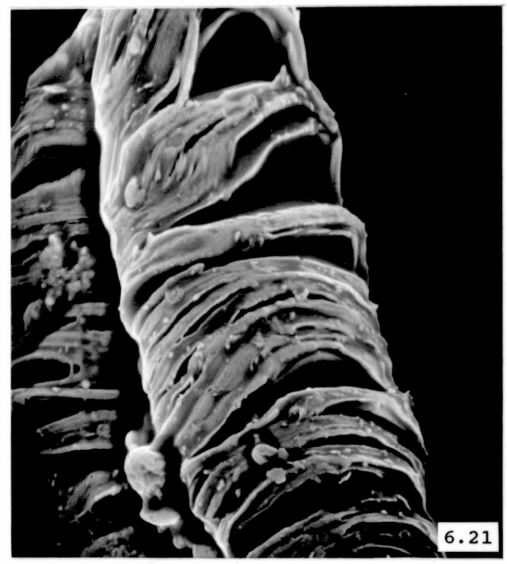


Fig. 6.18 Multilocular pores, setae and microspines of teneral adult female Pulvinaria vitis (x 3,400)

Fig. 6.19 Spiracular setae and single marginal seta of Pulvinaria vitis (x 1,000)

Fig. 6.20 Spiracular setae of Pulvinaria vitis (x 1,700)

Fig. 6.21 C-shaped curls of wax around the central spiracular seta of Pulvinaria vitis (x 5,000)

bases of the marginal, spiracular and anal-ring setae produce large quantities of wax.

Marginal setal bases (Figs. 6.19 and 6.22)

Marginal setae are found in all instars except the pupal and adult male and are located in one or more rows around the margin. Each seta is completely coated with a thick layer of wax secreted from the base (Fig. 6.19). This may help to secure the insect to the substrate, extend the sensory capability of the seta and/or deter natural enemies.

Spiracular setal bases (Figs. 6.5, 6.19, 6.20, 6.21 and 6.22)

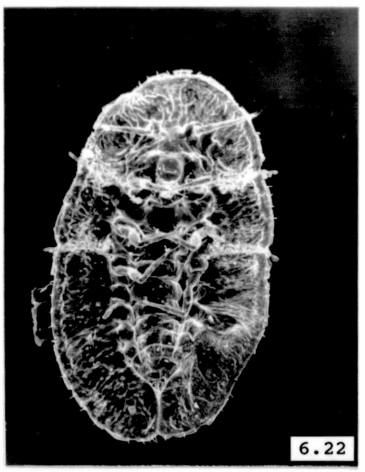
Spiracular setae are found in all instars except the pupal and adult male. They occur in groups of three on the margin, each group situated at the end of a spiracular furrow.

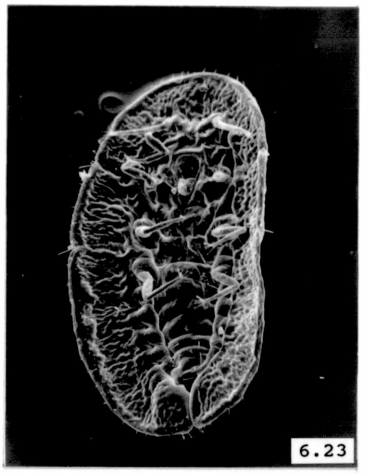
Rows of wax strands are produced on either side of the flattened seta which curl downwards and inwards (Figs. 6.19, 6.20 and 6.21). Glandular cells at the base of the seta secrete wax into a reservoir which communicates directly with the lumen of the seta (Foldi & Pearce, 1985). The wax in the lumen is secreted in regular bursts via micropores situated in 2 lines on opposite sides of the seta. The exact function of the wax produced is unclear but possibly it is associated with adhesion to the substrate, extending the sensory capabilities of the seta, preventing water from filling the spiracular furrow and to deter natural enemies.

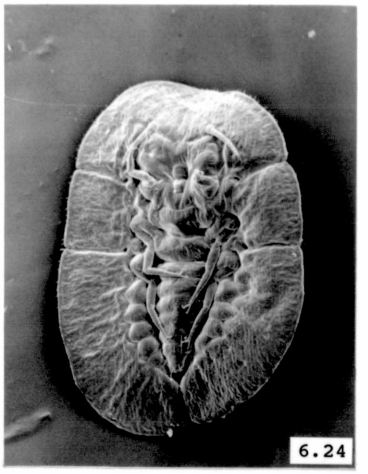
Anal ring and associated setae (Figs. 6.27 and 6.28)

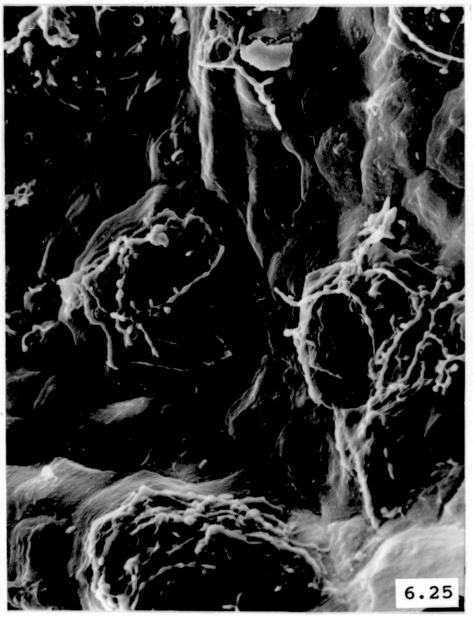
The anal ring and associated setae are found in all instars except the pupal and adult male. The anal ring is borne at the inner end of the anal tube, which is retracted into the abdomen. The eversible membranous anal tube is corrugated with parallel folds (Fig. 6.27). There are five pairs of setae attached to the anal ring in adult females of P. vitis; 3 large stout pairs, 1 slightly smaller pair and 1 very fine, small pair which is difficult to see. Adult female P. regalis have 4 pairs of anal-ring setae. A tube of densely matted, fine wax filaments is produced by thimble-like wax glands on the anal ring. Each of the anal-ring setae is sleeved with wax, which protude from the anal ring (Fig. 6.27).

The anal ring is extended out past the anal opercula to flick honeydew









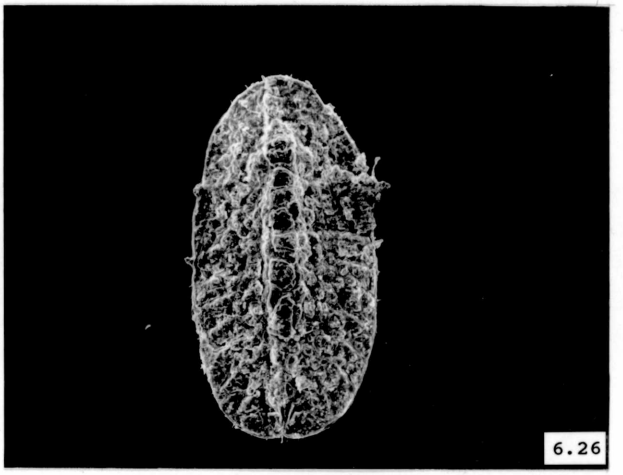


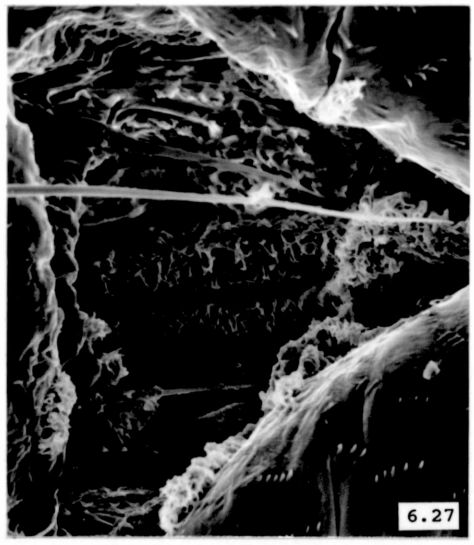
Fig. 6.22 Ventral surface of teneral adult female Pulvinaria vitis (x 56)

Fig. 6.23 Ventral surface of dewaxed teneral adult female Pulvinaria vitis (x 58)

Fig. 6.24 Ventral surface of dewaxed female third instar Pulvinaria regalis (x 52)

Fig. 6.25 Wax secretions on the dorsal surface of Pulvinaria vitis (x 250)

Fig. 6.26 Dorsal surface of adult female Pulvinaria vitis (x 56)



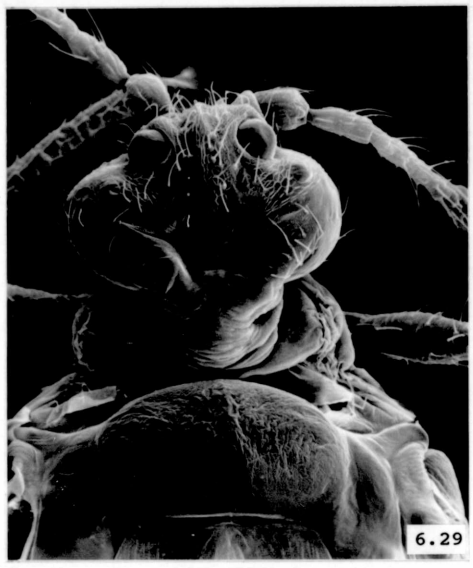


Fig. 6.27 Ventral view of the wax produced by the anal ring of teneral adult female Pulvinaria vitis; two pairs of fringe setae and a prevulvular seta are visable (x 1,500)

Fig. 6.28 Anal opercula of third instar female Pulvinaria regalis, partially opened (x 600)

Fig. 6.29 Dorsal view of the head of adult male Pulvinaria vitis (x 300)

Fig. 6.30 Lateral view of the head of adult male Pulvinaria vitis (x 300)

and waste products away from the body to avoid self contamination. The droplets of honeydew are first coated with particles from the anal ring. Newstead (1903) recorded droplets of honeydew being flicked a distance of 21 mm by "P. ribesiae".

6.3.4 Ovisac formation

Ovisac formation in adult females of P. vitis was found to start about two days before the first few eggs were laid and continue over a period of about 2 weeks in the laboratory. These processes probably take longer in the field, where environmental conditions are more variable.

The ovisac is formed from three types of wax, produced by three different morphological structures. The first part of the ovisac to appear is a sticky, felt-like outer wall, produced by the tubular ducts with slender filaments. The bulk of the ovisac is made from relatively non-sticky, loosely coiled or straight wax filaments produced by tubular ducts with thick inner filaments. Finally, numerous short curls of wax are produced by the multilocular pores, which coat the eggs. The first eggs are laid at the beginning of ovisac formation.

The function of the ovisac is to protect the eggs from the environment including rain, desiccation, honeydew contamination and natural enemies, and provide attachment to the substrate.

7 Biological observations on Pulvinaria vitis in Britain

7.1 Biology

7.1.1 Life cycle

Female Coccoidea are hemimetabolous with 3 or 4 instars; males if present, are holometabolous with 5 instars. The third and fourth male instar are termed the prepupa and pupa respectively. Female Coccoidea may be oviparous, ovoviparous or viviparous.

Newstead (1903) was the first to publish details of the life cycle of "Pulvinaria vitis (L.)" and "P. ribesiae Signoret" in Britain (given in Section 1.3.5) and all subsequent publications on the life cycle of these nominal species in Britain have been based on his work. The present study has shown, however, that the life cycle of P. vitis in Britain is considerably more flexible than had been previously reported.

The life cycle for the majority of females of P. vitis in Britain, both in the field and in reared cultures (discussed in Chapter 5), during 1988 and 1989, was as follows (see Fig. 7.1):

Ovisac formation and oviposition on outdoor plants occurred in the second half of May and early June; egg hatch occurred during June and July. In greenhouse conditions, however, oviposition and egg hatch occurred a month earlier, during April and early May respectively. The first instars dispersed over the host plant on leaves, petioles, stems and trunk. The first moult occurred during June or July and the second moult during July or August. The final moult occurred during September or early November. The resulting teneral adults migrated from the leaves and petioles to the stems or main trunk. Some teneral females migrated relatively long distances to find a secure situation to overwinter. The majority of specimens overwintered as adult females but some third instars were observed in January.

The timing of the life cycle of the males is similar to that of the females, except they have an extra moult during August. The glassy tests produced by second instar males were found on smaller twigs and branches from the end of July to the end of November. Immature males usually only moved a short distance away from the parent female, resulting in clusters of males near the post-reproductive female. The timing of male emergence varied considerably, even within the same population. It normally occurred from the end of September to early December.

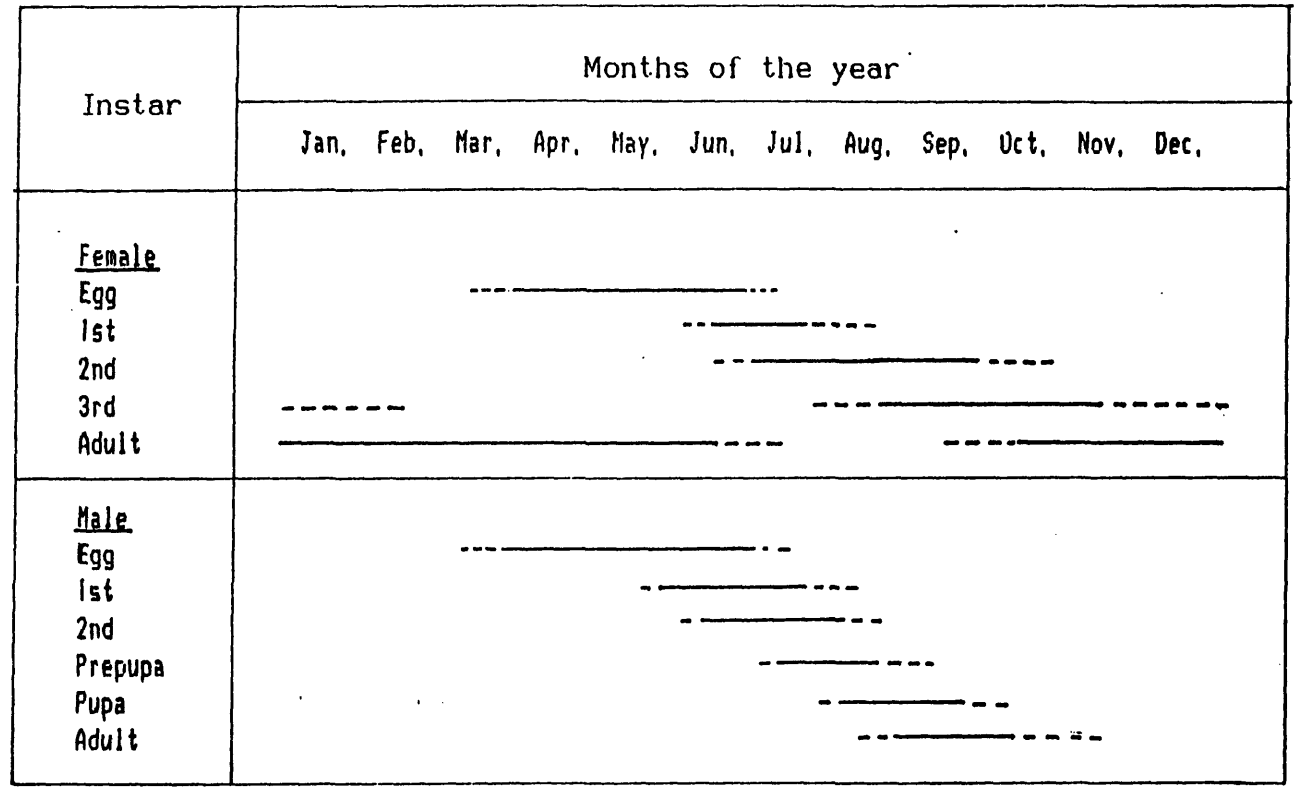


Fig. 7.1 Life cycle of Pulvinaria vitis in Britain

One experimental population of P. vitis on blackcurrant consisted of large numbers of both females and males; all the adult males emerged and dispersed during early October, while the majority of the females were still third instars. This poor synchronization would insure that, if the males were sexually functional, outbreeding would occur. The sexual function of the adult males, however, is uncertain (see Section 7.1.3).

The rate of development of individuals of P. vitis in the cultures varied with host-plant species. Development took about a month longer for scale insects on blackcurrant compared to individuals on birch and grapevine. The number of transfer replicates, however, was too small to determine if this was a host-induced effect. The variation in rates of development meant there was considerable overlap of different instars. Some of the cultures, which had originated in an unheated greenhouse but were subsequently reared outdoors, became third instars by the end of June, while some field populations were only just hatching from their eggs. All life-cycle stages were, therefore, present at the same time.

Although P. vitis is univoltine in the field in Britain, a second generation was produced by adult females on birch and blackcurrant plants that were kept in an insectary (16 hours light, 8 hours dark, 18-20°C) from the end of September 1988. They produced ovisacs and oviposited from the end of December to early January. All the adult female scale insects which produced the second generation were unfortunately parasitized, therefore, fecundity was low. The small numbers of crawlers which did emerge during January did not survive on the plants in the insectary. This may have been because the host plants were still dormant and may not have been in a suitable condition. Humidity in the insectary was low which may have also reduced the number of crawlers successfully establishing themselves on the host (see Section 7.1.2 below).

In conclusion, the life cycle appears to vary considerably, depending on environmental conditions such as climate and host-plant species. This could explain the differences in life cycle reported for the P. vitis complex in different geographical areas (see Section 1.3.5).

7.1.2 Egg hatch and first instar establishment

The timing of hatching, length of the hatching period and the total effective hatch from each ovisac in the cultures, varied considerably, even

within the same population. The first instars usually emerged early in the morning on bright, sunny days from the end of May to early July. The majority of crawlers emerged from the ovisac on the same day, although these were preceded by a small number on the previous day. The total effective hatch appeared to vary with the size of the adult scale insect and the size of the ovisac. It was drastically reduced by parasitism, although most parasitized female adult scale insects still produced viable offspring. The adult parasitoids emerged from the adult scale insect about a month after egg hatch, enabling them to parasitize second or third instars of the following scale insect generation.

The first instars initially congregated around the ovisac and on top of the adult scale insect. They appeared to rest until the temperature had risen before dispersing together. They were most active from midmorning until midafternoon. They were positively phototactic and moved rapidly up the plant, crowding the top-most leaves nearest the sun. When the plant was turned away from the sun they immediately moved towards the sun-lit side of the plant again. They were also photokinetic and stopped moving when placed in the dark. The crawlers clustered at the tips and outer margins of leaves in a moving mass. Only small numbers appeared to be removed by air currents, as the weather was generally calm when observations were made. In one instance, a large number of crawlers were observed to be removed when an exceptional large aggregation accumulated on the tip of a willow leaf and dislodged one another.

The crawlers initially settled to feed on the upper parts on the plant and subsequently moved downward so that they became more evenly distributed. The majority had settled by the second day; some even settled by the end of the first day. Crawlers which had not settled to feed by the end of the third day usually died. There was very high mortality amongst the crawlers before settling to feed; most appeared to die of desiccation.

The transfer experiments were considerably more successful during the summer of 1988 than during 1989 (see Section 5.3.4). One of the reasons for the very small numbers of first instars establishing on the plants during 1989 may have been the very dry weather during the period when the eggs were hatching and when the crawlers were settling to feed. Phillips (1963) has shown that the first instars of "P. vitis" require a high relative humidity in order to establish themselves on a host plant.

7.1.3 Adult male behaviour and sexual function

Observations were made on adult males of P. vitis reared in cultures and from field collected samples, using the following methods:

Twigs infested with male tests were collected in September; the excised end of the twig was bandaged with a damp cloth as soon as it was cut to prevent rapid desiccation. The infested twigs were placed into plastic boxes which were inspected daily for adult males and occasionally opened to allow ventilation to avoid condensation and prevent mould growth. The excised ends of some infested twigs were passed through a hole bored into a cork, which sealed a glass vial containing water (see Fig. 7.2). The cork prevented the scale insects from crawling down into the water and drowning. Scale insects survived for up to two months on such twigs.

After emerging from the pupa, each adult male remained beneath the wax test for at least two days, whilst secreting two wax filaments from the apex of the abdomen. In cold conditions, the adult males remained under the test for up to 5 days. On a bright morning, each male raised the test, emerged backwards, and initially rested beside the test before climbing upwards. After reaching an apical twig or leaf, each male opened the wings and jumped. The males were very poor fliers and usually simply spiralled downwards. They were active for between a few hours to about two days in the laboratory depending largely on humidity and temperature. The length of period of activity of adult males decreased with decreasing humidity; they were particularly susceptible to desiccation. They were also more active on sunny days when the temperatures were higher.

Bisexual (both sexes) cultures on birch and willow were observed in the laboratory between about 8.30am to 5.00pm over several days during October, 1988, to determine if the adult males were sexually functional. Despite approximately 15 hours of observation, no attempt at insemination was observed.

7.1.4 Sex ratio

Most small, low-density populations of P. vitis in the field appear to be unisexual (all female), regardless of host-plant species. In bisexual populations there were still usually a strong bias towards females; however, one population on hawthorn, London, 1990, was observed to consist of about 8 living teneral females and about 60 male tests, all the males appeared to be

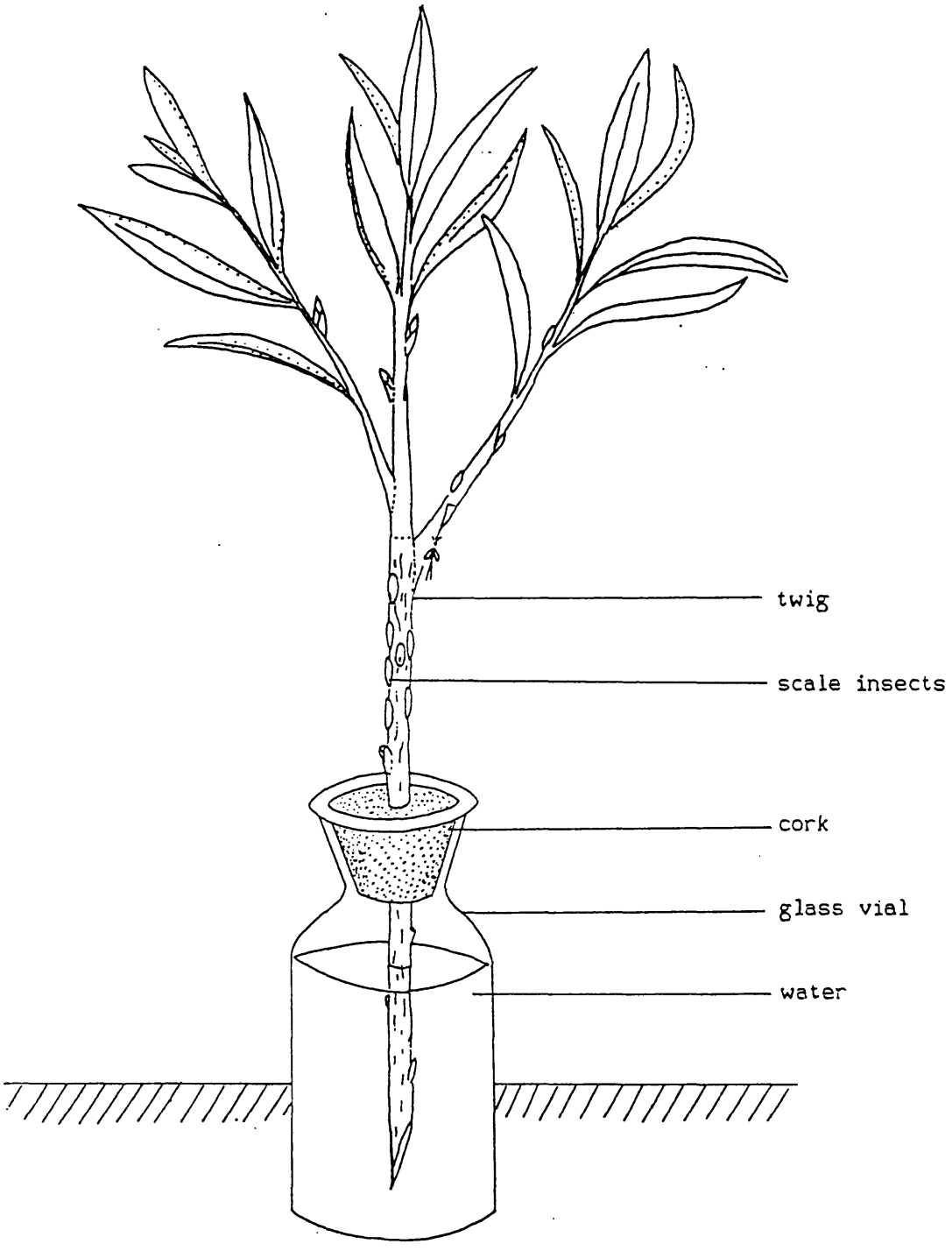


Fig. 7.2 Arrangement of host-plant twig, cork and glass vial used in the laboratory to make close observations of adult Pulvinaria vitis behaviour

the progeny of a single female. The sex ratio of populations of P. vitis in the field was observed to vary considerably between different years.

Males were usually only found in the field in high-density populations, occasionally in enormous numbers smothering parts of the host plant. The male tests were usually found on smaller twigs and branches. Males were present in a third of the cultures reared during this study. The sex ratio of the cultures varied with host-plant species but the number of replicates was too small to test if this was, inpart, induced by the host plant. Some males were produced in at least one culture originating from all the different source populations. Therefore, all the field populations from which the cultures were derived had the potential for producing males, although males were never observed in most of the field populations.

Bisexual populations may be more common than field observations suggest. Males may not be found because the mortality of immature males is higher than that of immature females. Immature males are smaller than females and may, therefore, be more susceptible to desiccation. Second and third instar females, however, are likely to have a higher mortality due to parasitism, than second and third instar males, as they are larger and are more likely to be chosen as hosts.

7.2 Natural enemies and associated insects

7.2.1 Natural enemies

The natural enemies of the P. vitis complex recorded worldwide are listed below in Table 7.1. There are two main types of natural enemy, the parasitoid and the predator. Parasitoids cause more mortality to the P. vitis complex than predators and contribute to maintaining populations of the complex at low levels in Britain. There are 28 species of primary parasitoid recorded for the P. vitis complex, belonging to four families of the order Hymenoptera: Aphelinidae (4 spp.), Chalcididae (1 sp.), Encyrtidae (20 spp.) and Pteromalidae (3 spp.). There are also 7 species of hyperparasitoid, belonging to 3 families of the Hymenoptera: Aphelinidae (3 spp.), Encyrtidae (3 spp.) and Pteromalidae (1 sp.).

The insect predators recorded for the P. vitis complex belong to 9 families in 6 orders of insect: Lepidoptera (Sesiidae, 1 sp.), Coleoptera (Coccinellidae 4 spp., Anthribidae 2 spp.), Heteroptera (Anthocoridae 1 sp., Miridae 1 sp.), Diptera (Chamaemyiidae 5 spp., Syrphidae 1 sp.), Neuroptera (Chrysopidae 1 sp.) and Thysanoptera (Phlaeothripidae 1 sp.). In

Table 7.1 Natural enemies of the Pulvinaria vitis complex

The list of natural enemies was obtained from field observations, rearing cultures of P. vitis in Britain and the following references: Newstead, 1903; Paramonova & Saakyan-Baranova, 1984; Peck, 1951, 1963; Kosztarab & Kozár, 1988; Kozár & Szugonjaev, 1979; Schmutterer, 1952; Trajapitzin, 1987.

Synonyms are included only when they have been recorded as a natural enemy of the P. vitis complex. The distribution of natural enemies has been greatly influenced by man, often in attempts at biological control of scale insects. Key to distribution P = Palaearctic N = Nearctic

Primary parasitoids	Distribution
Hymenoptera	
Aphelinidae	
♀♀ Coccophagus obscurus Westwood [= C. insidiator Dalman]	P
♀♀ Coccophagus lycimnia (Walker) [= C. lecanii Fitch]	P N
♀♀ Coccophagus semicircularis (Förster) NEW RECORD FOR BRITAIN	P
♀♀ Coccophagus scutellaris Dalman	P
Chalcididae	
Blastothrix sericea (Dalman)	P N
Encyrtidae	
Aphycus apicalis (Dalman)	P
Aphycus maculipes Howard	N
Aphycus pulvinariae Howard	N
Atropates collinsi Howard	N
Discodes aeneus Dalman [= Phaenodiscus aeneus (Dalman)]	P
Encyrtus albitarsis (Zetterstedt)	P
Encrytus fuscus (Howard)	N
Encyrtus merceti Masi	P
Encyrtus obscurus Dalman [= Eucomys obscura (Dalman)]	P
Encyrtus swederi Dalman [= Eucomys swederi (Dalman)]	P
Eumesion cornigerum (Walker)	P
Metaphycus melanostomatus Timberlake [= M. punctipes (Dalman)]	P
Metaphycus melanus Sugonjaev NEW RECORD FOR BRITAIN	P
Microterys cneus Trjapitzin & Sugonjaev	P
Microterys duplicatus (Nees) [= Encrytus duplicatus Nees]	P
Microterys ferrugineus (Nees) [= Encyrtus ferrugineus Nees]	P
Microterys flavus Howard	N
Microterys interpunctipus	N
Microterys lunatus (Dalman) [= Encrytus lunatus Dalman]	P
Trichomasthus albimanus Thomson	P
Pteromalidae	
Eunotus lividis Ashmead	N
Eunotus obscurus Girault	P
Pachyneuron concolor Förster	P
Hyperparasitoids	
Hymenoptera	
Aphelinidae	
♂♂ Coccophagus obscurus Westwood [= C. insidiator Dalman]	P
♂♂ Coccophagus lycimnia (Walker) [= C. lecanii Fitch]	P N
♂♂ Coccophagus scutellaris Dalman	P

Table 7.1 continued

Encyrtidae	
Cheiloneurus albicornis Howard	N
Cheiloneurus elegans Dalman	P
Cheiloneurus paralia (Walker) [= *C. formosus* (Boheman)]	P
Pteromalidae	
Pachyneuron concolor Förster	P

The following hymenopteran species do not usually attack Coccidae but have been recorded as parasitoids of the complex: Encyrtidae; *Ariagyrus schoenherri* (Westwood), *Ericydrus ventralis* (Dalman), *Pseudaphycus malinus* Gah. and *Acerophagus coccois* Smith.

Predators

Lepidoptera		
Sesiidae		
Coleoptera		
Coccinellidae		
?	*Adalia bipunctata* Linnaeus	P
	Exochomus sp.	N
	Hyperaspis binotata Say	N
	Hyperaspis proba proba (Say)	N
Anthribidae		
	Anthribus fasciatus Förster [= *Brachytarsus fasciatus* (Förster)]	N
	Anthribus nebulosus Förster [= *Brachytarsus nebulosus* (Förster)]	N
Diptera		
Chamaemyiidae		
	Leucopis annulipes Zetterstedt	P
	Leucopis membe Linnaeus	P
	Leucopis nigricornis Egger	P
	Leucopis pulvinariae Howard [= *Leucopomyia pulvinariae* (Howard)]	N
	Leucopis silesiaca Egger [= *Leucopomyia silesiaca* (Egger)]	P
Syrphidae		
	Syrphus ribesii Linnaeus	P
Heteroptera		
Anthocoreidae		
	Anthocoris sp.	P
Miridae		
	Deraeocoris ruber Linnaeus	P
Neuroptera		
Chrysopidae		
	Chrysopa carnea Stephens	P
Thysanoptera		
Phlaeothripidae		
	Haplothrips subtilissimus (Hal.)	P

Unidentified species of Arachnidae and four species of bird: *Parus major* (great tit), *P. caeruleus* (blue tit), *P. palustris* (marsh tit) and *P. ater* (coal tit) have also been recorded as predators.

addition, titmice (Paridae) and Arachnida have been recorded feeding on the P. vitis complex.

7.2.2 Importance of natural enemies for control of Pulvinaria vitis

Populations of P. vitis in natural situations in Britain are usually found in small, well-dispersed groups with relatively high levels of parasitism, mainly by species of Coccophagus and Metaphycus (see Section 7.2.3 below). In the far eastern U.S.S.R., populations of "P. betulae" in natural situations were reported to be heavily parasitized by Encyrtus swederi (Dalman) (Danzig, 1986). Accurate levels of parasitism of the P. vitis complex have only been recorded in crop situations. Paramonova and Saayan-Baranova (1984) reported that in Byelorussia, U.S.S.R., 50% of adult females and 56% of immatures of "P. ribesiae" on currants were parasitized, the commonest parasitoid being Coccophagus scutellaris Dalman, which accounted for 94% of adult parasitoids reared; C. lycimnia (Walker) accounted for a further 5% of the adult parasitoids reared.

The level of parasitism in the cultures in this study was highest at the end of the summer. Levels of parasitism of "P. vitis" in orchards in Canada was also highest at the end of the year and was reported to significantly reduce the number of scales that survive the winter (Phillips, 1963). Although parasitism levels can be high, the greatest mortality factor still appears to occur between egg hatch and first instar establishment.

Levels of parasitism reported for the Palaearctic region are generally higher than those reported in North America. For example, Phillips (1963) reported only 25% of overwintering adult females as being parasitisd by C. lycimnia (Walker) in Canada. It appears that the P. vitis complex is controlled more effectively in the Palaearctic region by parasitoids than in North America. This agrees with the assumption that the P. vitis complex was introduced to North America and therefore does not have such an established complex of natural enemies. C. lycimnia is very important in controlling other species of woolly scale in North America, which it appears to attack in preference to the P. vitis complex. In particular, the indigenous North American maple scale, Neopulvinaria innumerabilis (Rathvon), is often heavily parasitized by C. lycimnia (Forbes, 1907). The maple scale is larger and more common than the P. vitis complex and therefore may be a more suitable host for parasitoids.

The P. vitis complex is most serious as a pest in situations which promote unnaturally large populations of scale; for example, on crops grown in monocultures or in artificially sheltered environments such as greenhouses. Personal observations suggest that populations of the P. vitis complex have the potential for rapid increase, followed by an equally rapid crash to the original low population density. Parasitism appears to be a more important mortality factor when the scale insect population density is high. The levels of parasitism are higher in artificial situations than occur naturally; 100% parasitism occurred in cultures in a greenhouse in the present study. Parasitized individuals can still produce viable offspring but their fecundity is reduced by such an extent that the combination of parasitism and other mortality factors may render the population no longer viable.

Levels of mortality of "P. vitis" caused by predation were reported to be insignificant in North America (Phillips, 1963). Predation can, however, be locally important; for example, Newstead (1903) reported significant predation of overwintering female scales on currants in Britain by titmice (Paridae). Schmutterer (1952) reported that, in Germany, up to 95% of woolly scale eggs in one population were eaten by the larvae of Leucopis silesiaca Egger. Leucopis nigricornis Egger is important occasionally as an egg predator in the U.S.A.

Most natural enemies of the P. vitis complex are broadly polyphagous and most parasitoids attack scale insects belonging to several families. There are two species of Coccinellidae, however, which appear to be specific feeders on species of Pulvinaria and Neopulvinaria in North America (Phillips, 1963). The beetle larvae feed on the eggs and the adults feed on the immature scale insects. Both beetle species are rare and are thus insignificant to the control of the P. vitis complex. The larvae of Hyperaspis binotata Say were never recorded destroying more than 10% of "P. vitis" eggs (Phillips, 1963).

Thrips have not previously been reported as predatory on the P. vitis complex, however, Haplothrips subtilissimus (Halidy) was found in close association with P. vitis first instars on grapevine under glass and is probably predatory (Jenny Palmer, pers. comm.).

7.2.3 Parasitoids in Britain

The most common parasitoid of P. vitis in southern Britain was found in this study to be Coccophagus lycimnia (Walker). Adult C. lycimnia were present in Britain from June until early October. Two other species of parasitoid attacking P. vitis, recorded here for the first time in Britain, were locally common in the London area. Coccophagus semicircularis (Förster) was previously misidentified numerous times in the literature as C. scutellaris Dalman (Andy Polaszek, pers. comm.). The second new record is Metaphycus melanus Sugonjaev. Both C. lycimnia and M. melanus caused significant levels of parasitism in the cultures of P. vitis in the present study.

C. lycimnia is also reported to be a natural enemy of the P. vitis complex in mainland Europe (Borchsenius, 1957; Paramonova & Saakyan-Baranova, 1984; Kosztarab & Kozár, 1988) and North America (Phillips, 1963). It is an internal, nymphal parasitoid, reproducing both sexually and parthenogenetically and is bi- or trivoltine. Females develop as primary parasitoids but males develop as hyperparasitoids of coccids via their eulophid, aphelinid or encyrtid primary parasitoid-host species (Gauld & Bolton, 1988; Saakyan-Baranova, 1966; Saakyan-Baranova et al., 1971). C. lycimnia is cosmopolitan and broadly polyphagous on scale insect species belonging to the Coccidae and possibly the Cryptococcidae. Males are also reported emerging from Pseudococcidae (Clausen (ed.), 1978). Males are haploid resulting from unfertilised eggs. The eggs are stalked and sexually dimorphic (Gauld & Bolton, 1988). Pupation occurs within the host scale insect and each adult parasitoid emerges by cutting a circular hole in the integument of the host. The parasitoid overwinters as a larva.

Newstead (1903) reported Blastothrix sericea (Dalman) (Fig. 7.3) to be the most significant parasitoid of a population of the P. vitis complex on currants in Cheshire, England. B. sericea was not found in this present study. It is native to Europe where it primarily attacks Coccidae belonging to the Parthenolecanium corni (Bouché) complex and Eulecanium species (Clausen (ed.), 1978). It is bivoltine with development from egg to adult taking about 1 month. It is gregarious with up to 12 parasitoids emerging per host.

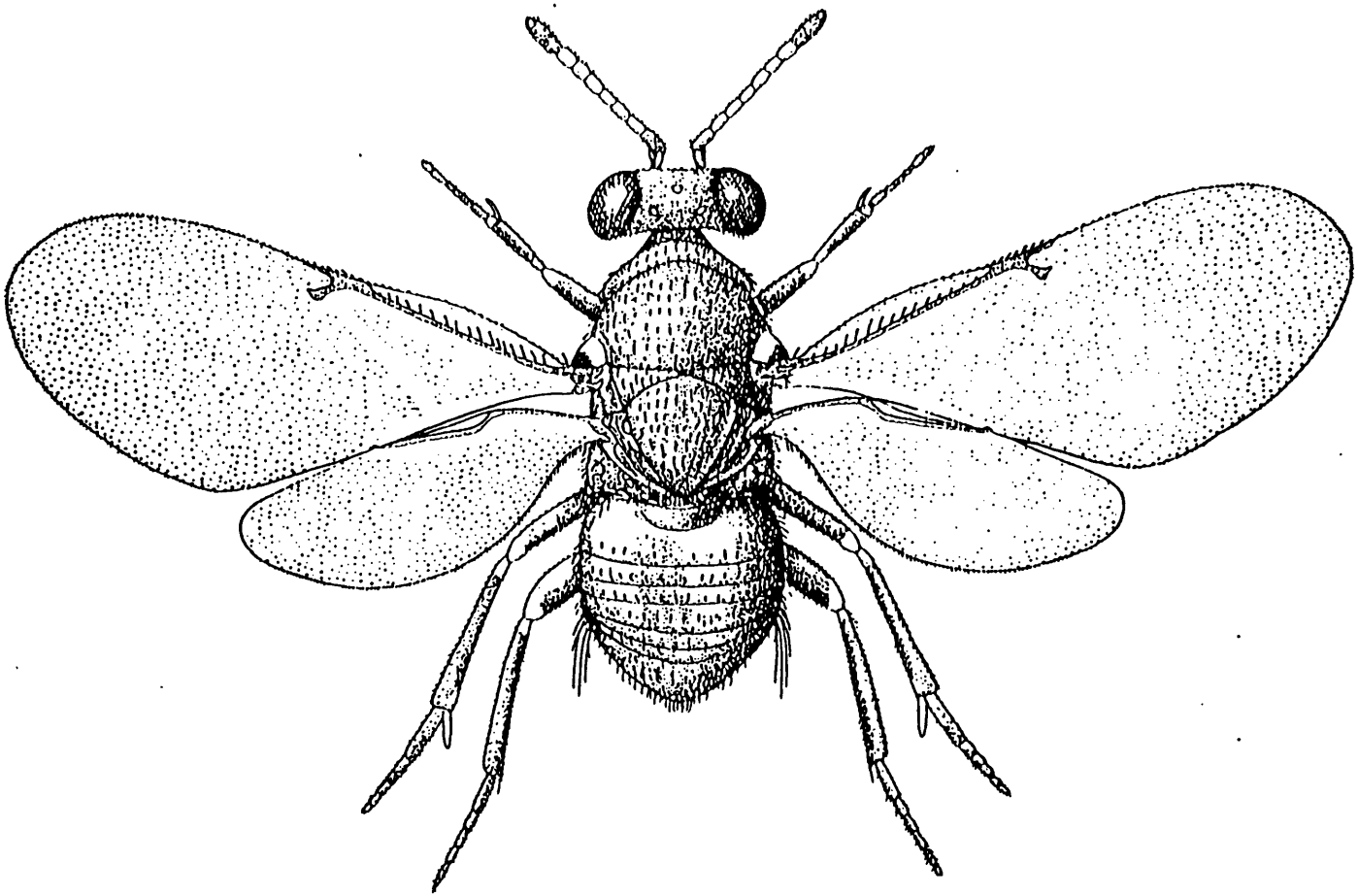


Fig. 7.3 Blastothrix sericea (Dalmon) (Encyrtidae)

Adult female parasitoid behaviour

Adult female Metaphycus melanus were observed climbing plants with their antennae continually waving and tapping the substrate. They moved around the edges of leaves and repeatedly back-tracked. Several potential host scale insects were examined before one was chosen. The parasitoid then climbed on to the dorsum of the scale and appeared to probe the dorsum with the ovipositor before it was inserted into the mid-thoracic or mid-abdomenal region. The parasitoid abdomen was moved slowly up and down during oviposition. After withdrawing the ovipositor, the wasp turned around and fed on the body fluids of the host scale at the site of the puncture, before departing in search of another host.

7.2.4 The potential for biological control of woolly vine scale in Britain

Control of scale insects with insecticides can be difficult due to their sessile nature, wax covering and intermittent feeding. Contact insecticides are best applied during the time of egg hatch to kill the active crawlers or systemic insecticides are required. Insecticides have the disadvantage that they can adversely affect the natural enemy population more than the pest scale insect population. For example, 25% of overwintering adult female "P. vitis" in unsprayed peach orchards were parasitized compared to only 4% in peach orchards sprayed with DDT (Phillips, 1963). Phillips et al. (1962) attributed a prolonged outbreak of "P. vitis" in peach orchards in Canada to the unwise use of insecticides which killed off all the natural enemies. Biological control is better than the repeated use of chemicals, as it is environmentally safer and cheaper in the long term.

The majority of parasitoid species attacking the P. vitis complex belong to the hymenopterous families Encyrtidae and Aphelinidae, which are the most important chalcidoid families for biological control of scale insects. Two parasitoid species attacking the P. vitis complex that have been used in biological control programs against Coccidae in North America are Coccophagus lycimnia and Blastothrix sericea. C. lycimnia has been used against several species of soft scale such as Saissetia oleae (Bernard) and B. sericea against Parthenolecanium corni and Eulecanium tiliae (L.) (Clausen (ed.), 1978).

There are many examples of successful biological control of economically important scale insect pests infesting long-lived woody plants by introducing

natural enemies. P. vitis, however, is indigenous to Britain and effective natural enemies are already present. Populations of P. vitis are normally maintained below economically important levels on outdoor crops largely by the weather and natural enemies. The occasional population explosion of scale insects on outdoor crops usually lasts only for a single season. Outbreaks of P. vitis are best controlled with selective insectides applied at the time of egg hatch, or by waiting for the natural enemies to reduce scale numbers to below the economic threshold. P. vitis can be a more serious pest in greenhouses, where, in favourable environmental conditions and in the absence of natural enemies, they can cause severe losses of fruit. In confined conditions such as a greenhouse, C. lycimnia and M. melanus can rapidly reduce large populations of P. vitis. The economic losses due to the scale insect complex in Britain, however, do not justify the expense of the research required to mass rear parasitoids for a biological control program. It is simpler and more cost effective to introduce the natural enemies from the field by collecting part of a plant infested with scale insect and placing it in the greenhouse. Almost all populations of P. vitis found outdoors have some level of parasitism. Secondly, the control of ants, which are beneficial to the scale insect colony (see Section 7.2.5, below), will also help reduce the numbers of scale insects.

7.2.5 Associated insects

Populations of P. vitis in Britain were often found in association with other insects, particularly with ants (Formicidae), social wasps (Vespidae) and the honey bee (Apidae) in the order Hymenoptera. These associations are usually beneficial to both the scale insect population and associated insect.

Ants have the closest association with populations of P. vitis, providing protection to the scale insects by warding off parasitoids and predators and in return they are supplied with sugar-rich honeydew. The removal of the sticky honeydew from the scale colony is of direct benefit to the scale insect population. Foldi (1983) demonstrated experimentally that dense colonies of Pulvinaria in the laboratory suffer from self contamination by honeydew unless ants are present to remove it. Many species of Diptera, especially hoverflies (Syrphidae), are also attracted to the sugary honeydew excreted by scale insects. Honeydew serves as a medium for the growth of sooty mould, which can contaminate the scale insect colony and cause a

reduction in the number of scale insects. Large populations of P. vitis hidden beneath layers of peeling bark on mature grapevines were personally observed in Hungary during the course of the present study. In such confined situations the scales could not flick the honeydew that they produced, away from themselves. The scales were attended by large numbers of ants, which were essential for the removal of the honeydew, thus preventing self-contamination of the colony. It is probable that without the presence of the ants the majority of the scales could not survive in such a confined place. Small numbers of ant-attended "P. vitis" were also found under peeling bark of mature hawthorn bushes.

Scale insect colonies that are ant attended have the problem of contamination by sooty mould eliminated. Ant-attended colonies of scale insect are often larger than colonies of the same scale on the same host which are not ant attended (Hamon & Williams, 1984). Honeydew can also have a more directly harmful effect on the colony. Large numbers of first instars have been personally observed to be physically trapped in honeydew excreted by immatures which had established themselves on the host plant earlier in the year.

Schutterer (1952) reported that, in Germany, populations of P. vitis were attended by Formica rufa pratensis Retzius, Lasius niger alienus Förster and Lasius niger niger Linnaeus. Ants were reported to build carton covers of chewed wood and debris over colonies of "P. betulae" in the far eastern U.S.S.R. (Danzig, 1986). The ant shelter provided some protection from the weather, but more importantly, shielded the colony from attack by natural enemies. Ants and other hymenopterous insects are often seen feeding directly on honeydew. Many species of ant and a species of Coccophagus (Aphelinidae) have also been observed to 'fondle' the scale insect in the area of the anal opercula with their antennae (Hamon & Williams, 1984; Cendana, 1937). The scale insect reacts by slowly extruding a droplet of honeydew from its rectum, which is consumed by the ant or parasitoid.

P. vitis is often found in association with other species of scale insect. An individual plant may be infested by 2 or more species of scale insect when other host plants in the vicinity are unaffected. These associations between the scale insect species appear to occur by chance; the scale insect species both share the same host-plant species, and a plant which is susceptible to infestation by one species is likely to be susceptible to

infestation by others. Scale insect species commonly found in association with P. vitis in Britain are: Coccidae, Eulecanium tiliae (L.) and Parthenolecanium corni (Bouché); Diaspididae, Chionaspis salicis (L.) and Lepidosaphes ulmi (L.).

8 Cytogenetics, sperm bundle morphology and endosymbionts

8.1 General introduction

Cytogenetic studies, sperm bundle morphology and endosymbionts are potentially useful in the separation of closely related taxa within species complexes, where separation based on more traditional morpholological structures is unreliable (Miller & Kosztarab, 1979). These related areas of study are discussed below for Pulvinaria vitis (L.) in Britain.

8.2 Karyotype analysis

8.2.1 Introduction

A large number of scale insect species have been karyotyped and the significance of cytogenetic studies to coccid systematics has been reviewed by various authors (Brown, 1977; Hughes-Schrader, 1948; Moharana, 1990; Nur, 1971: 1977: 1980; White, 1973). The diploid number for scale insects has been found to range from 4 to 64 and may vary considerably within any one family, for example, by up to a factor of 8 in the Pseudococcidae. Nevertheless, the majority of species within a family share the same (modal) chromosome number. The approximate number of species analysed, the range of diploid numbers and the modal number for 6 scale insect families are given below in Table 8.1 (Moharana, 1990; Nur, 1990).

Table 8.1
The modal and range of chromosome numbers found in 6 scale insect families

Family	Spp. no.	Diploid no.	Modal no.
Margarodidae	8	2-40	4
Pseudoccocidae	100	8-64	10
Coccidae	25	10-36	16
Eriococcidae	35	12-48	18
Phoenicoccidae	9	6-18	10
Diaspididae	140	6-18	8

It can be seen from Table 8.1 that the modal number of chromosomes for the Coccidae is 16. The diploid number obtained from embryo squashes for species of Coccidae belonging to the genus Pulvinaria collected from India

are: P. psidii Maskell 2n = 14, P. polygonata Cockerell 2n = 18, P. polygonata group 2n = 18 and Pulvinaria sp. 2n = 16 (Moharana, 1990).

Karyotyping has provided possible evidence for distinct taxa within the P. vitis complex. Large and small specimens identified as "P. betulae (L.)" and "P. ribesiae Signoret", have been reported to have somatic cell nuclei with 2n = 16 and 18 chromosomes respectively (Drozdovsky, 1966). They were collected from birch (Betula sp.) and blackcurrant (Ribes nigrum) in Moscow province, U.S.S.R. I have examined the original slide-mounted specimens from the U.S.S.R. but have found no consistent morphological differences between the two nominal species (discussed further in Section 3.3). The range of variation of the U.S.S.R. specimens labelled "P. betulae" and "P. ribesiae" fit into the range of variation found within P. vitis from Britain. There is a size difference between the two nominal U.S.S.R. species, "P. ribesiae" being smaller, but this is consistent with the host-induced morphological variation demonstrated in Chapter 5, by a host transfer experiment. Unfortunately, there are only a small number of specimens avaliable for examination that were reported to have 18 chromosomes.

Samples of P. vitis were collected from different populations on different host-plant species in Britain to see if taxa could be distinguished by karyotypic variation.

8.2.2 Methods

Source material

Second-instar males were collected during July and August. They were easily distinguished from the oval, convex females by their smaller, more elongate and slender appearance. They were found to be most suitable when collected before secretion of the wax test. They were preserved in freshly prepared cytological fixative (3 parts absolute methanol: 1 part glacial acetic acid). Males were very difficult to collect in the field and were obtained for this present study, from laboratory cultures. The cultures were started with adult females collected from the four sources listed below. The methods of collecting specimens in the field, transferring the ovisacs between host-plant species and rearing the cultures, are discussed in Section 5.2.3. The collection data and experimental host species on which they were cultured are as follows:

Original host	Locality	Date	Culture host
Prunus persicae 'Peregrine'	Misterton	1988	Ribes nigrum var. Ben Lomond
Betula pendula	Wisley	1988	Ribes nigrum var. Ben Lomond
Ribes sanguineum	London	1988	Crataegus monogyna
Ribes nigrum	London	1988	Betula pendula

Chromosome preparations

The methods used for the chromosome preparations follow Blackman (1980). They were originally intended for aphids and have been modified for use with scale insects. The first method described below is relatively quick and is suitable for freshly collected live specimens. The second method, using acid hydrolysis, is more laborious but produces clearer chromosome preparations with little or no cytoplasmic background. It can also be used for specimens preserved in cytological fixative. Most of the chemicals used in these two preparation methods are potentially highly dangerous and needed to be treated with great care. The health hazard and safety precautions necessary when using the chemicals listed below are given in the HMSO Compendium of Product Safety Data Sheets, COSHH Regulations.

Quick method

1. Second instar males were collected just prior to secretion of the wax test and placed into 75% potassium chloride (KCl) solution in a solid watch glass. This dissecting fluid is mildy hypotonic to aphid and scale body fluids. The scale was placed on its dorsum under a binocular microscope, to expose the softer ventral surface. The body was held secure by the margin with a finely pointed, mounted entomological pin. The cuticle of the opposite margin was dissected with a second mounted pin. This precedure was then repeated so that both margins were dissected. The abdomen was gently pressed to push out the gut and testes. The testes sometimes floated out of their own accord due to their covering of adipose tissue. The fat bodies were carefully dissected away from the two large oval testes unless endosymbionts were required, in which case the fat bodies were not removed. The size of the testes and quantity of adipose tissue varied considerably with the adipose tissue decreasing with testicle maturity (Fig. 8.9).
2. The testes were transferred, using a mounted pin, to freshly prepared cytological fixative, for 15 minutes to an hour. Care was taken not to

stick the testes too firmly to the pin as they were occasionally very difficult to dislodge.

3. A square coverslip was cleaned with a paper tissue or lint-free cloth so that it was free from dust and grease. It was examined against a dark background with side lighting to check that it was clean. Top quality, 1.0-1.2mm thick microscope slides were used which had been cleaned by boiling in chromic acid. This was washed off with running cold water overnight. The slides were stored in 95% ethanol with 2% concentrated HCl. They were transferred to 95% ethanol prior to use. The ethanol was wiped off from each side of the slide by a single stoke of a tissue or lint-free cloth. This was to ensure that it was dust free and that static electricity did not build up. The slide was placed on filter paper and a small drop (4-5mm diameter) of propionic acid was placed on it.

4. Using a finely pointed, mounted pin, a testis was lifted out of the fixative and placed into the propionic acid. The slide was placed on to the microscope stage and a check was made for foreign matter. The coverslip was also checked for dust and, if clean, lowered gently onto the slide. There was just enough liquid in the drop to spread evenly to the edges of the coverslip and no more. The slide was inverted on to filter paper and pressed down at both ends. A second filter paper was placed on to the back of the slide over the region of the coverslip, whilst the slide was held very firmly. A steady, verticle pressure was applied for 1-2 seconds with thumb or forefinger.

5. The slide was examined under a compound light microscope with phase contrast illumination. It was scanned initially with about 100-200 x magnification and promising cells with 400-600 x magnification. A good slide had no air trapped under the coverslip and could only dry out very slowly from the edges. If necessary to complete the examination, a little more 45% propionic acid was added with a pipette around the edges of the coverslip and any excess absorbed with a tissue. If a permanent preparation was desired, the slide was placed in a covered Coplin jar that had been swilled out with distilled water, so the preparation could keep for a few hours without drying out. The precedure to make permanent preparations is given below.

Acid hydrolysis method

The scales were first fixed by placing them, alive, into freshly prepared cytological fixative. They could be stored in this fixative for 2-3 months.

1. Second instar males were transferred to fresh fixative in a solid watch glass. The testes were dissected out in the manner described above (quick method, part 1).
2. A lens tissue thimble, made from a segment of a hair roller from which the spines had been removed, a lens tissue and an elastic band, was prepared as shown below in Fig. 8.1. The thimble stood in a large, solid watch glass filled with fixative to just below the rim. The testes were transferred to the thimble using finely-pointed, mounted pins.

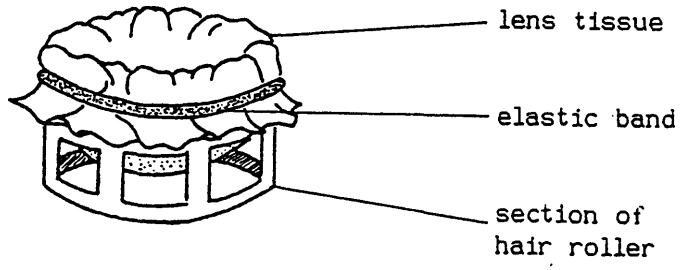


Fig. 8.1 Lens tissue thimble

3. The thimble and testes were removed and excess solution was drawn off by standing the thimble on filter paper for a second, before it was transferred to another watch glass containing 75% methanol and left for 5 minutes. During this time it was covered with an inverted watch glass to prevent contamination with dust.
4. The thimble and testes were transferred to another watch glass, containing 1 M hydrochloric acid (HCl) in an oven equilibrated at 65°C, for 5 minutes.
5. The thimble was then transferred to distilled water where the testes floated freely on the surface. The testes were transferred individually, using two mounted pins, to a drop of 45% propionic acid on a clean slide. They were squashed in the method described above (quick method, part 4). Care was necessary again to avoid introducing any foreign matter to the slide, which would interfere with squashing.

Permanent, stained slide preparations

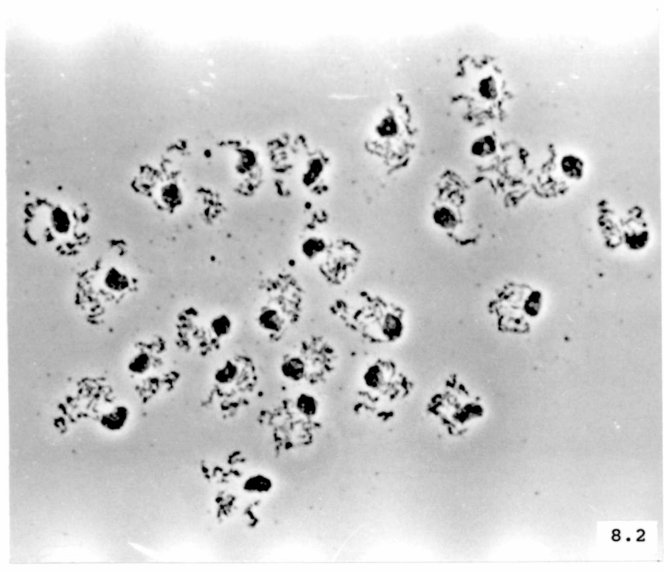
Exceptionally clear, condensed chromosome preparations, obtained by the quick or acid hydrolysis methods described above, were made into permanent slide preparations for future reference.

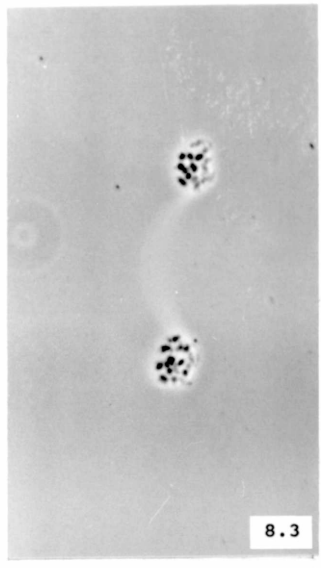
1. The slide was placed, coverslip uppermost, on a flat slab of solid carbon dioxide for 7-10 minutes. A sharp scalpel was then applied to the edge of the coverslip to flick it off. The slide was immersed briefly into 95% ethanol and the back of the slide wiped. The preparation was then placed in a closed cupboard or an oven at room temperature to dry for at least a week. During this time it was covered with a tissue to exclude dust.
2. The slide was then placed into Sorensen's phosphate buffer at pH 6.8 for 5 minutes.
3. The slide was then transferred to 5% Giemsa solution in pH 6·8 phosphate buffer, which had been left to stand for 1 hour before use. Preparations made by the quick method (without HCl treatment) only required about 10 minutes in the stain. The preparation made by acid hydrolysis required longer, about 30-40 minutes. Staining was monitored periodically under the microscope.
4. The preparation was differentiated for 2 minutes in distilled water.
5. The back and ends of the slide were wiped and it was then left to dry for 1-2 weeks at room temperature, again covered with a tissue.
6. The preparation was immersed in xylene for 10 minutes.
7. It was then mounted in DePeX and dried in an oven at 50°C for 24 hours.

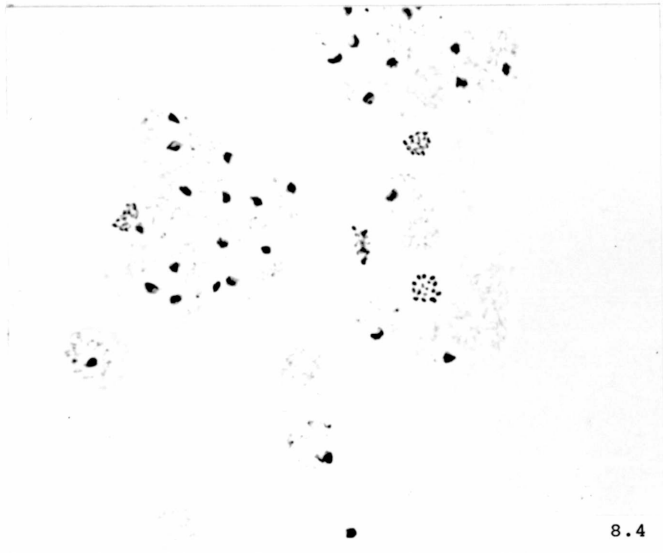
8.2.3 Results and discussion

Only a few ($n = 3 \sim 6$) individual specimens from each of the four samples were at exactly the right stage of spermatogenesis to be karyotyped. Chromosome squashes during spermatogenesis are shown in Figs. 8.2, 8.3, 8.4 and 8.5. All the specimens had a diploid chromosome number of 16, and all the chromosomes were of similar size (Fig. 8.4).

A diploid chromosome number of 16, corresponds to the number given for "P. betulae" by Drozdovsky (1966). Sixteen is also the modal number for the family Coccidae. During the host transfer experiment conducted in 1988 (see Section 5.1.3), it was demonstrated that specimens with 16 chromosomes can







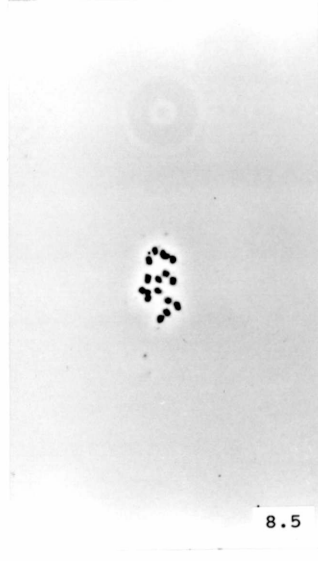


Fig. 8.2 Chromosome squash during spermatogenesis in Pulvinaria vitis

Fig. 8.3 Late second anaphase and telophase of spermatocyte meiosis

Fig. 8.4 Spermatocytes with condensed chromosomes

Fig. 8.5 Single spermatocyte showing condensed chromosomes 2n = 16

complete their life cycle on alder (Alnus glutinosa), blackcurrant, flowering currant (Ribes sanguineum), birch, hawthorn (Crataegus monogyna), grapevine (Vitis vinifera), willow and peach. Drozdovsky (1966) reported finding small specimens on currants in the U.S.S.R. with a diploid chromosome number of 18. No specimens were found with 18 chromosomes in the present investigation, although small specimens collected from currant were examined.

Is it possible for a variable, cosmopolitan, polyphagous species of Coccidae to have different karyotypes? Interspecific karyotype variation is relatively common in aphids. Where 10 or more samples or clones of what is regarded as a single aphid species have been examined, 75% showed karyotype variation (Blackman, 1980). For example, samples of the parthenogenetic species, Rhopalosiphum maidis (Fitch), on barley usually have 2n = 10, but on maize and Sorghum spp. have 2n = 8 (Brown & Blackman, 1988). Two other karyotypes (2n = 9 and 11) have also been recorded for clones of this species, although the clones can not be separated on morphology alone. Interspecific karyotype variation and instances of heterozygosity (by implication, highly adaptive genomes) are numerous in aphid species and groups which have partially or completely abandoned the sexual phase of the life cycle in favour of permanent thelytoky (Blackman, 1980). According to Blackman, species which have holocentric chromosomes and thelytokous reproduction, like many aphids and scale insects, might be expected to show interspecific karyotype variation. Most scale insect species have only been karyotyped from single samples or clones so the degree of variation which may occur is not known. It is therefore, not only possible for a variable, cosmopolitan, polyphagous species of Coccidae to have different karyotypes, but quite probable.

The specimens collected by Drozdovsky, labelled "P. ribesiae" and "P. betulae", cannot be consistently separated morphologically from each other or from P. vitis from Britain (see Section 3.3.2). No firm conclusion can be made concerning the relationship between the specimens with 16 and 18 chromosomes recorded by Drozdovsky (1966), until a greater number of specimens with 18 chromosomes have been studied morphologically, cytologically and ecologically.

8.3 Chromosome systems

8.3.1 Introduction

Scale insects are useful for genetic studies because a variety of different sex-determination mechanisms and chromosome behaviours (chromosome systems) occur within the group. Scale insect chromosome systems are more diverse than in any other comparably sized group of animals (Nur, 1980). Scale insects possess a variety of different types of both sexual and parthenogenetic reproduction which are reviewed by Nur (1980). The following chromosome systems are used by purely bisexually reproducing species: XX-XO, male haploidy, hermaphroditism, 2n-2n, lecanoid system and the Comstockiella system. The following chromosome systems are used by species in which at least some of the females are produced parthenogenetically: haploid and diploid arrhenotoky, deuterotoky, agonoid and gonoid thelytoky.

Species of the family Coccidae belonging to the genera Pulvinaria and Neopulvinaria have been reported to reproduce sexually and parthenogenetically using the following chromosome systems (Nur, 1980; Phillips, 1963): facultative diploid arrhenotoky (P. floccifera, N. innumerabilis), facultative and obligate deuterotoky (P. mesembryanthemi, P. hydrangeae, N. innumerabilis) and agonoid thelytoky (P. hydrangeae, "P. vitis", N. innumerabilis). Other genera of Coccidae are reported to reproduce only sexually, using the Comstockiella system.

Diploid arrhenotoky is a type of facultative parthenogenesis in which fertilised eggs develop into females and unfertilized eggs into males. The males begin to develop as haploids, but they become diploid through the fusion of the first two haploid cleavage nuclei (Nur, 1971: 1972). A few divisions later, one set of chromosomes becomes heterochromatic.

Deuterotoky is closely related to diploid arrhenotoky and is likely to have evolved from it (Nur, 1980). In both these types of chromosome system, the males are diploid and develop from unfertilized eggs. The main difference is that in deuterotoky some of the unfertilized eggs develop into females. The fate of the fertilised eggs in deuterotokous species is unknown, but it is probable that they all develop into females as in diploid arrhenotoky. Deuterotoky is only known from the Coccidae. Adult males of the species P. mesembryanthemi and P. hydrangeae are reported to be non-functional (Nur, 1980). The sexual function of males of P. vitis is also unknown (see Section 7.1.3). In many other species of Coccidae, males are

very rare and it is likely that all these species are deuterotokous (Nur, 1971: 1980). The presence of non-functional males may be explained by the process of evolution towards thelytoky, in which the suppression of heterochromatinization has not yet stabilised (Nur, 1980).

Agonoid thelytoky is also closely related to diploid arrhenotoky and is likely to have evolved from it when some of the eggs were modified so that heterochromatinization of one of the two haploid chromosome sets no longer took place. According to this interpretation, deuterotoky is likely to have been an intermediate state between diploid arrhenotoky and agonoid thelytoky. In agonoid thelytoky the number of chromosomes present in the first metaphase of oogenesis is less than the number present in the oogonia. In diploids of this type, a haploid number of bivalents are present in metaphase I. The maternal chromosome number is restored in Coccidae by the fusion of the first two cleavage nuclei, or the fusion of the female pronucleus with the second polar body.

"P. vitis" was reported to be obligate thelytokous in Ontario, Canada as males were unknown (Phillips, 1963), however, both bisexual and unisexual populations of P. vitis occur in Britain. The chromosome systems used by P. vitis in Britain were investigated in order to see if there were differences in the chromosome systems used by different populations. The chromosome system can be determined by looking at the sex of the progeny of uninseminated females.

8.3.2 Methods

All immature male scales were removed with forceps from the bisexual cultures of P. vitis on potted birch (Betula pendula), willow (Salix alba) and blackcurrant (Ribes nigrum) plants. The plants were isolated indoors in an insectary from the end of September to the beginning of November 1988 to ensure that the females were uninseminated. The experimental populations were originally derived from peach (Prunus persicae), Misterton, UK, 1988. The plants were moved outdoors in November for the female scales to overwinter, one week after all the adult males on the outdoor cultures had emerged. Only two or three adult females were present on each plant. In the following spring surplus adult females were removed with forceps, to leave a single, unparasitized adult on each birch and willow plant, and 4 scales on currant plants, each on a separate branch. The sex of the

offspring of each of the uninseminated females was then recorded. The offspring were sexed during the second instar, as this was the earliest stage at which they could be differentiated.

8.3.3 Results and discussion

Three uninseminated adult females on blackcurrant produced all female offspring and a fourth adult produced offspring of both sexes but predominantly female. The single adult female on birch produced all female offspring and the single adult on willow produced all male offspring. Individuals from a single population of P. vitis, therefore, were able to use three different chromosome systems; diploid arrhenotoky, deuterotoky and thelytoky. Obligate thelytoky has already been reported to occur in North America, as Phillips (1963) recorded uninseminated females only produced females. Unisexual populations also occur in California (R. Gill, pers. comm.). Thelytokous reproduction occurred in cultures during this study with P. vitis on grapevine, where all the cultures for two successive generations were female only. Diploid arrhenotoky also occurs in Britain, as at least one uninseminated female in the experiment above produced only males. Some uninseminated females also produced both females and small numbers of males, indicating deuterotoky.

It is not known for certain whether the adult males in P. vitis are sexually functional (see Section 7.1.3). Unisexual populations appear to be more common than bisexual populations in Europe and the sex ratio is often strongly biased towards females in bisexual populations. This may be due to higher mortality of male nymphs, which are smaller than females of the same age.

The results fit my personal field observations of separate unisexual and bisexual populations of P. vitis in Europe. This has been reported previously by various authors (Danzig, 1972, 1986; Gill, 1988; Green, 1920; Phillips, 1963; Jablonowski, 1916; Schmutterer, 1952). I have also observed, in Britain, Hungary and The Netherlands, single, isolated, post-reproductive females in the field, each surrounded by large numbers of male tests with no other female scales in the vicinity, indicating that diploid arrhenotoky is probably widespread. Most of the examples of probable diploid arrhenotoky observed occurred on willow.

Are the unisexual and bisexual populations of P. vitis sibling species or

races of a single species? Discrete unisexual and bisexual populations also occur widely within species of Diaspididae (Gerson, 1990) and opinions differ with regard to their taxonomic status. Some authors confer subspecific and even specific ranks to unisexual and bisexual populations that are rejected by others. For example, the oystershell scale, Lepidosaphes ulmi (L.), has been divided into five biological races (Bouchra, 1978) and into subspecies (Schmutterer, 1959) on the basis of the host-plant species and type of reproduction used. An abundant, polyphagous, parthenogenetic form was named L. ulmi ulmi and a rarer, oligophagous, bisexual form was named L. ulmi bisexualis Thiem. Biological as well as morphological differences have been reported to occur within the unisexual and bisexual populations of L. ulmi, as well as between the unisexual and bisexual populations. This has lead to the conclusion that there are several sibling species under the label of L. ulmi (Gerson, 1990). The situation could be resolved by culturing all these taxa under similar environmental conditions and examining any resulting differences in a similar way to that used in this present study.

The race of P. vitis that has been introduced into North America appears to be obligatorily thelytokous, as all the populations are reported to be unisexual (Gill, 1988; Phillips, 1963; Richards, 1964). It is possible that a unisexual race can have an advantage over a bisexual race when initially establishing in a new environment or extending geographical range; a parthenogentic female will not have to spend time and energy waiting or searching for a male before reproducing. Unisexual populations usually have a much wider distribution than bisexual populations of the same species (Vandel, 1928, 1931; Suomalainen et al., 1976), termed geographical parthenogenesis by Vandel (1928). For example, unisexual and bisexual races of the aphid Macrosiphum albifrons Essig are present in North America but only unisexual populations are found in Europe (Stroyan, 1981). The various races of the parthenogenetic polyploid boreo-alpine weevil, Otiorrhynchus dubius Stroem, have different distributions in Europe (Suomalainen et al., 1976). In northern Europe, it is parthenogenetic and tetraploid, whereas in central Europe it is diploid and sexual. All the parthenogenetic races of the weevil appear to be polyploid.

Introduced unisexual populations have the potential to revert to bisexual reproduction. The aphid group Therioaphis trifolii/maculata (Buckton) was introduced to North America from Europe. It was anholocyclic when first

introduced into southern U.S.A. as the sexual stage phase was abortive; however, it acquired the ability to produce viable sexuales and over winter as eggs in about 1960, in the course of its spread to more northerly latitudes (Manglitz et al., 1966). The mechanism for this change from anholocyclic to holocyclic is unknown.

Further evidence for the existence of different races of P. vitis are the differences in host-plant range, life cycle and phenology found in different geographical regions. This is to be expected due to the relative genetic isolation between widely separated demes. Significant genotypic differences even occur between demes of Nuculaspis californica (Coleman) (Diaspididae) on different twigs within individual host trees and between demes on neighbouring trees (Alstad & Corbin, 1990).

Different races or clones of the same species of aphid often have different host preferences. P. vitis has been a major pest on outdoor peach trees in North America but has not been recorded as an important pest of currant or grapevine. The reverse is true for Europe. This may be due to different varieties of these plants being grown on each continent. Differences in life cycle have also been used to separate the nominal species in the P. vitis complex, although these differences now appear to be environmentally determined (see Section 7.1.1). A partial second generation is possible at higher temperatures and the overwintering stage for P. vitis in Britain is considerably more variable than reported by Newstead (1903).

The biological and morphological differences that have been reported between different populations of the P. vitis complex, appear to be mainly environmentally induced rather than genetic. Therefore, the different unisexual and bisexual populations appear to be races of the same species, at least in Britain, rather than sibling species. The classification of medically important diptera have been studied more intensively than most groups of insects and there are several groups of sibling species, such as the Anopheles gambiae group, which are only distinguishable by differences in behaviour, cytology or ecology. Sibling species according to Mayr (1969), are near the indistinguishable end of the spectrum of morphological species differences, separable from the nearest relatives by 'minute' deviations. Their ranking is therefore somewhat arbitary.

Current evolutionary theory suggests that ecological diversification lags far behind morphological diversification and speciation (Gerson, 1990). This

does not appear to be the situation with L. ulmi or P. vitis, where there appears to be incipient biological rather than morphological speciation, implied by the unisexual and bisexual forms. This may be due to P. vitis exploiting crop plants that are usually grown as monocultures, with new varieties added continually. Such new challenges are more likely to be met by relatively rapid ecological adaptations rather than by slow morphological changes.

8.4 Gross morphology of the sperm bundle

8.4.1 Introduction

Scale insect sperm are very different, both morphologically and physiologically, from the sperm of most animal groups. Spermatozoa have been examined from about 25 species of scale insect belonging to the following families: Diaspididae, Pseudococcidae, Eriococcidae, Kermesidae, Coccidae and Margarodidae. All contain filamentous sperm occurring in motile bundles of 16, 32, 64 or more, surrounded by membranous sheaths. The spermatozoa are not divided into distinguishable regions such as a head, middle-piece and tail as in other groups of animals; neither do they contain any centrioles, mitochondria, golgi membrane derivatives or the 9+2 arrangement of microtubules in the motile apparatus. The motile bundles of scale insect sperm are highly modified and exhibit extraordinary variability. The number and arrangement of sperm present in a bundle and the accessory contents of the sheaths vary at specific level (Robinson, 1977: 1990). Robinson (1990) has discussed the implications of these unusual variations to the physiology, function, taxonomy and phylogeny of scale insect sperm bundles.

8.4.2 Methods

The specimens examined came from cultures derived from the four sources listed in Section 8.2.2. The method used was essentially the same as the quick method for chromosome preparations in Section 8.2.2. Fully-developed sperm bundles were found in mature second-instar males.

8.4.3 Results and discussion

The development of the male internal reproductive system is shown diagramatically in Fig. 8.9. It undergoes the greater part of its

development during the second instar and the testes contain fully developed sperm bundles by the end of this stage.

The gross morphology of the sperm bundles is shown in Figs. 8.6, 8.7 and 8.8. The bundles are typically long and thin with a sheath that is thickened anteriorly and sculptured to form a rigid corkscrew structure. The filamentous sperm are aligned in a helical arrangement in the posterior half of the bundle and some appear to be emerging from the apex (Fig. 8.8).

The gross morphology of the sperm bundles were identical between all the populations of P. vitis from Britain examined. The gross morphology and number and arrangement of sperm present in a bundle was found to be very similar between unrelated species in the family Coccidae; Parthenolecanium corni (Bouché) and Neopulvinaria innumerabilis (Rathvon) (Tremblay, 1977). The gross morphology of the sperm bundles appears to be more useful at higher levels of scale insect taxonomy, rather than at the specific level.

8.5 Endosymbionts

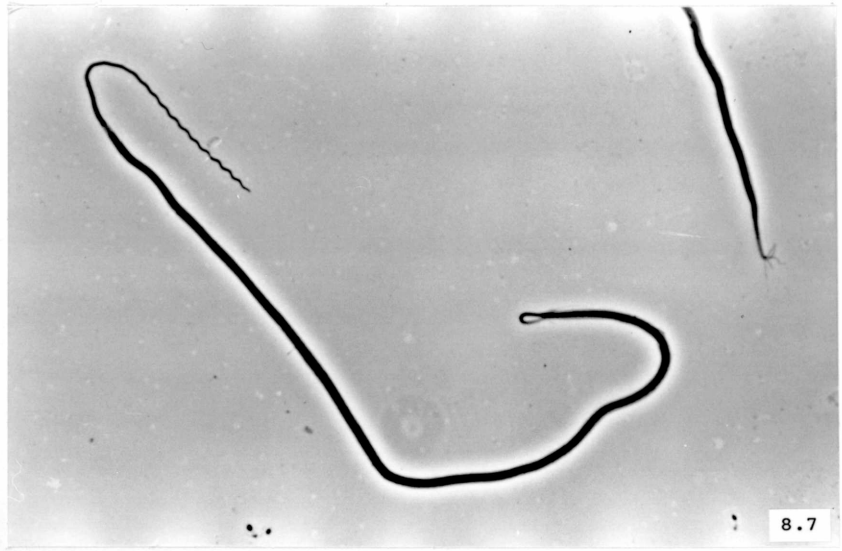
8.5.1 Introduction

Most scale insects contain intracellular, bacteria- or yeast-like symbionts in the cytoplasm of special cells called mycetocytes. The symbionts are reported to supply essential vitamens and minerals to the scale by the breakdown of excretory products and resynthesis using atmospheric nitrogen supplied by the scale's respiratory system (Tremblay, 1977). The heterogenous nature of the internal symbionts of the scale insects suggests a polyphyletic origin (Tremblay, 1977; Buchner, 1965). The potential usefulness of the various types of endosymbionts to scale insect systematics has been discussed by Tremblay (1977).

Yeast-like symbionts have been reported to occur in adipose tissue or in free, enlarged fat cells in several species of Coccidae. Endosymbionts have not been previously reported to occur in P. vitis, however.

8.5.2 Methods

The specimens examined came from the cultures derived from the four sources listed in Section 8.2.2. The method used was the quick method for chromosome preparations given in Section 8.2.2, above. Endosymbionts were searched for in the adipose tissue surrounding the testes, during karyotyping the specimen.



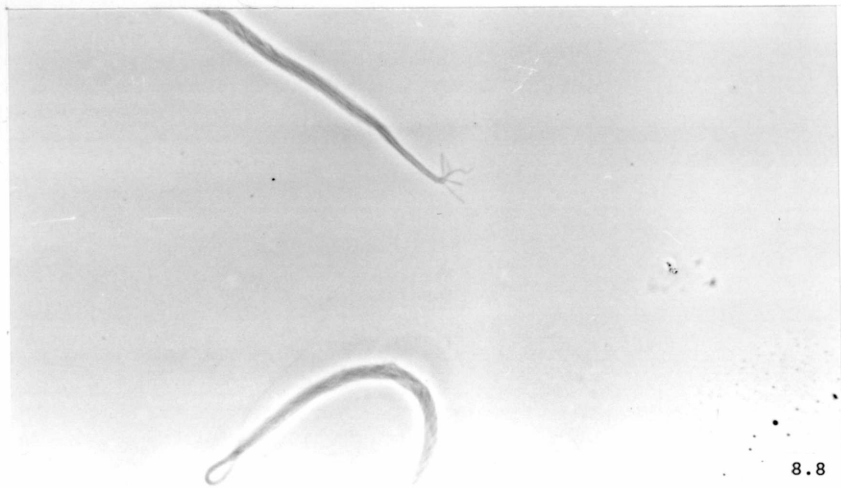


Fig. 8.6 Sperm bundles of Pulvinaria vitis

Fig. 8.7 Single sperm bundle

Fig. 8.8 Single sperm bundle showing the rigid corkscrew structure of the anterior portion, formed by the thickening and sculpturing of the sperm bundle sheath

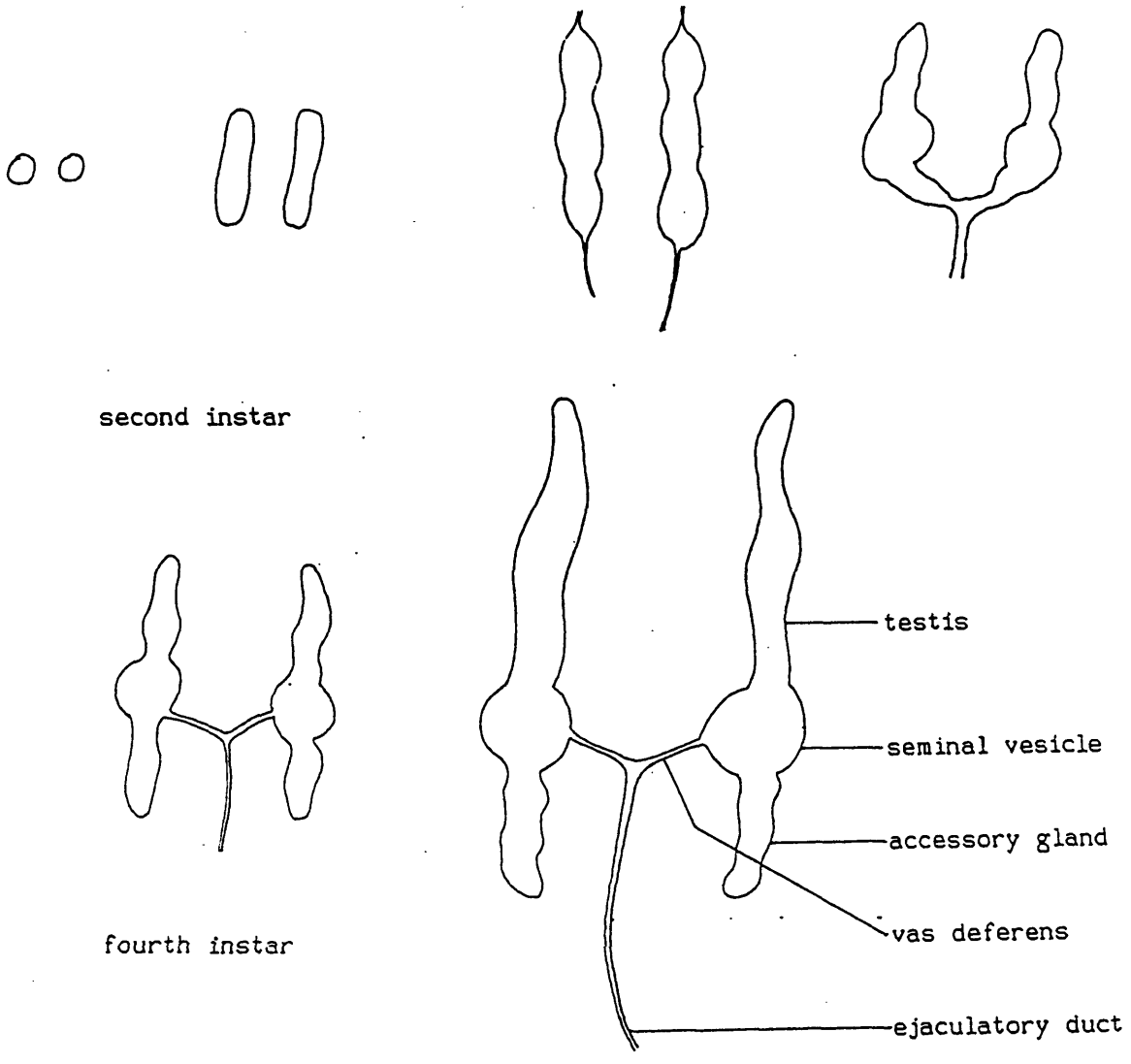


Fig. 8.9 Diagramatic representation of the development of the internal male reproductive system of Pulvinaria vitis

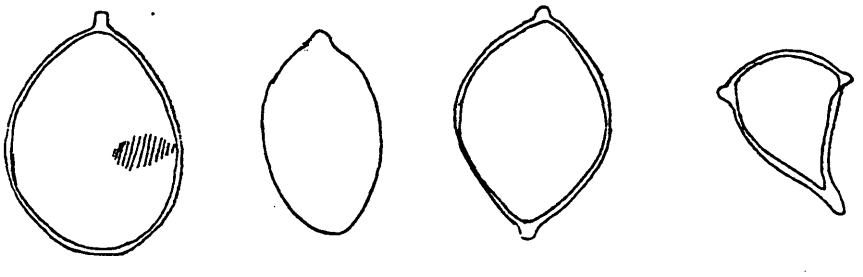


Fig. 8.10 Diagramatic representation of the yeast-like endosymbionts found in adipose tissue surrounding the testes of Pulvinaria vitis

8.5.3 Results and discussion

Specimens were examined from all four sources and all were found to contain intracellular yeast-like endosymbionts in the adipose tissue surrounding the testes. These endosymbionts are illustrated in Fig. 8.10. They were mostly oval with a small protuberance at each end; however, there was variation in shape and size. No bacteria-like endosymbionts were found.

Yeast-like endosybionts have been found in all the species belonging to the family Coccidae examined (Tremblay, 1977). Endosymbiont studies appear to be more useful for suggesting phylogenetic relationships between higher taxa.

8.6 General discussion

Cytogenetic studies, gross morphology of the sperm bundle and endosymbionts were not found to be useful in the separation of taxa within P. vitis in Britain. Karyotyping and chromosome studies are potentially the most useful techniques for the separation of taxa within the P. vitis complex and studies of the complex are needed from different geographical areas.

9 Gel electrophoresis

9.1 Introduction

Gel electrophoresis is a method of investigating the molecular structure of enzymes that has proved useful for distinguishing closely related taxa, where separation based on morphological differences is not possible or is unreliable. Electrophoresis has the advantage that enzymes are a direct expression of the genotype and are not influenced by environmental conditions. It is particularly useful, therefore, for distinguishing species which exhibit a large degree of environmentally induced morphological variation. Many enzyme systems show no variation between sexes or different instars, so identifications do not have to be based on a specific instar as morphological identifications often are.

The application of electrophoresis techniques to taxonomy has been reviewed by many authors (e.g. Avise, 1974; Lokovaara et al., 1976; Tomiuk et al., 1979), who have concluded that enzyme bands are valid taxonomic characters. Ayala et al. (1976) studied fruit flies of the Drosophila willistoni group, and found that all the species could be distinguished at one or more loci. Miles (1974) showed electrophorectically that in mosquitoes, 'subspecies' of the Culex pipiens group were valid species.

Electrophoretic studies usually closely agree with biometric studies of the same group; however, Nur (1977) found evidence for 17 taxa which he considered merited species rank, within 9 morphological species of mealybug (Pseudococcidae) using electrophoretic data. Electrophoretic data were not found to be useful at higher levels of classification in mealybugs (Miller & Kosztarab, 1979).

Electrophorectic differences between different populations may only represent interspecific variation and do not necessarily indicate the exis tence of more than one species: thus, results need to be interpreted with care. If electrophoretic differences are found between populations that agree with differences found in morphology, biology or cytogenetics, then it is probable that they are distinct species.

Theore tically, the method of electrophoresis is simple, involving the separation of a homogenate of individual organisms by passage through a supporting medium of starch or polyacrylamide gel. In starch gels, the pore size is controlled by the concentration of starch used; however, there is

still variation in pore size which can cause gel inconsistency and lead to erroneous results. Polyacrylamide gels are formed by chemical polymerisation and the size of the pores within the gels can be more precisely determined and band resolution is greater. Enzyme molecules pass through the gel lattice under the influence of an electric field at rates according to their size and charge.

Specific enzymes are revealed on the gel by the use of stains which are released by the catalytic action of the enzyme. Enzymes present are revealed as single or as a series of distinct bands which can be measured both quantitatively and qualitatively. The distance that the enzyme band travels from the starting point or origin on the gel, relative to the front, is known as its relative mobility (Rf) value. The occurrence and intensity of specific bands and their Rf values can be used as taxonomic characters. Enzymes that differ structurally because they are the product of different alleles but of the same locus, are known as allozymes. Enzymes with similar catalytic action, but which differ in size or structure and are products of different loci, are known as isozymes.

Wagner and Selander (1974) described esterases as the most polymorphic enzyme systems in aphids and, therefore, potentially the most useful for taxonomic investigations. In the present study, both horizontal starch gel and vertical polyacrylamide gel electrophoresis were carried out to investigate the esterase and other enzyme systems.

9.2 Methods

9.2.1 Material examined

Specimens of P. vitis were obtained for analysis by field collecting and from cultures. The methods of collecting specimens in the field and rearing the cultures are given in Sections 3.2.2 and 5.2.3 respectively. Only living specimens were used in the analysis. In addition to specimens of P. vitis, samples of Pulvinaria regalis Canard and Coccus hesperidum (L.) were also collected and analysed for comparison.

The specimens analysed were derived from the sources given below in Table 9.1.

Table 9.1

Collection data for soft scale samples used in the gel electrophoresis

Pulvinaria vitis (L.)

Betula pendula, Wisley, Surrey (reared on Ribes nigrum); Crataegus monogyna, Upper Richmond Road, London; C. monogyna, Embankment, East Putney, London; C. monogyna, Queens Club Gardens, London; Prunus persicae, Nottinghamshire (reared on Ribes nigrum); Ribes sanguineum, Ealing, London.

Pulvinaria regalis Canard

Acer pseudoplatanus, Embankment, East Putney, London; Aesculus hippocastanum, Embankment, East Putney, London; Tilia europea, Cromwell Road, London; T. europea, Holland Road, London.

Coccus hesperidum L.

Ilex aquifolium, Addison Crescent, London.

Table 9.2

Abbreviations used for the chemicals and biochemicals

ADP = adenosine 5'-diphosphate; ATP = adenosine 5'-triphosphate; ß-NAD/TPN = ß-nicotinamide adenine dinucleotide, triphospho-pyridine nucleotide, monosodium; bis-acrylamide = N,N'-methylene bis (acrylamide); EDTA = ethylenediaminetetra-acetic acid; MTT = 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAD^+ = ß-nicotinamide adenine dinucleotide; $NADP^+$ = ß-nicotinamide adenine dinucleotide phosphate; NBT = nitro blue tetrazolium; PMS = phenazine methosulphate; TCHA = tricyclohexylamine; TEM = tris (hydroxymethyl) aminomethane, ethylenediaminetetra-acetic acid + malic acid; TEMED = N,N,N',N'-tetramethylethylenediamine; Tris = tris(hydroxymethyl) aminomethane.

9.2.2 Electrophoretic methods

Chemicals and biochemicals (high purity grades) were purchased from Aldrich Chemical Co. Ltd., BDH Chemicals Ltd., Boehringer (Mannheim) and Fisons or Sigma (London) Chemical Co. Ltd. Abbreviations used for the chemicals and biochemicals are given in Table 9.2.

The methods for electrophoresis follow Seccombe and Brown (pers. comm.), which were modified from Eggers-Schumacher (1983), Watson (1982) and Loxdale et al. (1983). Five enzyme systems were analysed, using buffer system A (pH 8.0): Esterases, Alkaline phosphatase, Phosphogluco mutase, Phosphogluco isomerase and Trehalase. The consituents of the buffer solutions, gels and stains are listed in order of use in Tables 9.3, 9.4 and 9.5. All reagents were weighed out on a balance before starting the precedure, except for light-sensitive, temperature-sensitive or hydroscopic reagents. All solutions were stirred with a magnetic stirrer and, where necessary, heated with a thermostatic heating block.

Many of the reagents are potentially harmful and disposable rubber gloves were worn throughout the gel- and stain-making procedures to avoid skin contact. The polyacrylamide gel was prepared in a fume cupboard, as acrylamide is a cumulative neurotoxin. The health hazard and safety precautions necessary when using the chemicals listed below are given in the HMSO Compendium of Product Safety Data Sheets, according to the Control of Substances Hazardous to Health (COSHH) Regulations.

Starch gel

The precise contents and quantities of each mixture for this precedure are given in Table 9.3

1. The gel mould was made from 4 strips of glass or perspex shuttering, glued with starch gel on to a glass sheet. The moulds were prepared at the end of the previous starch gel-making session, but in the first instance, a small quantity of gel had to be specially prepared. The interior dimensions of the mould were 21 x 13 x 1.3cm. The corners of the mould were taped when dry with Scotch electrical tape.
2. The electrode buffer was prepared and then used to make the gel buffer. The buffers were stored in the fridge and could be used for up to 4 weeks.
3. To prepare the gel, the starch was sieved and gradually added to the

Table 9.3
Constituents of reagents used for starch gel electrophoresis

Electrode buffer system A pH 8.0	
Distilled or deionised water	200.00ml
Boric acid	23.60g
Sodium hydroxide	2.00g or as required for pH
Gel buffer system A pH 8.0	
Distilled or deionised water	1000.00ml
Magnessium chloride	0.05g
Citric acid	0.50g
Tris	2.70g or as required for pH
Electrode buffer	100.00ml
Starch gel	
Sigma hydrolysed starch	40.00g
Gel buffer	400.00ml
Homogenising solution system A	
Gel buffer	100.00ml
Triton x 100	0.05ml
Bromophenol blue (tracking dye)	2.0 grains
Fixative	
Distilled water	90.00ml
Methanol	90.00ml
Glacial acetic acid	20.00ml

Table 9.4
Constituents of reagents used for polyacrylamide gel electrophoresis
Quantities given are to make 12 gels.

Electrode buffer system A pH 7.45	
Tris (Sigma 7-9)	5.00g
Barbitone	27.60g
Distilled/deionised water	5000.00ml
Small pore gel system A pH 7.8 total acrylamide 6% total volume 270ml	
Acrylamide	15.633g
Bis-acrylamide	0.567g
Tris	2.310g
HCL (10.2 M)	1.45ml
HCL (1.0 M)	1.85ml or as required for pH
Ammonium phosphate	189.00mg
TEMED	100.00ul
Triton X100 (1.6% stock solution)	35.00ml
Distilled/deionised water	235.00ml

Table 9.4 continued

Large pore gel system A pH 7.8 total acrylamide 3.1% total volume 60ml	
Acrylamide	1.50g
Bis-acrylamide	0.375g
Tris	0.515g
HCL (1.0 M)	3.45ml
HCL (0.1 M)	as required for pH
Ammonium persulphate	42.00mg
TEMED	35.00ul
Triton X100 (1.6% stock solution)	15.00ml
Distilled/deionised water	45.00ml
Homogenising solution	
15% Sucrose	100.00ml
Bromophenol blue (tracking dye)	2.0 grains

Table 9.5
Constituents of stains
Quantities given are for a single portion of gel which are subdivided into slices.

Stain for esterases (Est)	
Acetone	1.00ml
1-naphthyl acetate	0.015g stored below 0°C
Fast Blue BB	0.30g stored below 0°C
Phosphate buffer 0.1 M pH 6.0	75.00ml
Stain for alkaline phosphate (AlPh)	
Magnesium chloride	0.01g
Sodium chloride	1.60g
Disodium a-naphthyl phosphate	0.05g stored below 0°C
Polyvinyl pyrrolidine	0.50g
Fast Blue RR salt	0.05g stored below 0°C
Tris buffer 0.05 M pH 8.5	100.00ml
Stain for phosphoglucо mutase (PgM)	
This recipe is for a pH 7.0 gel but works with system A pH 8.0	
a-D-glucose-1-phosphate (disodium)	0.60g stored below 0°C
ß-NADP/TPN	0.01g
NBT	0.02g
Magnesium chloride	0.20g
Glucose-6-phosphate dehydrogenase	1.0 flake store below 0°C
Tris buffer 0.1 M pH 7.1	100.00ml
PMS	3.00ml added after 1 hour

Table 9.5 continued

Stain for phosphoglucose isomerase (Pgl)
This recipe is for a pH 7.0 gel but works with system A pH 8.0

Fructose-6-phosphate disodium	0.02g store below °C
ß-NADP/TPN	0.01g
NBT	0.02g
Magnesium chloride	0.02g
EDTA	0.026g
Glucose-6-phosphate dehydrogenase	1.0 flake
Tris buffer 0.1 M pH 7.1	100.00ml
PMS	5.00ml added after 1 hour

Stain for trehalase (enough for 2 trays)
This recipe is for a TEM pH 7.4 gel but works with system A pH 8.0

D(+)Trehalase dihydrate	0.400g
NBT	0.032g
$NADP^+$	0.032g
Hexokinase	2.0 flakes
Glucose 6 phosphate dehydrogenase	2.0 flakes
Magnesium	0.160g
ATP	0.040g
PMS	5.00ml
Phosphate buffer 0.1 M pH 7	40.00ml

Phosphate buffer 0.1 M pH 6.0

Distilled water	1000.00ml
KH_2PO_4	23.85g
Na_2HPO_4	8.81g

Phosphate buffer 0.1 M pH 7

Distille water	1000.00ml
NaH_2PO_4	12.96g
Na_2HPO_4	11.44g

Tris buffer 0.1 M pH 7.1

Distilled water	500.00ml
Tris hydrochloride	7.02g
Tris	0.67g or as required for pH

Tris buffer 0.05 M pH 8.5

Distilled water	1000.00ml
Tris hydrochloride	7.02g
Tris	4.03g or as required for pH

gel buffer in a vacuum flask. Care was taken, when adding the starch, not to form lumps nor to coat the insides of the flask with powder; if lumps did occur, they were broken up with a glass rod. The mixture was heated and stirred continuously until the gel had thickened and steam bubbles were seen forming around the base of the flask.

4. The mixture was then degassed for 1 minute under vacuum, using an oil vacuum pump, and then poured into the mould. Air bubbles and lumps of starch were carefully removed using a spatula. The gel was then covered with Melanex film to exclude dust and prevent evaporation. Remaining starch gel was used to prepare more gel moulds. The gel was left overnight and then chilled to 0°C before use.

5. A perspex block with regularly spaced wells bored into it was placed on a tray of ice. The wells were numbered and labelled. Homogenising fluid, 20-50ul, was pipetted with a micropipette into the appropriate number of wells, and 1 or 2 specimens were added to each well. The specimens were macerated in the homogenising fluid using a small electric drill with a ground glass bit. The glass bit was washed in distilled water and wiped between each sample.

6. The gel was taken from the fridge and the Melanex film removed. A scapel was used to separate the gel edge from the mould shuttering. The shuttering was carefully removed and the gel sliced parallel to, and about 3 cm from, the long edge of the gel.

7. Each homogenate was then pipetted on to a piece of chromotography paper 0.5 x 1.0cm, using a new micropipette tip each time. An even covering of homogenate was required and any surplus was carefully blotted off to prevent excess homogenate spreading between samples by capillarity. Any pieces of unmacerated cuticle were also removed from the paper strips. The strips were inserted into the slit made in the gel; each was positioned close to, but not touching the adjacent strips. Up to 30 samples could be used on each gel. The two portions of the gel were then pushed together to close the slit and the mould shuttering was replaced and taped. More glass shuttering was placed carefully inside the mould along the far edge of the gel to accomodate for gel shrinkage during the electrophoretic run. Elastic bands were put around the mould to prevent the glass shuttering coming apart and distorting the gel. The Melanex film cover was replaced.

8. The gel was set up with the electrophoresis apparatus in the refrigerator as shown in Figs. 9.1 and 9.2. The baths were filled with cold electrode buffer. Wicks were made from chromatography paper (6 layers, 21 x 12cm) folded in half lengthwise and soaked in electrode buffer. The gel was placed lengthways on top of the baths and the wicks were positioned between the gel surface and the Melanex film. The wicks connected the gel surface with the buffer in the baths. The wicks were checked to ensure they were immersed along their entire length. An ice tray was then positioned on top of the gel to reduce heating. A platinum electrode was fixed in each bath, with the cathode on the side closest to the samples. The current was turned on and checked to ensure it was running properly before the refrigerator door was closed.

9. The gel was left to run at a constant 330V for 4 hours. The current was initially 70-100mA, but decreased to around 40mA during the run. If the current was too high, resistance could cause the gel to heat up, resulting in poor resolution of the bands. The progress of the run was followed by the movement of bromophenol blue tracking dye in the homogenising solution.

10. The stains and stain buffers were prepared (see precedure below) while the electrophoretic run was in progress. After the run was completed, the power was switched off and the gel was taken out of the fridge. The Melanex film and shuttering removed. The cathodic portion of the gel and the paper strips were discarded. The anodic portion was sliced into 6 or 7 layers with a wire cutting apparatus. Great care was required when slicing the gel and in teasing the layers apart in electrode buffer. The top layer of the gel was discarded. Each remaining gel layer was placed into a labelled plastic tray with the appropriate stain and left in the dark. Darkness was neccessary because some of the stains were light-sensitive. The staining time varied from half to several hours depended on the concentration of the enzyme present. After staining, the stain was decanted and the gels were washed with distilled water. The water was then drained away and fixative added. The gels were removed from the fixing solution after 5 minutes, wrapped in a plastic film and stored in the refrigerator for later study.

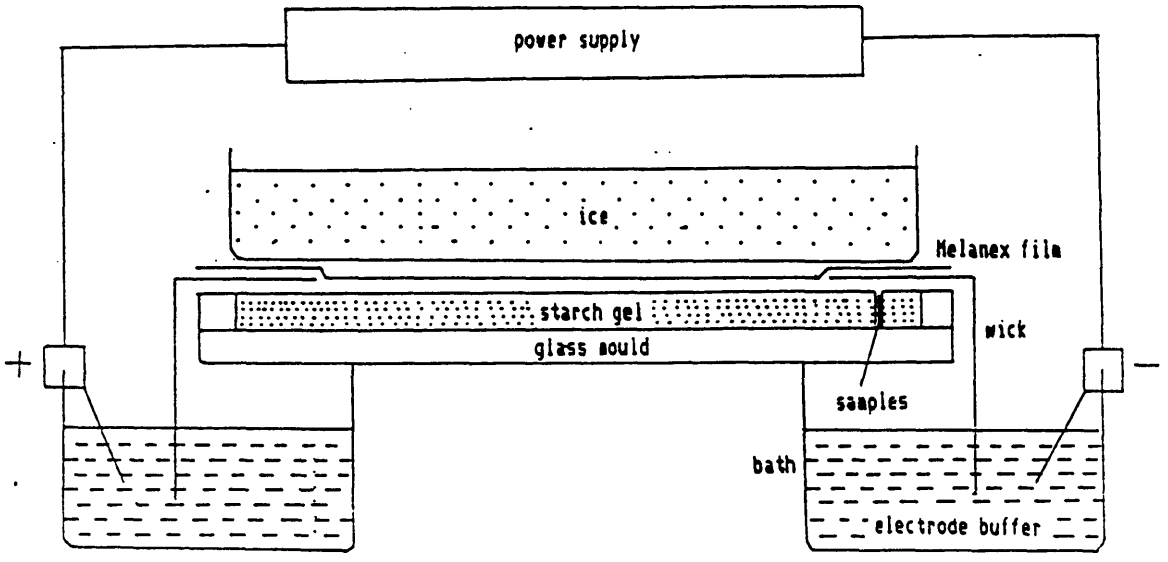


Fig. 9.1 Starch gel electrophoresis apparatus (side view)

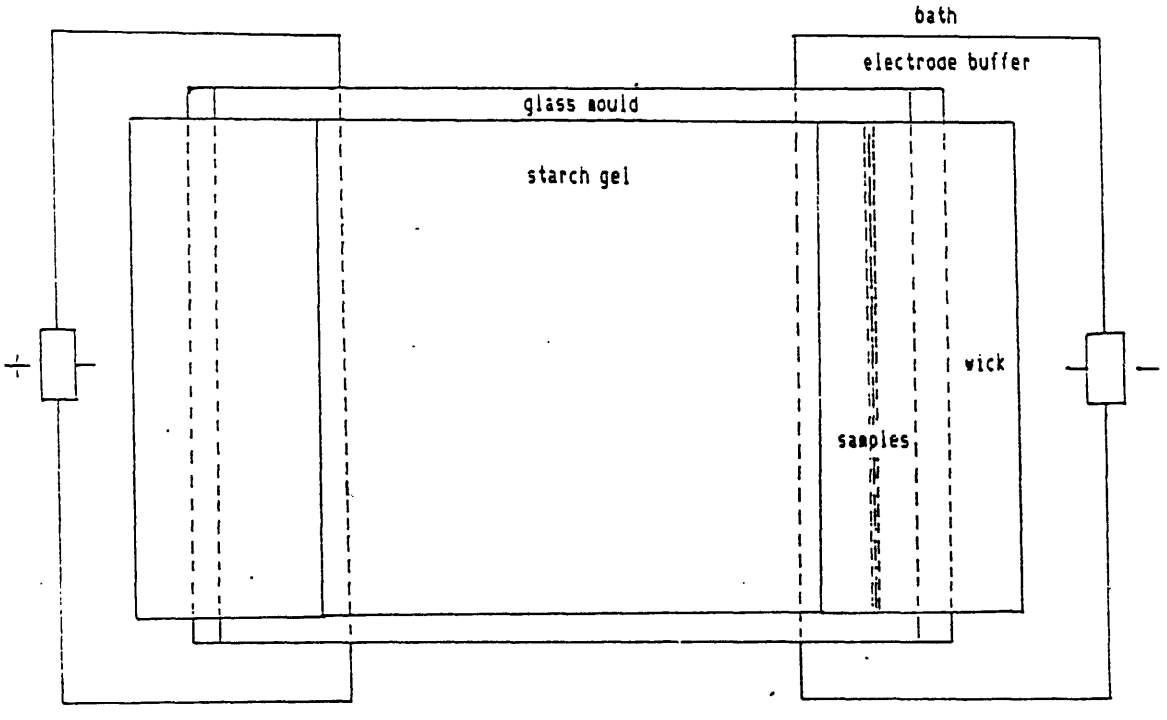


Fig. 9.2 Starch gel electrophoresis apparatus (top view)

Polyacrylamide gel

The precise contents and quantities of each mixture for this precedure are given in Table 9.4.

1. The polyacrylamide gels were contained within glass cassettes. Sixteen or 24 glass plates (8.1 x 8.1cm x 1.5mm) were first washed with detergent and water. They were then washed with acetone, rinsed with 95% alcohol and dried in a drying oven. The glass plates were moved using forceps, as fingerprints and dirt could effect the results. Each casette (Fig. 9.3) was made with two glass plates separated by plastic strips (8.1cm x 4 x 1.5mm), taped together with Scotch electrical tape. The plastic strips had to be flush with one end of the glass plates. Eight or 12 cassettes (as required) were placed in the casting apparatus, which had been washed with distilled water and allowed to dry.
2. In a fume cupboard, the acrylamide, bis-acrylamide and Tris were dissolved in distilled water. TEMED was added and the mixture made up to 230ml with distilled water. The 10.2M HCL was added and the pH taken. Drops of 1.0M or 0.1M HCl were added until the pH was exactly 7.8. Ammonium persulphate was added and the total volume made up to 235ml with distilled water.
3. The mixture was immediately degassed for 2 minutes, using a vacuum pump, and cold Triton X 100 was added to make up the final volume. The mixture was quickly poured into the casting apparatus, which holds the gels vertically. The cassettes were filled to a mark 12mm below the upper edge; the space was left to accommodate the large-pore gel. Butan-2-ol was pipetted on to the gel to a depth of about 5mm to exclude oxygen from the gels and aid polymerization. The gels were left to set at room temperature for one hour. The butanol was then decanted and the exposed gel surface washed twice with distilled water.
4. The large-pore gel was made up in the same way as the small-pore gel above, except that 1.0M and 0.1M HCl were used to balance the pH. The large pore gel was poured on to the small pore gel up to the top of the cassettes. The spacer combs were inserted into the large pore gel at the top of the cassettes. Butanol was again carefully poured onto the top of the acrylamide gel. The gels were left to set for another hour, after which the butanol was decanted and the spacer combs removed. The gels were stored in labelled plastic bags in a refrigerator at 4-5°C and were

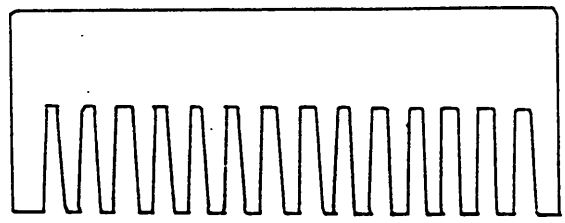


Fig. 9.3 Spacer comb for making wells in large pore gel

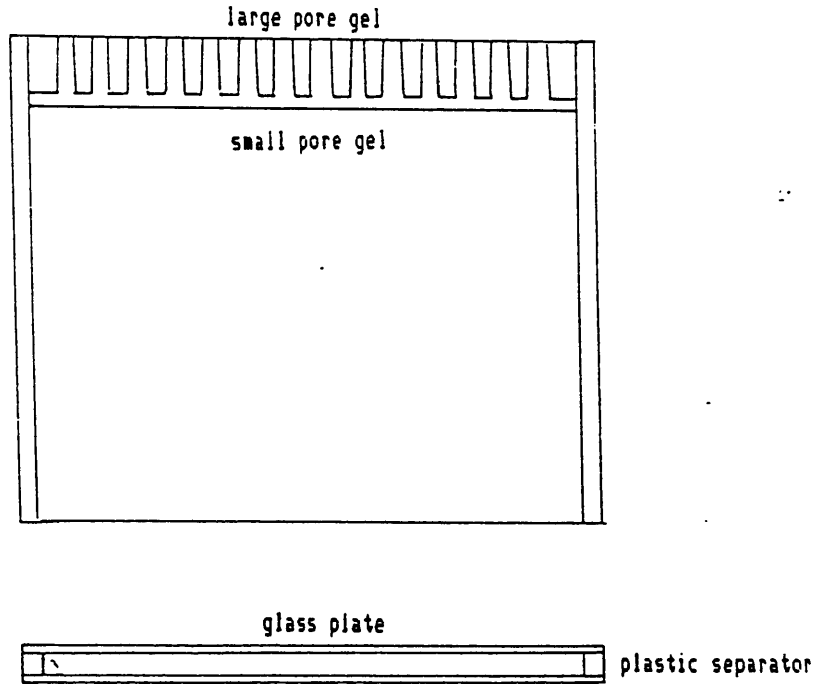


Fig. 9.4 Polyacrylamide gel cassette and gel (side and end view)

usable for up to 2 weeks.

5. Between 1 and 4 gels were placed in the electrophoresis apparatus shown in Fig. 9.5 and the electrode buffer was added. Care was necessary when inserting the cassettes, as the glass plates were easily cracked.
6. A homogenising device of a similar design to that used by Loxdale et al. (1983) was used to prepare the specimens. It consisted of of two perspex blocks, the bottom piece having regularly spaced, cylindrical wells (3 x 8mm) cut into it. The top piece had round-tipped metal rods arranged so that one fitted directly into the middle of each well. The movement of one block against the other created a pestle-and-mortar effect. The lower block was placed on ice and 10-15ul of homogenising solution was added with a micropipette to each well. The quantity of homogenising fluid was decreased with larger specimens. A single specimen was added to each well and then macerated using the upper block. Each well was numbered and the sample recorded. Each homogenate was inoculated into a well in the large pore gel using a microsyringe with a new tip for each homogenate.
7. One-dimensional electrophoresis was preformed at 5-10°C at a constant 150V, 100mA, using conventional vertical slab equipment (Fig. 9.5). The buffer was drip-circulated between the cathode and the anode chambers. The equipment was left to run for 2 hours, during which time the stains were prepared.
8. After the run, the power was switched off and the cassettes were carefully removed from the apparatus. Each gel was released from the cassette and placed into a labelled staining tray with the appropriate stain in the dark. After staining, the gels were fixed in 7% acetic acid for 5 minutes. The acetic acid was then decanted and replaced with fresh acetic acid for an hour before the gels were photographed if required.

Staining

This staining precedure was used for both the starch and polyacrylamide gels. The stains were prepared and filtered just before they were used. The gel slices were left in plastic trays containing the stain in the dark. The slices were left for 30 minutes in the esterase stain and for an hour in the other stains. After an hour, a stain activator (PMS) was added to all stains except the esterase stain. The stains were replaced with fixative

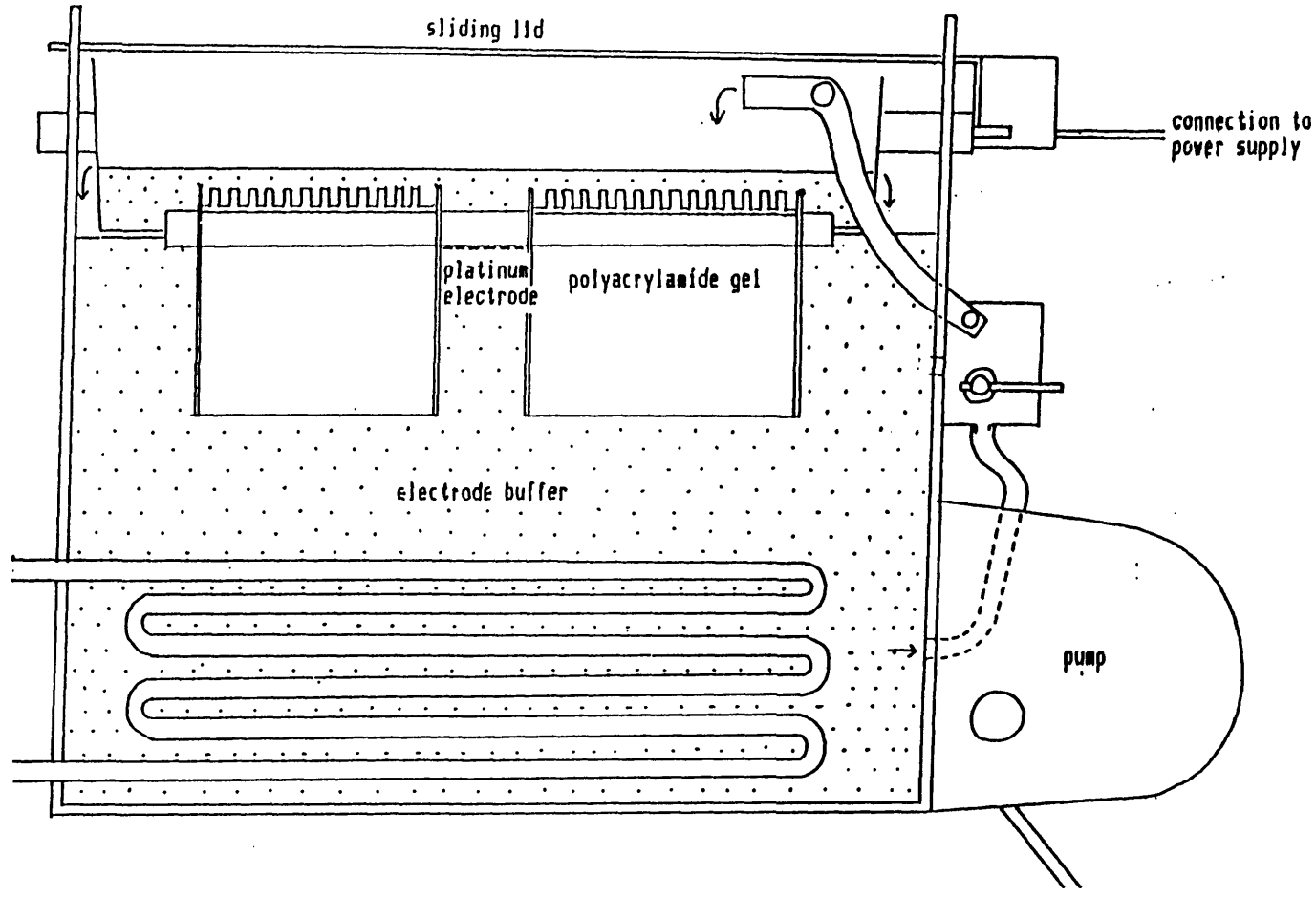


Fig. 9.5 Vertical polyacrylamide gel electrophoresis apparatus (side view)

after the isozyme bands had appeared.

The quantities given in the Table 9.5 for the stains are for each section of gel and can be multiplied as required.

9.3 Results

Four of the enzymes stained gave consistent staining although resolution of bands was poor for all except the esterases. The other enzymes that produced banding patterns were trehalase, phosphoglucomutase and phosphoglucose isomerase. Both the starch and polyacrylamide gels produced the same band resolution.

Description of banding patterns

Esterase (Fig. 9.6)

Specimens of P. vitis and of P. regalis both produced two or three-banded, black-staining esterase patterns with variation of definition and staining intensity between bands. All the samples of P. vitis analysed, from different localities and host-plant species, produced similar esterase isoenzyme patterns. P. regalis produced the first (slowest) esterase band which was the most intense. The second and third esterase bands of P. regalis approximately coincided with the first and second esterase bands of P. vitis. These two bands may be homologous between P. vitis and P. regalis.

Parasitized specimens of P. vitis produced very different esterase patterns to unparasitised specimens from the same sample. Parasitized specimens produced two different patterns, one was three-banded and the other was two-banded. In the three-banded pattern, the slowest band was diffuse, intense and black. The second and third bands appeared to correspond to the second and third esterase bands of the host scale insect. In the two-banded pattern, the slowest band was also diffuse and intensely black. The three bands for the host scale insect were also diffuse.

Specimens of C. hesperidum produced four- or five-banded, faint black staining patterns which were very different to the patterns produced by the Pulvinaria species.

Phosphoglucomutase

P. regalis produced a diffuse three-banded, purple-staining pattern; P.

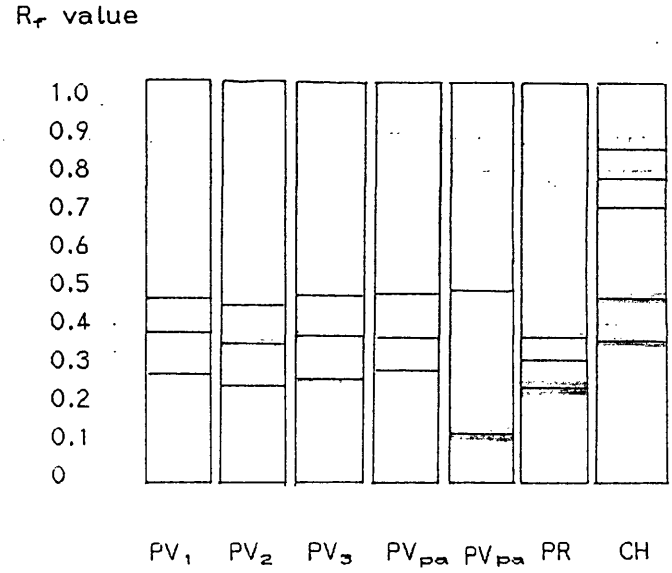


Fig. 9.6 Zymogram of characteristic esterase isoenzyme mobility patterns found in British samples of Pulvinaria vitis, P. regalis and Coccus hesperidum

Key:
PV_1 = P. vitis from Betula, Wisley, Surrey. $n = 10$
PV_2 = P. vitis from Crataegus, Upper Richmond Road, London. $n = 12$
PV_3 = P. vitis from Ribes, Ealing, London. $n = 8$
PV_{pa} = P. vitis from Crataegus, parasitized $n = 5$
PR = P. regalis from Tilia, Cromwell Road, London. $n = 9$
CH = C. hesperidum from Ilex, Addison Cresent, London. $n = 5$

vitis produced a two-banded and C. hesperidum a single band. The fastest phosphoglucomutase isoenzyme appeared to be homologous between all three species. A second band appeared to be homologous between the two Pulvinaria species and P. regalis had a third band which was the slowest.

Trehalase

P. vitis produced a large very diffuse, intense purple staining pattern although there was considerable variation in intensity between individual specimens. P. regalis produced an intense diffuse stain near the origin which may have simply been a result of body pigments. C. hesperidum occasionally produced a faint single-banded pattern.

Phosphoglucose isomerase

P. regalis produced a diffuse one-two banded pattern. P. vitis produced a slower diffuse one-banded pattern. C. hesperidum produced no pattern.

Alkaline phosphatase

P. regalis produced intense staining at the base line which was probably due to body pigments. Some specimens of P. vitis produced a very faint single purple band. C. hesperidum produced no pattern.

9.4 Discussion

Four of the enzyme systems tested, trehalase, phosphoglucomutase, phosphoglucose isomerase and esterase, gave relatively consistent staining with the latter system producing the clearest band resolution. The results show that some isoenzyme systems appear to be more suitable for taxonomy than others due to greater differences in interspecific mobility; for example, esterase and phosphoglucomutase.

No consistent differences were found in the electrophoretic data between the different British populations of Pulvinaria vitis (L.). There was no evidence for the existence of more than one taxa within P. vitis in Britain, although there was considerable variation in enzyme-isoenzyme band definition and staining intensity among specimens within each population. Trehalase was found to be particularly variable among individuals within each population of P. vitis. Interspecific variation in band definition and stain intensity was also found for P. regalis and C. hesperidum. Alstad and

Corbin (1990) found genetic differentiation in three polymorphic enzyme systems over extraordinarily short distances in a population of blackleaf scale, Nuculaspis californica (Coleman), infesting Ponderosa pine trees. Significant genetic differences were even found between demes on different twigs within individual host trees. The electrophoretic methods, used by Alstad and Corbin, were more sensitive and the sample sizes of scale significantly larger than the present study so the results are not directly comparable.

P. vitis, P. regalis and C. hesperidum can be distinguished from their banding patterns and there was greater similarity between the two Pulvinaria species than between the Pulvinaria species and C. hesperidum. Both of the Pulvinaria species stained for more enzymes systems than C. hesperidum. There was close similarity in the number of isoenzyme bands and their mobility between P. regalis and P. vitis, suggesting homology between the isoenzymes which may result from homologous loci.

Parasitism strongly influenced the enzyme-isoenzyme banding patterns of P. vitis. The effect of the parasitoid on the banding patterns of the host and the banding patterns of the parasitoid need to be determined in order that differences in banding patterns between populations can be properly interpreted. Field-collected specimens often contain hymenopterous parasitoids or fungal pathogens that can produce bands which may confuse the host allozyme pattern.

The number of samples of P. vitis in this present study was small and all were derived from a relatively small geographical area compared to the the whole range of the P. vitis complex; however, now that the esterase banding pattern is known for the complex in Britain, it can be compared to the banding pattern produced by specimens belonging to the complex from other parts of its geographical range to study relationships between populations.

10 Summary and conclusion

Summary

1. The number of biological species in the Pulvinaria vitis (L.) complex in Britain and how each species may be recognised were previously unknown. Consequently, there was considerable confusion in the literature concerning the biology and taxonomy of the nominal species within the complex.
2. British field-collected specimens show considerable continuous morphological variation which is, in part, associated with host-plant species and locality. The greatest variation occurs with enumerate characters which are strongly correlated with the size of the insect. Specimens of the P. vitis complex from other geographical areas fit into the range of morphological variation found in British specimens.

   Isometric growth occurs, but there is little allometric growth.
3. Parasitism by Coccophagus lycimnia (Walker) and Metaphycus melanostomatus Timberlake was not found to significantly influence morphological variation or to cause sterility in adult female P. vitis.
4. Field-collected specimens group according to the host-plant species from which they are collected using principal components analyses. The groups were confirmed with canonical variates analyses. The host-plant species were shown experimentally to significantly influence morphological variation of P. vitis. The characters which show the greatest variation were the numbers of pores, ducts and setae which have traditionally been used as diagnostic characters.

   The morphological variation of P. vitis is also due to genetic variation between demes as well as environmental conditions. The characters which show genetically-induced variation are similar to the characters which show environmentally-induced variation.
5. The morphology of P. regalis Canard and P. vitis seen under scanning electron microscopy is similar, but they differ in fine morphology. Spiracular pores produce short C-shaped curls of wax that fill the spiracular furrows allowing ventilation. Multilocular pores also produce short curls of wax which coat the eggs. The tubular ducts produce wax filaments that form the ovisac. The anal ring produces a mass of wax

filaments that form a sheath around the anal-ring setae which helps prevent self contamination with honeydew. The marginal and spiracular setae are also sheathed with wax which may help with adhesion, protection and sensory capability.

6. The life cycle of P. vitis in Britain is considerably more flexible than had previously been reported. The life cycle varies with environmental conditions such as temperature and host-plant species. This flexibility explains the discrepencies in the life cycle reported in the literature. A second generation is possible in a single year under suitable environmental conditions. The rate of development and sex ratio appear to vary with host-plant species. The highest mortality occurs between egg hatch and first instar establishment on the host. The sexual function of the adult males is questioned.

7. The most important natural enemies are hymenopterous parasitoids; the most common parasitoid of P. vitis in the London area is Coccophagus lycimnia (Walker). Two other parasitoid species of P. vitis are recorded for the first time to Britain; Coccophagus semicircularis (Förster) and Metaphycus melanus Sugonjaev.

8. The karyotype for somatic-cell nuclei of P. vitis in Britain is 2n = 16 chromosomes. The complex in Britain uses three different chromosome systems: diploid arrhenotoky, deuterotoky and thelotoky. Some populations use all three chromosome systems whereas some populations appear to be obligate thelytokous.

   P. vitis contains yeast-like endosymbionts in adipose tissue surrounding the testicles.

9. P. vitis, P. regalis and Coccus hesperidum L. can be distinguished from each other by their enzyme-isoenzyme banding patterns. Four enzyme systems produced results for P. vitis; trehalase, phosphoglucomutase, phosphoglucose isomerase and esterase. Esterase produced the most consistent and clearest resolution. Greater similarity was found in the banding patterns between P. regalis and P. vitis than between the Pulvinaria species and Coccus hesperidum. There was great similarity in the number of bands and their mobility between P. regalis and P. vitis suggesting homologous enzymes and isozymes possibly coded from homologous loci. Parasitism was found to greatly influence the banding pattern.

Conclusion

In conclusion, there is a single plastic species, P. vitis (L.), in Britain which exhibits considerable morphological, ecological, cytological, enzyme-isoenzyme and genetic variation. The morphological characters that are used in the taxonomy of the P. vitis complex show considerable plasticity varying with both host-plant species and deme. The other nominal species of Linnaeus (P. betulae, P. oxyacanthae and P. carpini) are considered to be synonyms of P. vitis as proposed by Newstead (1903). Much of the morphological and ecological variation is environmentally induced.

The great variability of the P. vitis complex has enabled it to become established throughout the temporate regions. Specimens belonging to the P. vitis complex from other geographical areas examined during this study were found to fit into the morphological range of P. vitis from Britain; however, there is ecological and cytogenetic evidence for distinct 'races' of P. vitis in different geographical areas.

Future work

A neotype for P. vitis needs to be designated and described. A neotype is not designated in this present study, as a thesis is not considered a suitable publication. A neotype for P. vitis will be formally designated by the present author in a subsequent paper. However, a morphological description and illustration of P. vitis from Britain is given in Appendix 10.1. Holotype or other type material avaliable, of the numerous taxonomic species in the P. vitis complex, need to be compared with the designated neotype of P. vitis, in order to see if they can be morphologically distinguished. It would also be useful to designate neotypes for the other nominal species of Linnaeus which can then be synonymised with the neotype of P. vitis. This would avoid any confusion in the future and ensure that new names were used for new species identified subsequently.

Electrophoresis is currently one of the best methods of studying variation in biology and would be the most useful technique to study 'races' and/or biotypes of the P. vitis complex. However, a proper understanding of the taxonomic and phylogenetic relationships of different populations can only be achieved by adopting a multidisciplinary approach, bringing together avaliable information on morphology, host plants, life cycles, and karyotype,

and taking these into account when interpreting the results of electrophoretic data.

There are many unresolved complexes of species in the Coccidae which require clarification, such as the Parthenolecanium corni (Bouché) group in Europe. The methods and results of this investigation should be useful to subsequent researches who are investigating these complexes.

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Appendix 1.1
The numbers of indigenous and exotic species of scale insect (Coccoidea) recorded in Britain

Family		Indigenous	Exotic	Total
Ortheziidae	Ensign scales	5		5
Margaridae	Giant scales	2	3	4
Pseudococcidae	Mealybugs	26	14	40
Coccidae	Soft scales	25	23	48
Aclerdidae	Flat grass scales	1	1	2
Phoenicococcidae			1	2
Kermesidae	Gall-like scales	2		2
Eriococcidae	Felt scales	12	4	16
Asterolecaniidae	Pit scales	4	1	5
Diaspididae	Armoured scales	12	45	57
		89	92	181

Main references: Kloet & Hincks, 1964; Williams, 1962: 1985a; the collection at The Natural History Museum, London.

Appendix 1.2
A checklist and keys to the British Coccidae

Checklist of British Coccidae

There are 48 species of Coccidae belonging to 21 genera recorded in Britain; 25 species are probably indigenous and 23 species are of exotic origin (Kosztarab & Kozár, 1988). Four of the exotic species are naturalized and well established outdoors; 2 are common and widespread in greenhouses and others are sporadically abundant. The majority of the exotic species however, are only rarely found, in greenhouses or on imported plants and plant produce. A species of Poaspis has recently been reported by F. Kozár, collected on Gramineae outside Brighton, England, but its identity is uncertain. Pulvinaria hydrangeae Steinweden and P. phaiae Lull are recorded here for the first time from Britain.

Indigenous species

1.	Eriopeltis festucae (Fonscolombe)	cottony grass scale
2.	Eriopeltis stammeri Schmutterer	Stammer's cottony scale
3.	Eriopeltis varleyi Manawadu	
4.	Eulecanium ciliatum (Douglas)	ciliate oak scale
5.	Eulecanium douglasi (Šulc)	currant soft scale
6.	Eulecanium franconicum Lindinger	heather soft scale
7.	Eulecanium tiliae (Linnaeus)	nut scale
8.	Exaeretopus formiceticola Newstead	
9.	Lecanopsis formicarum Newstead	Newstead's soft scale
10.	Lichtensia viburni Signoret	viburnum soft scale
11.	Luzulaspis dactylis Green	Green's soft scale
12.	Luzulaspis frontalis Green	long-headed soft scale
13.	Luzulaspis luzulae (Dufour)	woodrush scale
14.	Luzulaspis scotica Green	Scottish soft scale
15.	Palaeolecanium bituberculatum (Targioni Tozzetti)	bituberculate scale
16.	Parafairmairia gracilis Green	elongate soft scale
17.	Parthenolecanium corni (Bouché)	brown scale, European fruit lecanium
18.	Parthenolecanium fletcheri (Cockerell)	fletcher scale
19.	Parthenolecanium persicae (Fabricus)	European peach scale
20.	Parthenolecanium pomeranicum (Kawecki)	yew scale
21.	Parthenolecanium rufulum (Cockerell)	oak soft scale
22.	Physokermes piceae (Schrank)	spruce bud scale
23.	Poaspis sp.	
24.	Pulvinaria vitis (Linnaeus)	woolly/cottony vine scale
25.	Vittacoccus longicornis (Green)	long-horned scale

Exotic species * = naturalised outdoors in Britain.

26.	Ceroplastes cerifera (Fabricius)	Indian wax scale	
27.	Ceroplastes cistudiformis Cockerell	tortoise wax scale	
28.	Ceroplastes japonica Green		
29.	Ceroplastes rubens Maskell	red wax scale	
30.	Ceroplastes rusci (Linnaeus)		
31.	Coccus hesperidum Linnaeus	brown soft scale	*
32.	Coccus longulus Douglas	long brown scale	
33.	Coccus pseudohesperidum (Cockerell)	orchid soft scale	
34.	Coccus viridis Green	green scale	
35.	Eucalymnatus tessellatus (Signoret)	tessellated scale	
36.	Kilifia acuminata (Signoret)	acuminate scale	
37.	Parasaissetia nigra (Nietner)	nigra scale	
38.	Protopulvinaria pyriformis (Cockerell)	pyriform scale	
39.	Pulvinaria floccifera (Westwood)	cottony camellia scale	*
40.	Pulvinaria horii (Kuwana)		
41.	Pulvinaria hydrangeae Steinweden	cottony hydrangea scale	
42.	Pulvinaria mesembryanthemi (Vallot)	iceplant scale	*
43.	Pulvinaria phaiae Lull	cottony orchid scale	
44.	Pulvinaria psidii (Kuwana)	green shield scale	
45.	Pulvinaria regalis Canard	horse-chestnut scale	*
46.	Saissetia coffeae (Walker)	hemispherical scale	
47.	Saissetia oleae (Olivier)	black scale	
48.	Vinsonia stellifera (Westwood)	stellate scale	

Key to the genera of the British Coccidae
(Based on slide-mounted adult females)

1. Anal opercula, spiracular setae and marginal setae absent; rare, on Picea spp. only ..... Physokermes
   Anal opercula and marginal setae present; on plants other than Picea spp. 2
2. Anal opercula about 4 times as long as wide, body shape pyriform .. .. Protopulvinaria
   Anal opercula less than 4 times as long as wide, body shape varied 3
3. Second and third pairs of coxae conspicuously larger than the first pair of coxae ..... Kilifia
   Coxae, if present, all about the same size 4
4. Dorsum covered with large, conical, truncate setae; rare, on Gramineae ..... Eriopeltis
   Dorsum without large, conical, truncate setae; on Gramineae and other plants 5
5. Dorsum and venter with submarginal band of quinquelocular pores

(rarely with 3-6 loculi); spiracular setae absent; rare, on Carex spp. only ..... Vittacoccus
Dorsum and venter without submarginal band of quinquelocular pores; spiracular setae present or absent; on Carex and other plants ... 6

6. Spiracular setae numerous, stout, conical, almost hemispherical or bullet-shaped; derm around anal plates sclerotized or anal opercula on a sclerotized process 7
Spiracular setae usually 0-3 in each group, spine-like or bullet-shaped, occasionally undifferentiated from marginal setae; derm around anal opercula membranous or sclerotized, anal process absent 8

7. One pair of long setae between antennal bases; dorsum with triangular triocular pores and also often with quinquelocular pores; covered in a thick layer of oily wax, not star-shaped .... Ceroplastes
More than 1 pair of long setae between antennal bases, dorsum with simple pores; wax secretion star-shaped with 6 or 7 radiating arms ..... Vinsonia

8. Anal opercula each with a large discal seta; dorsal derm with polygonal reticulations or cell-like oval areas, membranous or heavily sclerotized, dorsal setae conical ..... Saissetia
Anal opercula without discal seta; dorsum with or without polygonal reticulations or cell-like oval areas, dorsal setae varied 9

9. Dorsum strongly sclerotized and polygonal or divided into plate-like areas 10
Dorsum membranous or sclerotized, neither polygonal or with plate-like areas 11

10. Dorsum with tessellations forming a mosaic of large polygonal plates giving a 'tortoise-like' appearance; dorsal setae slender, curved; marginal setae slender, simple or bifid ..... Eucalymnatus
Dorsum with tessellations forming a mosaic of small polygonal plates with a 'cell-like' appearance; dorsal setae cylindrical or capitate; marginal setae stout and frimbriate ..... Parasaissetia

11. Ventral submargin without tubular ducts; dorsal setae spine-like, cylindrical, capitate or clavate ..... Coccus
Ventral submargin with numerous tubular ducts, often in a band . 12

12. Single row of chisel-shaped marginal setae, each with a broad, flattened apex ..... Lichtensia
Marginal setae acute or frimbriate, not chisel-shaped 13

13. Legs with tibiotarsal articulatory sclerosis 16
Legs without tibiotarsal articulatory sclerosis 14

14. Medial spiracular seta larger than marginal setae 15
Spiracular setae subequal or shorter than marginal setae; dorsal setae slender or spine-like ..... Eulecanium

15. With a row of submarginal setae ..... (part of) Parthenolecanium

Without a row of submarginal setae ..... Palaeolecanium
16. With 12-20 pairs of submarginal tubercles .... (part of) Parthenolecanium
With less than 12 pairs of submaginal tubercles 17
17. Spiracular setae obviously differentiated from marginal setae, in groups of 2 or 3 19
Single or no spiracular seta differentiated from marginal setae 18
18. Legs and antennae long, anterior tarsi with an infolding of derm in the middle; spiracular seta single or not differentiated from marginal setae; setae fairly long on the dorsum, venter, margin and between antennae; transverse rows of setae on venter of abdomen; on Dactylis glomerata and Nardus stricta only ..... Exaeretopus
Legs and antennae short, stout, anterior tarsi without an infolding of derm; setae short, derm membranous, wrinkled with bands of microspines on abdomenal venter; subterranean on Gramineae .... Lecanopsis
19. Spiracular setae in pairs 20
Spiracular setae in groups of 3 ..... Pulvinaria
20. All coxae enlarged, hind coxae over half the length of trochanter + femur ..... Parafairmairia
Hind coxae less than half the length of trochanter + femur 21
21. With 9-31 interantennal setae; claw digitules broad, apical knob wider than middle of claw; anal ring with 6 setae; on Carex spp. only ..... Luzulaspis
With 35-70 interantennal setae; claw digitules slender, apical knob narrower than middle of claw; anal ring with 8 setae; on Gramineae ..... Poaspis

Genus Ceroplastes Gray, 1828 - The Wax Scales

There are about 157 species described in this genus worldwide (Gill, 1988) of which 5 have been recorded in Britain. All are exotic and only found on imported plants or occasionally in greenhouses. Chinese wax scale, C. sinesis Del Guercio, 1900, is included in the key although it has not yet been recorded in Britain. This is because it is a common, cosmopolitan pest species which is likely to be found in the future.

Key to species of Ceroplastes

1. Spiracular setae forming a single row along the margin between spiracular furrows; inner filament of ventral tubular duct as wide as duct ..... japonica
Spiracular setae not forming a single row along the margin between spiracular furrows (except some specimens of C. rusci); inner filament of ventral tubular ducts not as wide as duct 2
2. Tibiotarsal articulatory scleroses present 3

Tibiotarsal articulatory scleroses absent 5
3. Antennae with 6-7 segments, normally with 7; dorsal pores predominantly triocular ..... sinensis
Antennae with 6-7 segments, normally with 6; dorsal pores predominantly bilocular 4
4. Spiracular setae confined to spiracular furrows ..... cistudiformis
Spiracular setae not confined to spiracular furrows ..... rusci
5. Legs normal; most spiracular setae bullet-shaped ..... cerifera
Legs reduced; most spiracular setae almost hemispherical ..... rubens

Genus Coccus Linnaeus, 1758 - The Brown Soft Scales

There are about 72 species described in this genus worldwide and several rank as some of the most economically important species in the family Coccidae (Gill, 1988; Gill et al., 1977). Four species have been recorded from Britain and all are exotic although C. hesperidum has become naturalised and is well established both indoors and outdoors. The other species are only found on imported plants or occasionally in greenhouses.

Key to species of Coccus

1. Dorsal body setae straight, spine-like, cylindrical, clavate or capitate 2
Dorsal body setae curved, apices pointed or blunt 3
2. Dorsal body setae spine-like, apically pointed ..... hesperidum
Dorsal body setae short, cylindrical to clavate ..... viridis
3. Legs well developed; 8 segmented antennae; Body elongate, oval, often with almost parallel sides; anal plates with subdiscal setae; with 7-8 fringe setae ..... longulus
Body elongate, oval without parallel sides; Legs greatly reduced; 5-6 segmented antennae; anal plates without subdiscal setae; with 4 fringe setae ..... pseudohesperidum

Genus Eriopeltis Signoret, 1782 - The Small Reed or Cottony Grass Scales

There are about 24 species worldwide, most of Palaearctic origin (Gill, 1988; Kosztarab & Kozár, 1988) of which 3 are recorded from Britain (Manawadu, 1986). The species are difficult to separate morphologically since they appear to vary depending on the species of host from which they are collected. There has been much confusion in Europe between E. festucae and E. stammeri.

Key to species of Eriopeltis

1. Marginal setae on frons in 2 to 3 rows; rare, on Brachypodium pinnatumm only ..... varleyi
Marginal setae on frons in a single row 2
2. Marginal setae on each side of the body numbering fewer than 10 and without disc pores associated with their bases ..... stammeri

Marginal setae on each side of the body numbering 30 or more, most with 1-3 disc pores associated with their bases ..... festucae

Genus Eucalymnatus Cockerell, 1901 - The tessellated scales

There are about 15 species described in this genus worldwide which is apparently native to South America (Gill, 1988). Only a single species, E. tessellatus, is recorded from Britain. It is only found on imported plants or occasionally in greenhouses.

Genus Eulecanium Cockerell, 1893 - The Spherical Scales

There are between 36 to 60 species described in this genus worldwide (Gill, 1988; Borchsenius, 1957) of which 4 are native to Britain. E. tiliae is the only common species.

Key to species of Eulecanium

1. Marginal setae of various types; spine-like laterally and filamentous anteriorly and posteriorly ..... tiliae
   Marginal setae uniform, closely set, spine-like 2
2. With quinquelocular pores along dorsal margin of anal cleft; on Ericaceae only ..... franconicum
   Without quinquelocular pores along dorsal margin of anal cleft; not known from Ericaceae 3
3. Usually 1-4 marginal setae present between spiracular setae; spiracular setae about double the width of marginal setae, bullet-shaped, stout or slightly curved ..... douglasi
   No marginal setae present between spiracular setae; spiracular setae about the same width as marginal setae, curved ..... ciliatum

Genus Exaeretopus

There are about 10 species described in this genus worldwide (Kosztarab & Kozár, 1988) of which only E. formiceticola has been recorded from Britain.

Genus Kilifia (Signoret, 1873)

There are 6 species described in this genus worldwide of which only K. acuminata has been recorded from Britain on imported plants. K. acuminata is a polyphagous species distributed throughout the tropics.

Genus Lecanopis Targioni Tozzetti, 1868

There are about 14 species described in this genus worldwide (Kosztarab & Kozár, 1988) of which only L. formicarum has been recorded from Britain. All the species of Lecanopsis are subterranean feeding mainly on grasses and are often attended by ants.

Genus Lichtensia Signoret, 1873

Lichtensia was described as a monotypic genus and the validity of

subsequent generic assignments of species to this genus are uncertain (Kosztarab & Kozár, 1988). Some authors consider it a monotypic Palaearctic genus and the single species, L. viburni, is native to Britain.

Genus Palaeolecanium Šulc, 1908

A monotypic Palaearctic genus. P. bituberculatum is native to Britain.

Genus Parafairmairia Cockerell, 1899

There are about 4 species described in this genus worldwide (Kosztarab & Kozár, 1988) of which only P. gracilis has been recorded in Britain on grasses and Carex spp.

Genus Parasaissetia Takahashi, 1955 - The Nigra Scales

There are 5 species described in this Afrotropical genus of which only P. nigra has been recorded from Britain. P. nigra is a cosmopolitan, polyphagous pest species found in Britain on imported plants or occasionally in greenhouses.

Genus Parthenolecanium Šulc, 1908 - The Lecanium Scales

There are 15 to 20 species described in this genus worldwide (Gill, 1988; Kosztarab & Kozár, 1988) of which 5 are native to Britain. Only P. corni is common, and is an occasional pest of fruit trees and ornamentals.

Key to species of Parthenolecanium

1. With 12-17 pairs of submarginal tubercles; legs with tibiotarsal articulatory sclerosis ..... persicae
   With less than 12 pairs of submarginal tubercles; legs without tibiotarsal articulatory sclerosis 2
2. Marginal setae equal in length, or slightly shorter than lateral spiracular setae; often with less than 10 preopercula pores; on Cupressaceae ..... fletcheri
   Marginal setae definitely shorter than lateral spiracular setae; with more than 10 preopercula pores; on Cupressaceae and other plants . 3
3. Marginal setae relatively few and widely spaced, 3-6 between anterior and posterior spiracular setae ..... pomeranicum
   Marginal setae numerous and closely set, 8 or more between anterior and posterior spiracular setae 3
4. Marginal setae stout, spine-like with blunt apices, most setae about 12-15um long and 2um wide ..... rufulum
   Marginal setae variable, spine-like, slender or broad, blunt or pointed apices, most setae about 16-25um long and 1.5-2um wide corni

Genus Physokermes Targioni-Tozzetti, 1868 - The Conifer-bud Scales

There are about 12 species described in this genus worldwide (Gill, 1988) of which only P. piceae, found on Picea spp., has been recorded from Britain.

Genus Poaspis Koteja

There are 7 species described in this Palaearctic genus (Kosztarab & Kozár, 1988) of which a single unidentified species has been recorded from Britain. They are all grass feeders.

Genus Protopulvinaria Cockerell, 1894 - The Pyriform Scales

There are about 5 species described in this genus worldwide which is regarded as Oriental in origin (Gill, 1988). P. pyriformis has been recorded from Britain which is a polyphagous, tropicopolitan, pest species. It has only been recorded on imported plant produce in Britain.

Genus Pulvinaria Targioni Tozzetti, 1868 - Woolly/Cottony Scales

There are more than 200 species described in this genus of which 8 have been recorded from Britain. Only P. vitis is indigenous although P. regalis has become naturalised to such an extent that it is the most common woolly scale in urban areas in southern Britain. P. floccifera and P. mesembryanthemi are also well established outdoors on camellia and holly, and iceplants, respectively. P. hydrangeae and P. phaiae are recorded here for the first time from Britain. The other species are only rarely recorded on imported plants or plant produce.

Borchsenius (1952, 1953, 1957) subdivided the genus Pulvinaria into 8 genera within the tribe Pulvanariini. If his concepts were followed, P. floccifera (Westwood), P. phaiae Lull, P. psidii (Kuwana) and P. hydrangeae Steinweden would be placed in the genus Chloropulvinaria Borchsenius and P. mesembryanthemi (Vallot) would be placed in the genus Pulvinariella Borchsenius. Borchsenius' generic concepts, however, are not universally accepted by coccidologists because they are based partly on field characteristics and the morphological characters intergrade when considered on a world basis. For these reasons, these species are left in the genus Pulvinaria. The generic position of the species P. horii is uncertain.

Key to species of Pulvinaria

1. Marginal setae fairly long, spine-like, blunt, subequal to spiracular setae ..... mesembryanthemi
   Marginal setae spine-like with acute, expanded or frimbriate apices, shorter than central spiracular setae 2
2. A few or all marginal setae with expanded and fringed apices 3
   Marginal setae with pointed apices, never expanded or fringed 6
3. Marginal setae stout, apices usually strongly expanded, fringed or dentate; spiracles surrounded by a sclerotized oval plate which is occasionally faint and difficult to see ..... psidii
   Marginal setae mostly simple, occasionally frimbriate; spiracles not surrounded by a sclerotized oval plate 4
4. Dorsal submarginal tubercles absent; ventral submarginal tubular duct band continuous anteriorly around head; subdiscal seta present on

each anal operculum ..... hydrangeae
Dorsal submarginal tubercles present; ventral submarginal tubular duct band extending not much further than posterior spiracular furrows; subdiscal setae not present on anal opercula 5

5. Short dorsal tubular ducts present; with 10-20 preopercular pores anterior to anal plates; polyphagous ..... floccifera
Dorsal tubular ducts usually absent; with 60-80 preopercular pores .. .. phaiae

6. Antennae 9 segmented; preopercular pores with thick rims forming a broad medial band from anterior of anal plates to above the mouthparts ..... horii
Antennae 8 segmented; preopercular pores with narrow rims forming a medial band anterior to anal plates, not extending as far as above the mouthparts 7

7. Setae similar to prevulvular setae present on all abdominal segments; 5 long setae at base of each front coxa; multilocular pores each with a bar in central loculus and 8-10 peripheral loculi; submarginal tubercles absent ..... regalis
Only 3 pairs of prevulvular setae; 1-3 long setae at the base of each front coxa; multilocular pores each without a bar in the central loculus and 9-12 peripheral loculi; dorsal submarginal tubercles usually present ..... vitis

Genus Saissetia Deplanche, 1859 - The Black Scales

There are about 60 described species (Gill, 1988) that belong to this genus which is considered native to Africa. Two species have been recorded from Britain and both are cosmopolitan, polyphagous, pest species. They are restricted to indoor and greenhouse plants where they can be serious pests, particularly S. oleae on Ficus spp. and oleander.

Key to species of Saissetia

1. Ventral tubular ducts on submargin of two types; one type with inner filaments as wide or wider than ducts, the other type more normal with slender inner filaments ..... coffeae
Ventral tubular ducts on submargin only of the normal type with slender inner filaments ..... oleae

Genus Vinsonia Signoret

A monotypic genus, V. stellifera is an exotic species which has been recorded on imported greenhouse orchids.

Genus Vittacoccus Borchsenius

Two species have been described from the Palaearctic of which only V. longicornis has been recorded from Britain. It feeds on the leaves of Carex spp. and grasses.

Appendix 1.3
Keys to the European species belonging to the genus Pulvinaria Targioni Tozzetti

Kostarab, M. & Kozár, F. (1988) Scale Insects of Central Europe. Akadémiai Kiadó, Budapest: 456 pp (239).

1. Each spiracular band with more than 77 quinquelocular pores 2
   Each spiracular band with less than 77 quinquelocular pores 3
2. Legs more than 800µm long; labium 133µm long, 142µm wide; 105-370 (normally more than 200) disc pores in each spiracular band; only on poplar, Populus spp. ..... populi
   Legs less than 600µm long; labium 114µm long, 133µm wide; 78-115 disc pores in each spiracular band; polyphagous, feeds on a variety of trees ..... betulae
3. Median spiracular setae 60-84µm long; hind leg ca 850µm long; labium 95µm long, 109µm wide; only on currants, Ribes spp. ..... ribesiae
   Median spiracular setae 90-95µm long; hind leg ca 750µm long; labium 109µm long, 142µm wide; polyphagous, feeds on a variety of trees and shrubs ..... vitis

Danzig, E.M. (1987) Suborder Coccinea - Coccids or mealybugs and scale insects. In Keys to the Insects of the European USSR. Apterygota, Palaeoptera, Hemimetabola. Keys to the fauna of the USSR. Ed. G. Ya. Bei-Bienko. Akademiya Nauk SSSR, Zoologicheskii Institut. 84(1): 830-831.

1 (6). Female large, more than 5.0 long.
2 (3). Each spiracular slit contains 105-210 5-celled glands. Crimea, Northern Caucasus. On branches of poplar ..... populi Sign.
3 (2). Each spiracular groove contains less than 100 5-celled glands, rarely 115 glands.
4 (5). Distance between most marginal hairs shorter than hairs or as long as them. On birch, alder, poplar, Sorbus, etc. .... betulae (L.)
5 (4). Distance between most marginal hairs on sides of body markedly longer than hairs. South. On grapevine ..... vitis (L.)
6 (1). Female small, not more than 4.0 long.
7 (8). Each spiracular groove contains more than 60 5-celled glands. On branches of currant ..... ribesiae Sign.
8 (7). Each spiracular groove contains up to 50 5-celled glands. Caucasus. On thin roots of hawthorn and hornbeam .... terrestris Borchs.

Borchsenius, N.S. (1957) Fauna of the USSR, Insecta, Homoptera, Coccoidea. Coccidae: 493 pp. (228-229). Translated from Russian by D. Williams and C. Malumphy

1 (12). Length of adult female 5mm or greater than 5mm.
2 (11). All claw digitules of equal size.
3 (8). Spiracular furrows contain up to 100, rarely 115 quinquelocular pores.
4 (7). Marginal setae collected into 2 more or less clear rows. In some places they are situated in chequer-board pattern.
5 (6). Intervals between the majority of marginal setae shorter than or equal to the length of the marginal setae ..... betulae (L.)
6 (5). Intervals between the majority of marginal setae in the lateral parts of the body noticeably greater than the length of the marginal setae, some of the intervals exceed the length by 2-3 times ..... vitis (L.)
7 (4). Marginal setae in one row ..... oxyacanthae (L.)
8 (3). Each spiracular furrow contains of more than 150, often 200-370 quinquelocular pores, rarely some of them contain 150.
9 (10). Intervals between the majority of the marginal setae greater than the length of the setae by 1½-2 times; top of the ovisac without longitudinal ridges ..... populi Sign.
10 (9). Intervals between the majority of the marginal setae exceed the length of the setae by 3-5 times; top of ovisac bears small clear longitudinal ridges ..... costata Borchs.
11 (2). Claw digitules are unequal, one being perceptively thicker than the other ..... tremulae Sign.
12 (1). Length of adult female 4mm or less than 4mm.
13 (16). Marginal setae are 1½-2 times longer than the lateral spiracular setae.
14 (15). Marginal setae numerous, being collected into 2 rows. On the lateral parts of the body between the spiracular setae there are approximately 30 setae ..... salicicola Borchs.
15 (14). Few marginal setae, being collected in a single row. On the lateral parts of the body between the spiracular setae there are 4-5 setae ..... kirgisica Borchs.
16 (13). Marginal setae are of approximately the same length as the lateral spiracular setae.
17 (18). Marginal setae collected into 1 row ..... populeti Borchs.
18 (17). Marginal setae collected into 2 rows or are situated in a chequer-board pattern.
19 (22). Central spiracular setae 3-3½ times longer than the lateral ones.
20 (21). Each spiracular furrow contains of more than 60 quinquelocular pores ..... ribesiae Sign.
21 (20). Each spiracular furrow contains of up to 50 quinquelocular pores ..... terrestris Borchs.
22 (19). Central spiracular seta is 2 to 2½ times longer than the lateral ones ..... rhizophila Borchs.

Appendix 1.4
Morphological variation found in a single population of Pulvinaria vitis (L.) collected from grapevine under glass in Bickleigh, Devon, England, 1988.

Length of hind coxa, trochanter, femur, tibia and tarsus 620-890µm, mean 775µm (n = 20)
Length and width of labium 98-129µm x 110-141µm (n = 10)
Length of anterior central spiracular seta 93-116µm (n = 10)
Length of anterior lateral spiracular seta 28-56µm (n = 10)
Length of marginal seta approximately equal to length of lateral spiracular seta
Length of body of teneral female 2.21-4.55mm (n = 10)
Length of lateral marginal setae greater than the interval length between them
Number of spiracular pores in anterior spiracular furrow 51-108 (n = 10)
Marginal setae present in a double row

Many of the specimens collected from the scale population on grapevine do not key out clearly with any of the keys given in Appendix 1.3. Almost all the characters used in the keys show considerably variation, some vary with the maturity of the scale. The keys rely heavily on the host-plant species, however, if only the morphological characters are considered, the first key (Kosztarab & Kozár, 1988) identifies individuals from the scale population as the nominal species, P. betulae, P. ribesiae and P. vitis. The second key (Danzig, 1987) identifies teneral specimens as P. ribesiae and P. terrestris. The body length of post-reproductive females would be longer than the length of teneral females given above. In this case, specimens would key out as P. populi and P. betulae. The third key (Borchsenius, 1957) identifies teneral specimens as P. ribesiae, P. terrestris, P. rhizophila and larger post-reproductive specimens as P. betulae.

Appendix 1.5

The introduction and economic importance of Pulvinaria vitis (L.) in North America

P. vitis was introduced into North America at the end of the nineteenth century and was subsequently described as a new taxon on several different host-plant species and confused with several of the indigenous woolly scales. There has been much uncertainty concerning the systematics of the Pulvinaria species in North America and the complexes involved are still not all resolved satisfactorily today.

When first introduced, P. vitis appears to have been misidentified as Neopulvinaria innumerabilis (Rathvon). Putnam (1880) gave an extensive account of the biology of "N. innumerabilis" on peach in Iowa. Since peach is not a host plant of N. innumerabilis, and for other reasons, Phillips (1963) concluded that Putnam was dealing with P. vitis. Another possibility is that the woolly scale on peach was P. rhois Ehrhorn but little is known of the distribution of P. rhois due to confusion with P. vitis. Three years later, Comstock (1883) recorded P. vitis on grapevine and P. salicis (Bouché) on willow. Comstock also suggested that P. salicis might prove synonymous with N. innumerabilis and that the latter might be synonymous with P. vitis. P. salicis was synonymised with P. vitis by Newstead (1903). Today, N. innumerabilis is easily separated morphologically from P. vitis and has different principal host-plant species.

The first positive record of P. vitis in N. America according to Phillips (1963) was in 1897 when it was described as P. innumerabilis var. occidentalis Cockerell. The original description was poor and unillustrated and gives no precise type data. It was noted that the woolly scale was widespread in western Washington on currant, hawthorn, plum, pear, mountain ash, willow, poplar, gooseberry and alder. P. vitis was therefore, already widespread and well established outdoors in parts of North America by the end of the nineteenth century.

King (1901a) reported P. occidentalis to be a pest of red- and white-currants in Chilliwack, British Columbia in 1899, and suggested that it might prove to be identical to "P. ribesiae" in Europe. Further nominal species of the P. vitis complex were described as P. ehrhorni king, 1901, on willow from Mountain View, California; P. coulteri Cockerell, 1905, on Rosa sp. in Coulter,

Middle State, Colorado and P. occidentalis subalpina Cockerell, 1910, on Betula glandosa in Tolland, Colorado (Gill, 1988: Phillips, 1955: Steinweden, 1946: Williams and Kosztarab, 1972). P. ehrhorni, N. innumerabilis, P. occidentalis and P. vitis were later listed in a world catalogue of Coccidae by Fernald (1903) with extensive references. Several of the references and host-plant species given for N. innumerabilis by Fernald should be referred to P. vitis.

There was a gradual awareness of the problem of misidentifications by Sanders (1909) who described the 'deplorable state of knowledge of this group', which he considered a 'result of injudicious and hasty conclusions, combined with scattered, meagre, unillustrated descriptions'. Sanders synonymised 10 species with P. vitis including P. occidentalis. Unfortunately, he also synonymised N. innumerabilis and its synonyms with P. vitis which were then referred to as P. vitis in the North American literature.

Several papers were published in 1923 referring to P. vitis and its control (Harmon, 1923). It was a widespread pest of gooseberry, quince and pear in Washington (Frank, 1923); and peach in Ontario, Canada where it was misidentified as P. amygdali Cockerell. P. vitis was also reported on hawthorn in New York State (Felt, 1923) and in British Columbia (Ruthmann, 1924).

During most of the first half of the twentieth century, P. vitis and P. rhois were frequently misidentified as P. amygdali. There was also confusion between N. innumerabilis and P. acericola (Walsh & Riley) which were repeatedly misidentified as P. vitis (Britton, 1926: Davis, 1937: Dietz & Morrison, 1921: Forbes, 1907: Frank, 1923: Gillette et al., 1925: Hutchings, 1927).

A severe outbreak of P. vitis (misidentified as P. amygdali) was reported on peach in western New York State (Harmon 1927) and on peach and quince near lake Ontario (Felt, 1925). By 1926, P. vitis was generally distributed in peach orchards throughout Ontario in the economically important Niagara peach belt (Ross & Caesar, 1928). These infestations were confined to an area five to seven miles in width along the south shore of lake Ontario in Niagara, Orleans, Monroe, and May counties which was continuous with areas infested with P. vitis in Ontario in 1925/1926 and between 1946 to 1954 (Phillips, 1963). It was recognised that the identity of the scale on peach

was uncertain, and that it differed from P. amygdali (possibly P. rhois) as known from Georgia and California, and more closely resembled P. vitis from Europe (Parott & Harmon, 1927). As the name P. vitis was already used for N. innumerabilis, the name P. amygdali continued in use for P. vitis.

Steinweden (1946) recognised that the identities of P. rhois and P. amygdali were confused with each other and that N. innumerabilis had been misidentified as P. vitis. P. ehrhorni and P. coulteri were synonymised with P. vitis and it was indicated that P. occidentalis was also probably synonymous with P. vitis. Phillips (1955) concluded that the woolly scale on peach in Ontario was identical to P. vitis in Europe, and distinguished the immature stages of P. vitis from those of N. innumerabilis. Phillips (1958, 1963) later published a detailed study of the life cycle and ecology of P. vitis in Ontario. Although the taxonomic confusion between the N. American woolly scale species had been largely resolved, P. vitis was still being misidentified as N. innumerabilis and P. rhois as P. amygdali, in several publications (McNay & Creelman, 1958; Essig, 1958).

P. ellesmerensis Richards (1964) was originally described from lake Hazen, Ellesmere Island, Canada on Salix arctica. I have examined a paratype of this species deposited in the BMNH collection which differs from the original description in the following ways: the paratype has 8-segmented antennae, not 7-segmented; multilocular pores each with 7-14, mean 10 loculi, not with only 12 loculi; preopercular pores simple, not cribriform; and anal ring with 4 larger pairs of setae, not 3 pairs of setae. The number of antennal segments can vary between specimens within a population of soft scales and the other characters above can be difficult to record on some specimens. The paratype examined also differs from the original illustration in that the ventral submarginal band of tubular ducts do not extend around the head region. The paratype fits into the morphological range of P. vitis. It is possible that the holotype and paratype are different species although both were collected from the same population and no other species of Pulvinaria are known from the area. Richards remarked in the original paper that P. ellesmerensis was most closely related to P. salicicola Borchsenius which belongs to the P. vitis complex. It differed from "P. salicicola" only by the number of marginal and submarginal setae present between the anterior and posterior spiracular setae. The latter character is particularly variable and unreliable in the determination of species.

Richards found all developmental stages overwintering and concluded that it may take up to five years for P. ellesmerensis to complete development. I do not consider finding all developmental stages overwintering to be evidence for development to take any longer than a year. The P. vitis complex has been reported to overwinter in all developmental stages in parts of Russia where it only takes a year to complete development. Richards also reported P. ellesmerensis to be uniparental which is in agreement with reports of P. vitis in other parts of N. America. Richards also considered P. ellesmerensis to be mainly viviparous because he found only a few eggs together with many first instars without egg shells within an ovisac; however, the egg shells are so small and delicate that they are almost impossible to detect amongst the waxy filaments of the ovisac. First instars of P. vitis initially remain in the ovisac after hatching and many become trapped in the waxy filaments and die within the ovisac.

The paratype of P. ellesmerensis at the BMNH can not be separated morphologically from P. vitis but the possible synonymy can not be proposed until the holotype of P. ellesmerensis has been examined.

More recently, "P. vitis" was reported as an ocassional pest of grapevine in California and control measures given (Stafford & Doutt, 1974), however, the identity of the scale is uncertain as it was referred to as Cottony maple scale which is N. innumerabilis, and males were reported. There is still confusion between N. innumerabilis and P. vitis on grapevine. P. vitis is presently known throughout the western United States, New York and Canada. It has only been reported as a significant economic pest on peach in New York State and Ontario.

Appendix 1.6
Systematic list of the host-plant species worldwide of the Pulvinaria vitis (L.) complex

The host-plant list was compiled from the following references: Borchsenius, 1957; Danzig, 1959, 1967, 1986; Kosztarab, 1959; Kosztarab & Kózar, 1988; Lindinger, 1912; Newstead, 1903; Phillips, 1963; Richards, 1964; Schmutterer, 1952 ; Steinweden, 1946. Additional host data was obtained from collections in the depositories listed on page 30, and from personal collecting.

The plant classification follows the system of Mabberly (1987), the species names follow Index Kewensis (1988). Common names are given in parentheses after the scientific names. Synonyms are only included if they were originally recorded as the host species.

Key: ? = doubtful record

Class: DICOTYLEDONAE (MAGNNOLIOPSIDA)
Subclass: HAMAMELIDAE
Order: JUGLANDALES
Family: Juglandaceae
Juglans regia (walnut)
Order: FAGALES
Family: Fagaceae
Fagus slyvatica (beech)[= F. silvatica]
Family: Betulaceae
Alnus glutinosa (alder)
Alnus incana
Alnus maximowiczii
Alnus viridis
Betula ermani
Betula glandula
Betula mandshurica
Betula nana
Betula platyphylla
Betula pendula (silver birch)[= B. alba]
Betula pubescens (downy birch)[= B. alba, B. verrucosa]
Betula tauschii
Carpinus betulus (hornbeam)
Corylus avellana (hazel)
Ostrya carpinifolia (hop hornbeam)
Subclass: DILLENIIDAE
Order: MALVES
Family: Tiliaceae
Tilia europea (lime)
Order: SALICICACEAE
Family: Salicaceae
Populus alba (white popular)[= P. candicans]
Populus canescens (grey popular)

Populus hybrida
Populus nigra var. italica (black popular)
Populus tremula (aspen)
Salix alba (white willow) 'tristis' (weeping willow) var. vitellina
Salix arctica
Salix x-boydii
Salix britenzensis
Salix caprea (great sallow, goat willow)
Salix hastata
Salix herbacea var. Wehrhahrii
Salix hultenii
Salix lanata (woolly willow)
Salix lapponum [= S. helvitica]
Salix nigricans [= S. helvitica]
Salix pentandra
Salix repens (creeping willow)[= S. rosmarinifolia]
Salix reticulata (net-leaved willow)
Salix sachalinensis
Salix viminalis

Subclass: ROSIDAE
Order: ROSAES
Family: Rosaceae

Cotoneaster integerrimus (wild cotoneaster)[= C. integemma]
Cotoneaster microphyllus (small leaved cotoneaster)[=C. microphylla]
Crataegus chlorosarca
Crataegus laevigata (midlands hawthorn)[= C. oxyacantha, C. oxyacanthoides, Mespilus oxyacantha]
Crataegus monogyna (common hawthorn)[= Mespilus monogyna]
Cydonia oblonga (quince)[= C. vulgaris]
Malus sylvestris (crab apple)[= Pyrus malus, Pirus malus]
Mespilus germanica (medlar)
Prunus armeniaca (apricot)
Prunus cerasus var. montmorency (sour cherry)
Prunus damascena (damson)
Prunus domestica (wild plum)
Prunus persica var. nectarina, 'Peregrine' (peach)
Prunus pissardi
Prunus salicina vars. Italian prune, Damsom, First (Japanese plum)
Prunus spinosa (blackthorn, sloe)
Pyracantha auraniaca (pyracantha)
Pyracantha coccinea (firethorn)
Pyrus communis vars. pyraster, sativa (wild pear)[= P. pyraster]
Rosa sp. (rose)
Rubus fructicosa (bramble, blackberry)
Sarothamnus scoparius (broom, gulfweed)[= Cytisus scoparius]
Sorbus aria (common whitebeam)
Sorbus aucuparia (mountain ash, rowan)[= Pyrus aucuparia]
Sorbus commixta

Sorbus torminalis (wild service tree)
Sorbus wilsoniana
Spiraea betulifolia
Spiraea salicifolia (bridewort)
Family: Grossulariaceae
Ribes alpinum (alpine or mountain currant)
Ribes divaricatum 'Worcesterberry' (American gooseberry)
[= Grossularia divaricatum]
Ribes lacustre
Ribes nigrum vars. Balwin, Ben Nevis, Ben Lomond (black currant)
Ribes rubrum (British red currant)[= R. satiuum]
Ribes sachalinensis
Ribes sanguineum (flowering currant)
Ribes sylvestre (red or white currant)
Ribes uva-crispa (gooseberry)[= R. grossularia]
Family: Saxifragaceae
Saxifraga sp.
Family: Hydrangeaceae
Deutzia sp.
Order: FABELES
Family: Leguminosae
Medicago lupulina (black medick)
Robinia pseudoacacia (false acacia, locust tree)
Order: CORNALES
Family: Cornaceae
Cornus sp. (dogwood)
Order: SANTALALES
Family: Loranthaceae
? Loranthus europaeus
Order: CELASTRALES
Family: Celastraceae
Euonymus europaeus (spindle tree)[= E. europea]
Euonymus latifolias (broad leaved spindle)[= E. latifolia]
Euonymus verrucosus (warty-barked spindle)[= E. verrucosa]
Family: Aquifoliaceae
Ilex sp. (holly)
Order: RHAMNALIS
Family: Vitaceae
Vitis lambrusca (fox grape)
Vitis lambruscana
Vitis vinifera vars. black Hamburg, Concord, Agawarm, Precouvé de Maligeri (grape vine)
Order: SAPINDALES
Family: Hippocastanaceae
Aesculus hippocastanum (horse-chestnut)
Family: Aceraceae
Acer platanoides (Norway maple)
Acer pseudoplatanus (sycamore)

Subclass: ASTERIDAE
  Order: SCROPHULARIALES
    Family: Oleaceae
      Fraxinus excelsior (ash)
      Fraxinus mandschurica

Appendix 1.7
Summary table of the host plants of the Pulvinaria vitis (L.) complex

Class	Subclass	Order	Family	Genera.Species
Dicotyledonae	Hamamelidae	? Juglandales	? Juglandaceae	? 1. 1
		Fagales	? Fagaceae	? 1. 1
			Betulaceae	5.15
	Dilleniidae	Malves	Tiliaceae	1. 1
		Salicaes	Salicaceae	3.21
	Rosidae	Rosaes	Rosaceae	14.29
			Grossulariaceae	1. 9
			Saxifragaceae	1. 1
			Hydrageaceae	1. 1
		Fabeles	Leguminosae	2. 2
		Cornales	Cornaceae	1. 1
		? Santalales	? Loranthaceae	? 1. 1
		Celastrales	Celastraceae	1. 3
		Rhamnalis	Vitaceae	1. 3
		Sapindales	Hippocastanaceae	1. 1
			Aceraceae	? 1. 2
	Asteridae	Scrophariales	Oleaceae	1. 2
Total	4	12	18	36.94

Appendix 2.1
Recently published variations in the morphological terminology for Coccidae

Term used in this report	Recently published alternatives
DORSUM	
Dorsal setae	dorsal body setae, spines or spinules
Submarginal tubercle	submarginal duct tubercle, two-ringed duct or inverted duct tubercle
Disc pore	dorsal, circular or small disc pore
Preopercular pore	paraopercular, discoidal or large disc pore
Filamentous duct	Micro or minute duct or bilocular pore
Anal opercular	anal plate
Anterior margin	anterolateral or cephalolateral or anterior lateral margin
Posterior margin	posterolateral, caudolateral or posterior lateral margin
Anal fold	anogenital invagination
Ventral thickening	ventral inner margin, ventral or slender ridge or innerside thickening sclerosis
Fringe setae	anal invagination or anogenital setae
Apical setae	apical and subapical setae
Subapical setae	slender ridge or inner margin setae
Subdiscal seta	apical seta removed from apex
MARGIN	
Marginal setae	marginal spines or hairs
Submarginal setae	paramarginal setae
Spiracular setae	spiracular or stigmatic spines or setae
VENTRUM	
Ventral setae	ventral hairs or ventral body setae
Prevulvular setae	pregenital segment setae
Tarsal digitule	ungual digitule
Multilocular pores	disc pores
Spiracular pores	quinquelocular, pentalocular, circular, disc, or stigmatic pores
Spiracular furrow	spiracular depression or stigmatic/stigmal furrow or channel
Tubular duct	cylindrical or cleistostomatic duct
Duct	outer duct or ductlet
Inner filament	inner duct or ductlet
Microduct	obscure bordered pore or tubular microduct

Appendix 2.2
Morphological description of the Genus Pulvinaria Targioni Tozzetti

Field description

Postreproductive female Pulvinaria are distinguished in the field by a characteristic elongate, white, waxy ovisac produced behind the body. The female scale is pushed forward and upwards as the ovisac is produced so that she rests at an angle to the surface of the host. The old female usually remains attached to the anterior of the ovisac after oviposition is completed but detaches in some species. An exception to this are three species of Pulvinaria from Australia, P. dodonaeae Maskell, P. flavicans Maskell and P. salicorniae Froggatt, which produce an ovisac that partially or completely covers the dorsum. The generic placement of the three latter species is, however, uncertain (T. Qin, pers. comm.).

Description of slide-mounted adult female

Dorsum. Derm varies from lightly sclerotised to heavily sclerotised with age. Mounted females oval to circular. Anal opercula with varying numbers of apical and ventral inner ridge setae. Fringe setae number also variable. Well-developed anal ring with numerous wax pores and 4 pairs of setae, rarely 3 or 5 pairs. Submarginal tubercles present or absent. Preopercular usually developed forming a band anterior to the anal opercula. Dorsal pores and body setae variable. Tubular and filamentous ducts present or absent.

Margin. Marginal setae usually spine-like with variable apices. Spiracular setae usually present and differentiated from marginal setae. Usually in groups of 3 with the central seta longest.

Venter. Antennae 6-9 segmented, usually 8. Legs well developed with tibiotarsal articulatory sclerosis (except P. ericicola McConnell). Spiracular pores mostly with 5 peripheral loculi forming narrow bands in the spiracular furrows. Multilocular pores in vulvar area and in transverse rows on abdomenal segments. Tubular ducts numerous, usually two types; one type with thick inner filaments in 2-3 forms and a second type with slender inner filaments. The tubular ducts are found in a submarginal band and in the median area of the venter. Microducts numerous, scattered but concentrated in submargin. Body and submarginal setae variable.

Appendix 3.1
Collection data for field-collected specimens of the P. vitis complex examined in the present study, arranged according to country and host plant

Key: Dep. = Depository; Ref. = Reference number on slide label; SN = number of slides with identical collection data
Abbreviations used for the depositories are those given in Section 3.2.1.

Dep.	Ref.	SN	Host	Locality	Collector	Date
UNITED KINGDOM						
NHM	CM34	6	Alnus glutinosa	RHS Gdns, Wisley, Surrey	Malumphy	8.12.87
NHM	CM102	1	Alnus glutinosa	RHS Gdns, Wisley, Surrey	Malumphy	. 6.88
NHM	CM307	4	Alnus glutinosa	Tonbridge, Kent	Malumphy	18. 5.89
MNHN		4	Alnus glutinosa	Uxbridge, Middlesex	Waterston	. 5.30
NHM		2	Alnus glutinosa	Uxbridge, Middlesex	Waterston	. 5.30
NHM	BM53/754	2	Alnus glutinosa	Midhurst, Sussex	Hall	26. 5.46
NHM	BM58/578	1	Alnus glutinosa	Midhurst, Sussex	Hall	26. 5.46
NHM	BM81/539	3	Alnus glutinosa	Parsons P., Oxford	Varley	12. 5.56
NHM	BM81/539	1	Alnus glutinosa	Silwood Park, Berks	Boratynski	23. 5.50
NHM	CM39	16	Betula pendula	Wisley, Woking, Surrey	Malumphy	17. 2.88
NHM	CM319	1	Betula pendula	Wisley, Woking, Surrey	Malumphy	31. 5.89
NHM	CM222	2	Betula pendula	St. Albans, Hertshire	Malumphy	24. 1.89
NHM	CM14	1	Betula pendula	Putney Heath, London	Malumphy	8.11.87
NHM	BM45/121	1	Betula pendula	Camberly, Surrey	Newstead	6. 6.17
NHM	BM40/180	1	Betula	Camberly, Surrey	Green	22. 9.17
NHM	1074	3	Betula pendula	Bagshot, Surrey	Boratynski	10. 5.56
NHM	BM65/1	1	Betula pubescens	Kew Gargens, London	Eastop	4.11.62
NHM	BM45/121	1	Betula	Delamere, Cheshire	Newstead	1890
NHM		2	Betula	Richmond Park, London	Laing	16. 6.29
NHM	BM40/180	2	Betula	Llangammarch, Brecon	Green	. 9.25
NHM	BM19/113	1	Crataegus laevigata	Farnborough, Hampshire	Marriot	22. 5.19
NHM		2	Crataegus laevigata	England	Marriot	29.10.14
NHM	CM30	2	Crataegus monogyna	Regents Park, London	Malumphy	28.11.87
NHM	CM32	5	Crataegus monogyna	Regents Park, London	Malumphy	2.12.87
NHM	CM38	38	Crataegus monogyna	Putney, London	Malumphy	12. 1.88
NHM	CM40	14	Crataegus monogyna	Putney, London	Malumphy	15. 2.88
NHM	CM110	3	Crataegus monogyna	Putney, London	Malumphy	3. 5.88
NHM	CM294	2	Crataegus monogyna	Wallyndon, Surrey	Malumphy	9. 4.89
NHM	CM264	1	Crataegus monogyna	Hackbridge, London	Malumphy	5. 3.89
NHM	CM313	2	Crataegus monogyna	Lewisham, London SE13	Malumphy	21. 5.89
NHM	CM10	9	Crataegus monogyna	W. Kensington, London	Cox	26.10.87
NHM	CM26	11	Crataegus monogyna	W. Kensington, London	Malumphy	16.11.87
NHM	CM201	4	Crataegus monogyna	W. Kensington, London	Malumphy	20.10.88

Appendix 3.1 continued

NHM	CM206	2	Crataegus monogyna	Wareham, Dorset	Malumphy	1. 9.88
NHM	CM233	1	Crataegus monogyna	London W11	Malumphy	22.10.88
NHM	BM45/121	1	Crataegus	Lewisham, London	Newstead	.6.1891
NHM	BM69/1	1	Crataegus	Cookham	Williams	15. 7.69
NHM	BM40/	2	Crataegus	Selham, Sussex	Green	.6.1899
NHM	CIEA12126	1	Crataegus	England	Tremewan	. 5.80
NHM	BM81/539	1	Crataegus	Silwood Park, Berks	Boratynski	7. 6.51
NHM	BM45/121	2	Euonymus	Hastings, Sussex	Butterfield	. 6.18
NHM	BM63/3	1	Populus candicans	Kew Gardens, London	Eastop	. 8.62
NHM		1	Populus	East Malling, Kent	Massee	. 6.29
NHM	CM111	2	Prunus persicae	Misterton, Notts.	Malumphy	6. 5.88
NHM	CM112	16	Prunus persicae	Misterton, Notts.	Malumphy	8. 5.88
NHM	CM212	2	Prunus persicae	Misterton, Notts.	Malumphy	2.11.88
NHM	CM114	7	Prunus persicae	Windsor Rd., Berks	Malumphy	16. 5.88
NHM	CIEA3826	1	Prunus persicae	Evesham		. 5.70
NHM	BM45/121	2	Prunus persicae	Rootsford, Cheshire	Newstead	1891
NHM	BM40/180	1	Prunus persicae	Wisley, Surrey		. 7.24
NHM	BM81/539	2	Prunus persicae	Silwood Park, Berks	Boratynski	
NHM	BM45/12196	3	Prunus persicae	England	Douglas	
NHM	1628	1	Prunus spinosa	Sunningdale, Berks	Martin	3.10.76
NHM	CM42	19	Pyracantha coccinea	West Yeul, Epsom	Malumphy	26. 1.88
NHM	CM228	2	Pyracantha coccinea	Bridgewood, Surrey	Malumphy	20.10.88
NHM	CM230	2	Pyracantha coccinea	Billet Rd., London E17	Malumphy	24.10.88
NHM	CM180	9	Pyracantha coccinea	Nailsworth, Glouc.	Jackson	24. 6.88
NHM	BM45/121	1	Pyracantha coccinea	England	Newstead	
NHM	P84229	1	Pyracantha	Suffolk		. 7.84
NHM	BM69/1	2	Pyracantha	Malvern, Worcester		
NHM	BM58/578	3	Pyracantha	Wisley, Surrey	Wilson	23. 5.44
NHM	BM45/121	1	Pyracantha	England	Newstead	
NHM		2	Pyracantha	Ealing, London	Banner	. 6.84
NHM	CM218	3	Ribes nigrum	St. Albans, Herts	Malumphy	24. 1.89
NHM	CM207	7	Ribes nigrum	Swanage, Dorset	Malumphy	1.11.88
NHM	CM187	2	Ribes nigrum	Cardiff, S. Glamorgan	Malumphy	19. 7.88
NHM	CM205	1	Ribes nigrum	Salisbury, Wiltshire	Malumphy	31.10.88
NHM	CM312	2	Ribes nigrum	Hamdle, Hants	Malumphy	19. 5.89
NHM	CIE16688	1	Ribes nigrum	Coventry	Buck	24. 1.85
NHM	CM37	7	Ribes sanguineum	W. Kensington, London	Cox	14. 1.88
NHM	CM41	6	Ribes sanguineum	W. Kensington, London	Malumphy	27. 1.88
NHM	CM120	1	Ribes sanguineum	W. Kensington, London	Malumphy	26. 5.88
NHM	CM118	4	Ribes sanguineum	S. Kensington, London	Malumphy	25. 5.88
NHM	CM200	1	Ribes sanguineum	S. Kensington, London	Malumphy	20.10.88
NHM	CM160	3	Ribes sanguineum	Ealing, London	Malumphy	1.10.88
NHM	CM292	2	Ribes sanguineum	Ealing, London	Malumphy	9. 4.89

Appendix 3.1 continued

NHM	CM291	2	Ribes sanguineum	Ealing, London	Malumphy	19.11.88
NHM	CM171	3	Ribes sanguineum	Cardiff, S. Glamorgan	Malumphy	.10.88
NHM	CM209	1	Ribes sanguineum	Swanage, Dorset	Malumphy	1. 9.88
NHM	CM231	2	Ribes sanguineum	Vauxhall, London	Malumphy	24.10.88
NHM	CM229	5	Ribes sanguineum	Billet Rd., London E17	Malumphy	24.10.88
NHM	BM45/121	1	Ribes sanguineum	Chester	Newstead	. 9.07
NHN		1	Ribes sanguineum	Chester	Newstead	.10.07
NHM	CIEA13448	1	Ribes sanguineum	Plumrose Hill, Lydney	Evans	. .81
NHM	CIE1034a	4	Ribes sanguineum	Penhurst, E. Sussex	Hall	26.10.45
NHM	CM220	3	Ribes sylvestre	St. Albans, Herts	Malumphy	24. 1.89
NHM	CM205	2	Ribes sylvestre	Salisbury, Wiltshire	Malumphy	31.10.88
NHM	BM53/754	1	Ribes sylvestre	Stamford Brook, London	Uffon	. 5.49
NHM	BM68/27	1	Ribes sylvestre	Kirkwell, Orkney		. 8.66
NHM	CM208	3	Ribes uva-crispa	Swanage, Dorset	Malumphy	1.11.88
NHM	P85284	1	Ribes uva-crispa	Plawsworth, Durham		. 7.85
NHM	BM81/539	5	Ribes uva-crispa	Silwood Park, Berks	Boratynski	26. 5.55
NHM	BM81/539	2	Ribes uva-crispa	Silwood Park, Berks	Boratynski	2. 6.55
NHM	BM67/1	2	Ribes	Kent	Fitchew	21. 5.49
NHM	BM40/180	1	Ribes	Scotland	Morrison	
NHM	BM68/27	1	Salix britenzensis	Aldershot, Hants	Knight	. 4.68
NHM	BM62/3	9	Salix caprea	Camberly, Surrey	Green	23.11.21
NHM	CM204	6	Salix hastata	Westbury, Bristol, Avon	Malumphy	31.10.88
NHM	CM203	2	Salix helvatica	Westbury, Bristol, Avon	Malumphy	31.10.88
NHM	CM173	4	Salix herbacea	Westbury, Bristol, Avon	Malumphy	12. 7.88
NHM	CM215	6	Salix	Roath, Cardiff, S. Glam.	Malumphy	22. 1.89
NHM	3	2	Salix	Wisley, Surrey	Harris	30. 5.44
NHM	CIE5464	2	Salix	Thursley Common, Surrey	Sankey	. 6.60
NHM	BM45/121	2	Salix	Cheshire	Rorestead	1891
NHM	VC104	3	Salix	Papadil, Rhum	Strayan	2. 9.69
NHM	BM40/180	2	Salix	Camberly, Surrey	Green	24.11.21
NHM	BM45/121	2	Salix	Cheshire	Newstead	13.3.1891
NHM	BM45/121	1	Salix		Newstead	
NHM	BM45/121	4	Saxifraga	Prestatyn, Wales	Newstead	3. 8.18
NHM	BM40/180	1	Saxifraga	Prestatyn, Wales	Newstead	3. 8.18
NHM	CM194	2	Sorbus aria	Richmond, London	Malumphy	16.10.88
NHM	CM195	1	Sorbus aria	Richmond, London	Malumphy	.10.88
NHM	CM196	2	Sorbus aucuparia	Richmond, London	Malumphy	16.10.88
NHM	BM68/27	2	Sorbus aucuparia	London WC1	Brooks	27. 7.68
NHM	BM81/539	1	Sorbus aucuparia	Silwood Park, Berks	Boratynski	
NHM	BM81/539	3	Sorbus aucuparia	Silwood Park, Berks	Boratynski	27. 5.49
NHM	BM40/180	1	Sorbus aucuparia	Southhampton, Hants	Killington	19. 4.30
NHM	CIEA8824	1	Spiraea	England	Adams	. .76
NHM	CM36	68	Vitis vinifera	Bickleigh, Devon	Malumphy	12.12.87

Appendix 3.1 continued

NHM	CM107	1	Vitis vinifera	Bickleigh, Devon	Malumphy	21. 5.88
NHM	CM117	1	Vitis vinifera	Bickleigh, Devon	Malumphy	21. 5.88
NHM	CM105	2	Vitis vinifera	St. Edmonds, Suffolk	Redman	12. 7.88
NHM	CM183	2	Vitis vinifera	St. Edmonds, Suffolk	Redman	12. 7.88
NHM	CM214	5	Vitis vinifera	St. Edmonds, Suffolk	Malumphy	6.11.88
NHM	CM190	2	Vitis vinifera	London	French	. 7.88
NHM	P81/157	1	Vitis vinifera	London		
NHM		1	Vitis vinifera	London SW19	Harris	7.10.66
NHM		3	Vitis vinifera	Bristol, Avon	Pipping	. 4.66
NHM	CIEA5499	1	Vitis vinifera	London SW3	Dewhurst	1. 5.72
NHM	BM45/121	1	Vitis vinifera	Stonehouse, Devon	Biquell	1898
NHM		1	Vitis vinifera	Waltham Cross, Herts	Geo.M.	19.11.30
NHM		1	Vitis vinifera	England	Douglas	
NHM	9/76	3		Shepherds Bush, London	Mensah	. 5.76
NHM		2		England	Davies	11. 7.37
NHM	BM45/121	1		Cheshire	Newstead	13.7.1891
CANADA						
NHM	BM58578	4	Prunus persicae	Ontario	Vineland	1. 6.54
NHM	CIE2888	1	Prunus persicae	Ontario, Niagra penn.	Putnam	12. 1.49
CDFA	49C108	1	Prunus persicae	Ontario, Niagra penn.	Putman	12. 1.49
DENMARK						
NHM	8/3	1	Betula	Bornholm, Finnedalen	Hansen	1.10.18
NHM	740	1	Fagus		Heiberg	
NHM	746	1	Ribes uva-crispa	Roskilde	Bardenfleth	9.6.11
NHM	745	1	Vitis vinifera	Copenhagen	Rosmassen	28.4.1893
FRANCE						
MNHN	101341	1	Vitis vinifera		Viky	16.10.04
MNHN	101342	1	Vitis vinifera		Viky	16.10.04
MNHN	101352	1	Vitis vinifera	Fouway		
MNHN	10136	1	Vitis vinifera	Bordeaux		9. 6.12
MNHN	52332368	1	Vitis vinifera	Mechtras (Kabylie)	Lepipe	5. 5.27
MNHN	52333391	1	Vitis vinifera	Mechtras (Kabylie)	Lepipe	5. 5.27
MNHN	52334369	1	Vitis vinifera	Mechtras (Kabylie)	Lepipe	5. 5.27
MNHN	52335	1	Vitis vinifera	Mechtras (Kabylie)	Lepipe	5. 5.27
MNHN	52336	1	Vitis vinifera	Mechtras (Kabylie)	Lepipe	. 4.27
MNHN	52337	1	Vitis vinifera	Mechtras (Kabylie)	Lepipe	. 4.27
MNHN	101301	1	Vitis vinifera	Provins	Kunckel	. .00
MNHN	101302	1	Vitis vinifera	Provins	Kunckel	. .00
MNHN	101303	1	Vitis vinifera	Provins	Kunckel	. .00
MNHN	98181	1	Vitis vinifera	Tournenhen	Croin	. 5.84

Appendix 3.1 continued

MNHN	98182	1	Vitis vinifera	Tournenhen	Croin	.	5.84
MNHN	101331	1	Vitis vinifera		Youne	9.	6.06
MNHN	101332	1	Vitis vinifera		Youne	9.	2.06
MNHN	101291	1	Vitis vinifera	Environs de Paris	Poisson	.	.06
MNHN	101292	1	Vitis vinifera	Environs de Paris	Poisson	.	.06
MNHN	10129	1	Vitis vinifera	Environs de Paris	Poisson	.	.06
MNHN	10129	1	Vitis vinifera	Environs de Paris	Poisson	.	.06
MNHN	10129	1	Vitis vinifera	Environs de Paris	Poisson	.	.06
MNHN	10129	1	Vitis vinifera	Environs de Paris	Poisson	.	.06
NHM	CM100	10	Vitis vinifera		Gavroche	28.	3.88
MNHN	101351	1	Vitis vinifera			2.	4.07
GERMANY							
NHM	BM145121	1		Hommerstein Weinberg	Rh.		1850
NHM	9153	2		Carlsruhe			
HUNGARY							
IPP		1	Acer platanoides	Budapest	Kosztarab		
IPP		1	Acer pseudoplatanus	Gellérthegy, Budapest	Kosztarab		
IPP		1	Aesculus hippocastanum	Lipotmezö, Budapest	Balás		
IPP		1	Aesculus hippocastanum	Bemrakpart, Budapest		11.	6.76
IPP		1	Aesculus hippocastanum	Keszthely	Kosztarab		
IPP		1	Aesculus hippocastanum	Pécs	Kosztarab		
IPP		1	Alnus glutinosa	Zalaszentgrot	Kosztarab		
IPP		1	Carpinus betulus	Arboretum, Budapest	Kosztarab	.	5.50
IPP		1	Corylus avellana	Kamaraerdö, Budaörs	Babbnigg		
IPP		1	Corylus avellana	Arboretum, Budapest	Jenser	3.	5.51
IPP		1	Crataegus laevigata	Kamaraerdö, Budaörs	Kosztarab		
IPP		1	Crataegus laevigata	Veszprém		1.	7.69
IPP		1	Crataegus monogyna	Kamaraerdö, Budaörs	Kosztarab		
IPP		1	Crataegus monogyna	Arboretum, Budapest	Kosztarab		
IPP		1	Crataegus monogyna	Pécs	Balás		
IPP		1	Crataegus monogyna	Sima	Kosztarab		
IPP		1	Crataegus monogyna	Tatatouároskert	Balás		
IPP	358	1	Euonymus europaeus	Bakonygyepestöl		14.	6.75
IPP	426	1	Euonymus europaeus	Hegyoldal, Budapest	Kosztarab	6.	7.75
IPP	467	1	Euonymus europaeus	Misinatetö, Pécs	Kosztarab	9.	7.75
IPP		1	Euonymus europaeus	Zalaszentgrot	Kosztarab		
IPP		1	Euonymus verrucosus	Látohegy, Budapest	Balás		
IPP		1	Euonymus verrucosus	Gonc	Kosztarab		
IPP		1	Euonymus verrucosus	Pécs	Kosztarab		
NHM	H181	1	Euonymus verrucosa	Tokaji-hegy	Malumphy	16.	6.89
IPP		1	Populus canescens	Budatétény	Kosztarab		

Appendix 3.1 continued

IPP		1	Populus nigra	Kelebia	Erdos	
IPP		1	Prunus domestica	Kámon	Kosztarab	
IPP		1	Pyrus communis	Látohegy, Budapest	Kosztarab	
IPP		1	Pyrus communis	Kamaraerdö, Budaörs	Kosztarab	
IPP		1	Ribes rubrum	Budatétény	Domokos	
IPP	840	1	Ribes rubrum	Ördögárok, Budapest	Kozár	7. 4.78
NHM	H104	1	Ribes	Str. Örtogārol, Budapest	Malumphy	11. 4.89
IPP		1	Salix alba	Budapest	Balás	
IPP	405	1	Salix pentandra	Bátorliget	Kosztarab	28.6.75
IPP		1	Salix repens	Ágasegyháza		1. 5.52
IPP		1	Salix repens	Kiskunság National Park		. 5.
IZAS	862	3	Salix rosmarinifolia	Fülophiza	Kozár	16. 6.78
IPP		1	Salix	Kiskunság National Park	Tabdi	
IPP		1	Salix	Arboretum, Budapest	Kosztarab	
IPP		1	Sorbus torminalis	Farkasvölgy, Budapest	Balás	
IZAS	1438c	1	Vitis vinifera	Balatonfüred	Kozár	25. 3.81
IPP		1	Vitis vinifera	Szigetcsép	Kurus	
IZAS	144c	1	Vitis vinifera	Aszòfö	Santha	. 3.81
IRELAND						
NHM		1	Ribes nigrum	Wicklow, Delgany	Hammond	14. 6.78
JORDAN						
NHM		1	Vitis vinifera		Mustaba	. 5.86
NEW ZEALAND						
NZAC	783	1	Populus nigra	Christchurch	Baker	23.11.71
NZAC	84.355	2	Prunus persicae	Earnscleugh	Kemp	6.11.51
NZAC	84.350	1	Prunus armeniaca	Earnscleugh	Kemp	2.11.51
NZAC	84.352	2	Prunus armeniaca	Alaxandra	Kemp	7. 8.52
NZAC	84.353	2	Prunus armeniaca	Earnscleugh	Kemp	15.12.50
NZAC	84.354	1	Pyrus communis	Roxborough, Coal Creek	Mayo	22. 8.52
NZAC	84.356	3	Ribes	Christchurch	May	2.11.51
NZAC	2188/1981	2	Vitis vinifera	P. Suensson Gore	Williams	9. 2.81
NZAC	84.353	2	Vitis vinifera	Earnscleugh	Kemp	15.12.50
PORTUGAL						
NHM	BM45121	1		Villa Nova da Gaya	Morgan	30.9.1891
UNITED STATES OF AMERICA						
CDFA		1	Betula	Oakland, California	Allen	19. 3.56
CDFA	52E27	1	Crataegus	San Gabriel, California	Daniels	30. 4.52
CDFA	62D23/48	3	Crataegus	Hayward, California	Sweight	18. 4.62

Appendix 3.1 continued

CDFA	85E28/14	2	Crataegus	Stockton, California	Moretto	24. 5.85
CDFA	83C14/25	3	Crataegus	Tulelake, California	Greenbank	11.3.83
CDFA	62E438	4	Populus nigra	W. Petaluma, California	Lange	2. 5.67
NHM	BM40180	1	Prunus persicae	Geneva, New York	Harmon	
CDFA		1	Prunus persicae	Berkeley, California	Laing	20. 4.45
CDFA		3	Prunus pissardi	Burlingame, California	Lauder	28. 7.58
CDFA		3	Pyracantha	Monroe, Oregon	Westcott	4.11.80
CDFA		5	Pyracantha	Monroe, Oregon	Long	7.11.80
CDFA		4	Pyrus communis	Camino, California	Wilson	1. 4.59
CDFA		3	Salix	Petaluma, California	Kobayashi	3.4.86
CDFA		2	Salix	Petaluma, California	Kobayashi	20.5.85
NHM	BM58229	1	Vitis vinifera	Washington DC	Hall	5. 6.22
NHM	BM58229	1	Ribes sanguineum	Seattle, Washington	Smith	15.11.43
NHM	BM58229	1	Salix	Hooper, Utah	Knowlton	29. 5.29
NHM	BM24457	1		West Point, Washington	Cockerell	

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IZAS	118.73	1	Alnus glutinosa	Kaliningrad region	Ivanova	29. 5.73
IZAS	213.50	1	Alnus	Estonia		3. 6.35
IZAS	124.73	1	Betula pubescens	Kaliningrad region	Ivanova	7. 7.73
IZAS	38.72	1	Betula pubescens	Kaliningrad region	Ivanova	1. 9.72
IZAS	133.73	1	Betula pubescens	Kaliningrad region	Ivanova	11. 7.73
IZAS	58.70	2	Betula ermanii	Sakhalin	Danzig	20. 8.67
IZAS	220.71	1	Betula ermanii	Sakhalin	Ivanova	3. 7.69
IZAS	250.71	1	Betula tauschii	Sakhalin		9. 6.69
IZAS	19	1	Betula	Leningrad region	Danzig	27. 6.57
IZAS	365.50	1	Betula	Novgorod		26. 6.11
IZAS	28	1	Betula	Leningrad region	Danzig	22. 6.57
IZAS	212.82	1	Betula	Teberda, Caucasus		16. 7.82
IZAS	4	3	Betula	Moscow	Drozdovsky	9.6.58
IZAS	5	1	Betula	Moscow	Drozdovsky	8.7.60
IZAS		1	Betula	Moscow	Drozdovsky	30.5.58
IZAS		1	Betula	Moscow	Drozdovsky	14.5.60
IZAS	787.55	1	Carpinus betulus			31. 2.11
IZAS		1	? Corylus	Moscow	Drozdovsky	3.6.58
IZAS	19.65	1	Crataegus	Moldaria		1. 8.63
IZAS	42.52	2	Cydonia	Baku, Izerbaijan		3. 5.32
IZAS	16.65	2	Cydonia	Moldaria		4. 6.64
IZAS	6.67	1	? Cydonia			20. 3.67
IZAS	21.63	6	Fraxinus mandschurica	Southern Primorye	Danzig	12. 6.62
IZAS	148.67	1	Mespilus	Izerbaijan		9. 6.67
IZAS	49.70	1	Populus hybrida			11. 6.69

Appendix 3.1 continued

IZAS 139.65	2	Populus	Kishenev, Moldaria		14. 5.65
IZAS 18.65	1	Populus	Kishenev, Moldaria		. 6.64
IZAS N1	1	Populus	Erevan, Armenia		20. 6.66
IZAS N2	1	Populus	Erevan, Armenia		25. 8.63
NHM 38/74	6	Populus	Kizakhstan, Semipalatinsk		. 6.50
IZAS 254.53	1	Populus		Danzig	17. 8.53
IZAS 57.57	3	Populus		Danzig	19. 6.75
IZAS 3.64	1	Populus	Southern Primorye	Danzig	8. 6.63
IZAS 354.50	2	Ribes alpinum	Caucasus, Georgia		10. 7.29
IZAS 233.71	1	Ribes nigrum			8. 6.70
IZAS 1617b	3	Ribes rubrum	Bugulma	Kozár	5. 8.81
IZAS N91	1	Ribes sachalinensis		Ivanova	3. 9.69
IZAS 207.82	1	Ribes	Teberda, Caucasus	Danzig	24. 6.82
IZAS 176.68	1	Ribes	Minsk (White Russia)		23. 5.68
IZAS 7/8	2	Ribes	Moscow	Drozdovsky	18.6.58
IZAS 9	1	Ribes	Moscow	Drozdovsky	18.7.58
IZAS 10	1	Ribes	Moscow	Drozdovsky	5. 8.58
IZAS 11	1	Ribes	Moscow	Drozdovsky	5. 8.58
IZAS 12	1	Ribes	Moscow	Drozdovsky	18.8.58
IZAS 249.53	1	Ribes			7. 8.53
IZAS 60.66	1	Ribes			15. 9.66
IZAS 79.73	1	Ribes		Danzig	12. 9.68
IZAS 179.70	1	Salix hulterii	Sakhalin	Ivanova	25. 6.68
IZAS 6	1	Salix	Moscow	Drozdovsky	29.5.58
IZAS 190.82	1	Salix	Terberda, Caucasus	Danzig	9. 7.82
IZAS N3	1	Salix	Izerbaijan		11. 6.70
IZAS 27	1	Salix	Leningrad region	Danzig	22. 7.57
IZAS 52.52	1	Salix	Carpathian mountains		
IZAS 15.72	1	Salix		Danzig	22. 6.71
IZAS 219.82	2	Salix			15. 7.56
IZAS 122.73	1	Sorbus aucuparia	Kalningrad region	Ivanova	22. 5.73
IZAS 107.73	1	Sorbus aucuparia	Kalningrad region	Ivanova	22. 5.73
IZAS 152.67	1	Sorbus commixta	Sakhalin		12. 6.67
IZAS 30.68	2	Spiraea betulifolia	Southern Sakhalin Park	Danzig	20. 8.67
IZAS N88	1	Spiraea	Sakhalin	Ivanova	4. 8.69
IZAS 140.65	1	Vitis vinifera	Kishenev		31. 8.65
IZAS 17.65	1	Vitis vinifera	Kishenev		7. 2.65
IZAS 102.72	1	Vitis vinifera		Kozár	5. 4.72
IZAS 56.52	1	Vitis vinifera			19. 6.52
IZAS 84.54	1	Vitis vinifera			. .54
IZAS 52.73	1			Borchsenius	27. 5.56
IZAS 23.56	1				9. 8.55
IZAS 31.73	1				17. 6.73

Appendix 3.2

Host-plant species and localities of collection sites and reported infestations of Pulvinaria vitis (L.) in Britain during 1987-90

Key: Ref. No. = reference numbers to slide-mounted specimens
Population density indicates the number of adult scales found on each plant; 1 = 1-5; 2 = 6-15; 3 = 16-30; 4 = 30+ scales
* = populations used in the host transfer experiments

Ref. No.	Host species	Locality	Population density
34	Alnus glutinosa	Royal Horticultural Society's Garden, Wisley, Woking, Surrey	1
307		Golden Stable Wood Reserve, North Rith Farm, Tonbridge, Kent	1
14	Betula pendula	Putney Heath, Putney, London	1
222		Saint Albans, Hertshire	1
412		Silwood Park, Ascot, Berkshire SL5	1
413		St. Vincent's Hospital, East Cote, Pinner, Middlesex HA5	1-2
39/317/319/321		Wisley, Woking, Surrey	1
-		Llanishen, Cardiff, South Glamorgan, WALES	1
233	Crataegus monogyna	Pottery Lane, Notting Hill, London W11	4
316		Putney Hill, Putney, London	1
10/26/201		Queens Club Gardens, West Kensington, London	2
38/97/110/315		Dryburgh Rd and Upper Richmond Rd, Richmond, London	3-4
30/32		Outer Circle, Regents Park, London	1
-		Hammersmith bridge, Hammersmith, London	2
264/294		Nature Reserve, Hackbridge, Wallyndon, Surrey	1
410		Addison Rd, Kensington, London	2
411		Kensington High St, Kensington, London	2
312		Lee High Rd, Lewisham, London SE13	1
414		St. Vincent's Hospital, East Cote, Pinner, Middlesex HA5	1
-	Populus sp.	Royal Botanical Gardens, Kew, London, Surrey TW9	1
409	Prunus damascena	Kidbrooke Gardens, Blackheath, London	1
114	Prunus persicae var. Nectarine var. Peregrine	Ferhill Park, Windsor Rd, Windsor, Berkshire SL4	3

111/112/212		Station St, Misterton, Doncaster, Nottinghamshire DN10	2
230	Pyracantha coccinea	Billet Rd, Walthamstow, London E17	4
42		Hartford Rd, East Ewell, West Epsom, London	3
-		Burghill Rd, Westbury-on-Tryn, Bristol, Avon B510	2
-		St. James Avenue, Hampton Hill, Middlesex TW12	3
228		Bridgewood Road, Worcester Park, Surrey	2
-	Ribes nigrum	Jenks Avenue, Knives, Nr. Stourbridge, West Midlands DY7	3
218		Ruscombe Drive, Park St, St. Albans, Hertshire AL2	3
207		Queens Rd, Swanage, Dorset	2
312		Sylvan Lane, The copse, Hamble, Hants. SO3	2
187	var. Ben Nevis	Hanon Rd, Roath, Cardiff, South Glamorgan, WALES	3
229	Ribes sanguineum	Billet Rd, Walthamstow, London E17	3
118		Kensington Palace Gardens, Kensington, London	1
209		Queens Rd, Swanage, Dorset	1
121/171		Llanishen, Cardiff, South Glamorgan, CF4	3
231/291		Mayfield, Ealing, London	3
10/37/41/120/202		Queens Club Gardens, West Kensington, London	2
231		Trigon Road, Vauxhall, London	4
-		Martindale Rd, Woking, Surrey GU21	3
219	Ribes slyvestre	Ruscombe Drive, Park St, St. Albans, Hertshire AL2	3
208	Ribes uva-crispa	Queens Rd, Swanage, Dorset	3
-	Salix canata	Roman Road, Steyning, Sussex	2
204	Salix hastata	Passage Rd, Westbury-on-Tryn, Bristol, Avon	1
203	Salix helvitica	Passage Rd, Westbury-on-Tryn, Bristol, Avon	1
	Salix herbacea		
173	var. Wehrhahrii	Passage Rd., Westbury-on-Tryn, Bristol, Avon	1
215	Salix sp.	Lake Road East, Roath, Cardiff, South Glamorgan, WALES	4
194	Sorbus aria	Castle Gate, Richmond, London	1
195	Sorbus aucuparia	Lower Mortlake Rd, Richmond, London	1
333		Llanishen, Cardiff, South Glamorgan, WALES	1
-	Vitis vinifera	Morningside Road, Worcester Park, Surrey KT4	1
-		Lower Rd, Bookham, Leatherhead, Surrey KT23	1
112	var. Blackhamburg	Station Street, Misterton, Doncaster DN10	1
36/107/117		Yearlstone Manor, Chilton, Bickleigh, Tiverton, Devon EX16	4
105/214		Great Livermere, Bury St. Edmunds, Suffolk IP3	4

Appendix 3.3 Thirty-character morphological data, recorded from field-collected specimens.

Key: Ref. = reference number to collection data given in Appendix 3.1; Pos. = position of specimen on slide where l = left, c = centre, r = right, t = top and b = bottom.

Full 30 character data set

The numbers for the morphological characters are those used in Section 3.2.5

Ref	Pos.	Morphological character																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	29	14	15	16	17	18	19	20	30	21	22	23	24	25	26	27	28
CM34a		246	181	159	89	381	250	165	232	303	10	7	95	46	766	80	11	13	0	6	0	1	7	74	59	28	41	53	9	11	2
BM81/539a	l	287	184	177	100	390	303	226	200	335	7	8	98	41	597	75	9	11	0	6	0	1	7	54	68	30	52	65	12	3	7
BM81/539a	r	497	414	187	100	381	284	161	277	335	7	10	108	43	827	60	9	13	0	7	0	0	7	51	76	36	53	72	8	11	2
BM81/539b		570	466	187	94	435	310	200	271	326	10	8	89	48	831	80	7	12	0	3	0	0	3	59	80	54	75	81	20	10	6
BM81/539c		577	406	155	84	390	245	161	232	284	7	8	103	46	817	80	9	13	0	5	0	1	6	41	61	32	42	42	14	7	7
CM307d	l	432	281	156	78	373	249	171	218	264	9	9	98	41	826	80	8	10	4	8	2	2	16	60	42	22	29	40	10	1	1
CM307d	r	368	265	124	78	319	249	171	187	233	7	6	96	27	803	80	10	7	4	7	1	1	13	67	59	30	30	36	12	0	0
CM307a	r	327	205	135	74	327	249	163	179	233	6	7	89	37	768	75	9	6	3	3	3	2	11	55	32	18	24	25	1	0	2
CM307c	tl	457	338	148	93	334	264	163	202	241	5	6	80	32	838	80	9	10	2	6	0	0	8	50	34	26	34	50	5	0	2
CM307c	bl	378	239	140	78	373	241	160	194	257	7	5	100	44	755	80	10	9	5	8	0	2	15	34	41	12	23	31	0	0	0
CM39a		288	234	190	103	432	297	206	271	355	11	8	103	48	763	80	8	17	1	6	0	0	7	41	71	35	47	50	9	2	1
CM39b		316	268	203	113	450	358	226	319	416	10	10	102	59	767	70	9	27	0	9	0	4	13	88	168	40	55	80	6	13	0
CM39c		315	269	190	97	445	290	213	271	319	8	8	89	57	850	80	7	18	3	9	0	1	13	50	69	51	60	58	2	1	1
CM39d		319	206	181	97	393	297	200	274	335	10	10	98	44	818	80	9	21	0	10	0	1	11	66	75	42	55	62	5	27	3
CM39e		302	215	210	103	413	310	213	277	348	11	11	98	51	796	80	8	16	0	6	0	3	9	74	93	46	51	70	16	17	1
CM39f		353	255	200	110	477	310	248	310	400	14	10	105	72	775	80	9	26	5	4	1	1	11	81	106	52	65	72	23	28	2
CM39g		353	245	194	100	503	316	239	284	355	14	8	110	61	800	80	9	19	3	9	0	1	13	68	102	39	41	66	12	37	4
CM39i		296	202	197	105	451	310	219	264	332	13	9	94	57	795	80	9	13	6	5	3	2	16	66	100	31	53	63	13	6	2
CM39j		287	201	194	90	442	284	206	261	335	10	11	100	53	779	80	9	13	7	9	0	1	17	70	86	31	48	59	2	7	1
CM39l		323	237	194	100	435	306	232	271	355	11	7	98	59	763	80	9	25	3	7	0	3	13	37	68	37	49	59	8	5	4
CM14		248	179	181	97	397	277	210	226	297	8	10	85	35	761	80	9	11	1	8	0	3	12	83	72	22	40	55	5	5	5
9342		274	184	181	84	413	284	174	264	329	8	7	57	26	802	80	10	11	1	6	0	2	9	73	69	30	49	66	5	4	2
BM81/539		755	658	203	116	468	310	239	270	350	6	7	62	30	771	80	9	18	2	7	0	2	11	73	119	45	65	68	65	44	0
Laing a		539	564	200	97	471	329	210	303	355	5	6	58	29	854	80	9	16	2	8	0	1	11	55	81	30	42	61	22	8	9
Laing b		477	429	213	106	497	310	214	316	355	8	7	66	25	890	80	8	24	0	11	0	0	11	52	129	30	32	39	30	50	4
CM222a	tl	206	134	174	84	368	277	194	226	303	7	10	95	36	746	75	9	9	3	7	3	5	18	60	53	25	30	42	0	1	0
CM222a	tr	239	166	194	100	406	297	200	277	342	11	8	93	51	810	80	9	10	6	8	2	4	20	67	83	43	58	73	13	12	0
CM222a	bl	229	149	187	97	419	200	284	252	316	10	7	98	43	797	80	9	11	4	7	0	2	13	65	70	33	40	53	19	35	0
CM222a	br	286	177	180	103	413	303	190	258	323	8	8	89	43	799	80	9	10	8	8	2	2	20	88	70	34	56	77	9	10	1
CM222b	tl	194	119	168	90	381	310	180	226	297	8	11	82	38	760	80	9	11	2	9	1	1	13	90	51	31	39	53	10	11	3
CM222b	bl	268	176	180	110	406	316	200	258	342	8	7	90	57	754	75	9	14	4	9	0	1	14	60	67	37	47	72	12	28	0
CM222b	br	245	161	194	103	400	303	200	258	342	10	8	87	51	754	80	9	12	0	9	1	2	12	58	68	34	54	57	9	19	1
CM222c	l	233	168	180	110	426	303	200	245	342	8	10	95	30	716	80	9	12	0	8	1	1	10	79	75	18	37	45	8	25	1
CM222c	r	268	182	180	103	413	258	194	252	329	8	8	89	46	766	80	8	10	4	6	1	1	12	54	69	37	56	62	5	4	0
CM38b		215	139	168	90	352	252	213	239	310	10	10	84	30	771	80	9	12	1	6	0	1	8	48	64	21	34	42	6	7	5
CM38c		192	94	155	90	390	258	206	219	264	10	8	82	18	830	80	8	15	1	7	0	1	9	31	71	21	34	51	1	8	2
CM38h		212	156	155	87	387	252	200	226	284	10	10	89	34	796	80	9	11	0	5	0	3	8	49	61	25	42	41	3	1	8
CM38j		226	150	158	90	368	258	216	206	277	8	8	95	28	744	80	8	10	0	6	1	1	8	40	94	18	34	40	5	9	6
CM38k		223	147	155	87	381	258	194	226	310	7	11	82	25	729	80	9	10	3	6	0	1	10	27	60	27	35	43	2	15	7
CM40a		261	203	164	77	390	287	226	232	300	10	10	100	30	773	80	8	14	1	6	3	3	13	23	63	21	37	38	4	8	8
CM40b		197	135	155	97	368	258	197	206	258	10	10	87	33	798	70	9	10	1	4	0	1	6	25	46	21	34	44	6	14	1
CM40d	l	211	157	152	74	329	261	177	200	252	8	7	77	21	794	70	9	13	1	6	0	1	8	18	61	22	24	42	6	3	6
CM40d	c	205	154	155	90	361	245	181	200	264	5	7	67	25	758	75	8	12	1	4	3	4	12	27	44	28	32	38	1	2	7
CM40d	r	235	161	148	77	368	239	167	200	258	4	8	90	30	775	75	9	13	0	7	0	0	7	36	48	22	33	41	5	6	1

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CM30a		260	152	171	90	391	290	239	271	323	11	11	113	44	839	80	9	17	2	7	0	0	9	42	115	24	41	47	0	5	12
CM32a		260	163	187	97	445	290	219	277	348	8	10	97	31	796	80	11	14	0	6	0	2	8	55	98	22	33	47	2	6	2
CM32b		252	150	168	90	426	290	223	252	345	11	10	100	43	730	80	9	15	1	7	0	1	9	62	81	21	29	38	0	5	7
CM30b		223	139	177	94	439	290	226	258	316	10	8	89	34	816	75	7	16	1	8	0	2	11	43	68	23	29	41	1	2	2
.6.1899	tr	295	283	123	65	274	219	151	180	252	7	7	82	20	714	60	9	9	2	6	1	1	10	35	46	16	21	25	9	12	0
.6.1899	cr	312	304	110	65	297	239	161	187	235	7	7	105	30	796	80	8	11	0	5	0	3	8	30	38	15	19	27	4	19	0
.6.1899	tl	361	272	132	65	329	239	171	200	258	5	7	103	28	775	75	8	13	2	5	0	1	8	42	38	15	18	25	1	5	0
.6.1899	cl	302	232	155	71	300	239	161	194	235	5	7	90	28	823	80	8	12	1	4	1	2	8	32	54	14	29	37	3	8	0
.6.1899	br	366	326	142	65	329	226	148	194	239	5	8	95	28	812	80	9	11	1	7	0	2	10	27	41	16	22	30	0	0	0
CM10a		219	139	171	77	374	264	197	232	281	8	7	79	23	826	80	10	14	2	6	0	2	10	26	52	23	42	43	11	9	5
CM10b		194	113	181	84	345	271	181	223	271	8	10	92	25	823	80	7	11	1	6	0	2	9	41	67	20	35	50	1	2	4
CM201a	tl	193	138	163	78	350	268	187	226	264	7	9	86	23	856	80	10	8	4	7	1	2	14	43	63	31	42	57	5	9	3
CM201a	tcr	180	133	171	78	373	249	184	218	272	9	10	91	30	801	80	9	8	3	7	0	4	14	25	64	15	52	53	0	1	2
CM201a	tr	214	128	156	78	373	264	179	233	272	9	7	71	17	857	80	9	9	0	6	0	2	8	48	64	26	51	68	3	6	4
CM201a	br	156	98	156	74	350	241	175	202	264	7	7	73	23	765	80	8	9	0	6	1	1	8	32	50	28	39	56	0	4	1
CM201b	tcr	165	106	156	78	327	241	156	194	241	7	9	90	27	805	75	8	9	3	7	1	1	12	23	50	16	34	49	2	9	1
CM201b	bl	232	131	171	86	388	280	194	233	280	9	10	95	33	832	80	9	8	5	4	0	4	13	17	57	20	26	38	0	0	4
CM201b	bl	196	128	163	78	397	272	187	226	280	11	9	79	36	807	80	8	9	4	3	3	2	12	35	66	36	54	68	8	2	6
CM201c	tcr	157	97	148	70	354	233	156	194	249	7	10	59	25	779	80	9	9	4	5	1	5	15	39	47	21	31	45	2	9	2
CM264	tl	262	179	187	101	420	303	214	241	319	7	11	97	46	755	80	7	10	5	5	1	2	13	31	58	26	37	45	0	4	2
CM264	tr	266	160	171	78	381	272	195	233	303	7	6	94	31	769	80	9	7	6	5	3	2	16	62	61	25	33	42	1	2	3
CM264	bl	275	207	179	93	404	288	210	260	334	7	6	97	32	778	80	10	10	3	6	3	1	13	61	58	24	30	47	0	1	3
CM264	br	282	204	179	86	373	257	202	249	300	10	10	90	28	830	80	9	10	2	6	3	3	14	53	57	17	30	35	0	0	1
CM313		239	184	175	93	381	280	210	210	280	7	10	79	47	755	80	8	12	4	6	0	3	13	27	57	34	48	53	0	11	9
BM45121a	tl	441	384	187	86	390	277	252	232	316	7	9	100	38	734	80	6	15	4	3	0	2	9	37	67	13	19	27	2	3	8
CM112q		419	303	193	90	471	316	219	277	339	11	8	133	41	817	80	9	10	9	7	1	1	18	64	90	35	45	75	3	6	5
CM112o		461	323	197	97	477	319	223	284	335	9	8	128	43	848	80	9	15	6	4	2	0	12	43	93	37	54	56	3	2	5
CM112n		433	293	200	90	506	329	219	323	377	13	13	102	48	857	80	10	16	7	3	2	0	12	64	102	39	50	69	4	9	7
CM114g	l	465	335	197	97	516	310	219	310	364	11	7	95	36	852	80	11	14	4	8	1	1	14	45	119	38	53	56	3	23	6
CM114g	r	361	321	187	77	458	271	206	284	342	8	8	100	38	830	80	9	9	7	6	2	1	16	60	88	28	39	50	6	6	2
CM114f	l	437	259	193	97	477	316	226	297	348	11	7	102	38	853	80	11	12	2	6	1	1	10	62	110	33	50	54	13	13	6
CM114f	r	481	297	187	90	484	310	226	310	355	8	8	115	54	873	65	11	12	3	9	1	1	14	64	129	36	50	58	2	2	10
25/76		188	106	148	78	358	218	179	202	264	6	10	84	36	765	80	8	9	3	6	1	3	13	51	34	16	19	30	0	0	1
CM230a	tl	234	155	167	90	397	264	194	237	288	10	7	98	43	823	80	9	7	7	7	0	3	17	50	89	29	51	52	12	17	4
Cm230a	tc	221	145	163	86	389	257	202	233	276	11	9	101	23	844	80	9	7	3	10	0	1	14	26	75	30	42	67	15	14	2
CM230a	tr	208	138	148	78	358	237	187	218	272	7	9	85	27	801	75	9	9	7	4	1	1	13	17	56	22	41	56	3	8	0
CM230a	bl	223	165	171	86	443	226	190	249	284	9	12	94	20	877	80	9	12	3	7	1	1	12	31	54	25	42	46	4	7	4
CM230a	bcl	223	152	160	86	428	257	194	241	280	13	9	78	22	861	80	10	11	6	5	3	1	15	34	93	31	50	67	12	12	6
CM230a	tcr	211	146	163	86	381	257	202	241	288	11	9	85	34	837	75	8	11	5	8	0	0	13	28	72	37	39	54	3	3	2
CM230a	br	215	146	167	78	373	257	187	226	264	7	7	68	20	856	75	9	8	5	7	2	1	15	38	60	33	48	75	4	19	1
CM230b	tl	255	165	163	86	428	280	194	241	280	9	10	95	43	861	80	8	11	6	8	1	2	17	30	83	44	53	63	11	21	3
CM230b	tc	247	172	179	93	436	280	218	260	303	11	8	74	23	858	80	9	10	3	7	1	2	13	45	93	39	62	70	5	26	5
CM230b	tr	245	160	163	86	373	249	202	233	288	9	5	81	23	809	75	8	10	1	8	1	1	11	27	80	35	32	53	5	16	9
CM228a		165	104	132	70	311	233	179	179	226	9	6	73	26	792	80	9	11	2	3	2	1	8	34	49	22	32	44	0	2	7
CIEA16688	bc	258	187	168	77	387	281	171	252	316	11	8	98	46	797	80	8	8	1	6	0	0	7	64	82	29	42	51	1	0	5
CIEA16688	tc	239	162	155	77	335	245	161	213	264	8	7	98	46	807	60	9	12	0	8	1	1	10	91	71	17	21	36	2	0	8
CIEA16688	t	283	194	168	77	387	258	168	245	297	7	10	103	48	825	80	9	11	2	7	1	4	14	67	93	30	40	53	1	0	4
CM207a	tl	338	223	194	93	430	264	202	264	311	7	10	100	30	849	70	9	10	3	7	3	1	14	67	76	30	46	66	29	6	3
CM207a	tr	316	207	179	93	428	272	194	257	303	11	9	97	26	848	80	10	11	3	9	1	1	14	39	85	37	45	72	14	18	6
CM207a	bl	296	188	171	78	389	272	190	241	288	10	7	75	23	837	80	9	12	4	6	3	3	16	29	67	46	50	58	8	6	4
CM207a	br	279	201	163	78	397	272	171	249	288	7	14	91	30	865	80	9	8	2	5	0	3	10	30	92	26	52	64	13	12	5
CM207b	tl	257	172	163	78	358	280	202	233	272	7	9	91	26	857	80	8	10	5	5	0	0	10	33	73	32	43	56	16	7	2
CM207b	tr	308	212	187	93	451	288	210	249	292	10	12	78	37	853	80	8	9	3	5	2	2	12	41	81	35	45	59	20	8	5
CM207b	bl	290	177	171	86	373	280	194	241	280	9	7	98	27	861	80	9	10	4	8	2	0	14	51	80	33	47	51	13	5	6
CM207b	br	283	184	179	93	397	272	202	241	296	9	9	103	32	814	80	9	8	7	8	1	2	18	51	93	37	45	58	12	20	5
CM207f	l	150	81	129	55	257	239	155	187	226	5	7	74	17	827	80	8	10	3	5	3	0	11	33	54	22	33	44	5	2	0

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CM207f	r	143	74	129	65	284	226	135	161	232	7	7	66	23	694	80	8	8	1	5	0	1	7	41	25	21	25	38	0	1	1
CM218b	bl	268	183	160	75	389	241	198	226	280	11	10	90	28	807	80	9	8	2	7	1	0	10	91	63	44	53	59	9	4	5
CM218b	br	228	169	187	86	436	296	187	249	303	11	10	85	17	822	80	9	8	7	7	1	2	17	127	77	38	50	62	8	0	13
CM218b	tl	263	183	200	86	463	303	202	253	311	11	11	102	34	814	80	9	7	5	6	2	3	16	157	86	45	71	83	36	8	0
CM218b	tc	262	164	156	82	346	241	187	202	264	9	6	90	23	765	80	9	9	2	6	0	2	10	83	55	23	26	43	9	2	5
CM218b	tr	263	193	194	78	381	303	191	241	311	11	11	96	30	775	80	9	9	5	8	2	4	19	143	88	34	46	65	5	1	12
CM218c	tl	265	206	187	86	397	280	202	241	311	11	7	98	42	775	80	9	8	3	5	1	4	13	124	77	29	42	61	17	5	5
CM218c	tc	254	194	187	93	428	280	210	249	327	11	9	92	44	761	80	7	11	5	10	0	3	18	135	85	31	56	62	16	2	7
CM218c	tr	219	135	163	70	400	303	187	226	264	9	9	89	21	856	80	10	9	2	8	2	4	16	118	84	30	40	46	2	2	8
CM218c	bl	261	174	187	93	400	280	226	241	303	11	9	97	30	795	80	10	10	2	6	2	2	12	113	63	33	41	50	8	0	7
CM218c	br	200	134	171	86	373	257	202	226	272	9	7	85	27	831	75	9	8	2	6	1	3	12	141	59	20	40	47	3	0	8
CM37a		270	169	161	97	387	277	213	251	310	8	8	103	43	810	80	9	10	3	8	1	1	13	36	76	24	38	48	4	7	9
CM37d		281	187	168	84	410	271	181	252	310	8	8	95	41	813	80	9	10	3	8	0	2	13	51	65	36	33	48	3	4	12
CM41a	l	213	152	161	94	400	297	213	235	271	7	15	93	39	867	80	12	10	3	8	0	0	11	51	76	28	34	44	2	0	0
CM41a	c	205	140	168	110	355	258	213	239	277	10	15	100	38	863	80	9	10	1	6	0	1	8	56	66	15	23	43	3	3	5
CM41a	r	200	140	161	110	323	264	203	232	277	11	13	103	36	838	75	9	10	3	5	0	0	8	68	72	19	30	39	2	1	5
CM41b	l	187	132	148	77	355	277	194	219	271	8	11	93	34	808	80	9	11	0	7	0	2	9	54	55	15	27	32	2	2	8
CM41b	r	219	152	168	77	387	271	177	245	290	7	10	100	31	845	80	8	9	0	5	0	7	12	61	59	27	33	45	2	4	3
CM41c	r	174	110	143	61	348	239	155	213	258	7	9	92	38	826	80	9	8	5	8	0	3	16	51	59	15	19	32	0	3	3
CM120	l	212	152	161	84	374	274	184	248	290	7	7	97	44	855	80	9	13	1	7	1	3	12	54	67	14	28	31	5	7	7
CM120	r	213	148	161	84	381	252	184	226	297	7	10	84	43	761	80	8	10	1	8	3	3	15	50	50	20	21	30	1	2	5
CM118d		166	110	161	84	374	258	197	226	303	7	7	103	33	746	80	12	10	2	5	2	1	10	58	48	20	27	41	4	2	7
CM118b		306	206	168	77	419	290	226	245	316	8	8	103	46	775	80	9	11	3	7	2	3	15	95	52	26	33	40	0	0	5
CM118a		308	210	184	90	393	258	174	252	316	7	8	100	39	797	80	9	10	4	4	0	1	9	75	61	24	27	42	2	0	3
CIE1034a1		226	141	168	74	381	290	200	242	310	7	15	90	30	781	80	9	11	0	5	1	2	8	21	52	26	27	44	0	0	1
CIE1034a2		223	148	168	84	355	258	187	223	274	10	11	97	33	814	75	10	15	3	5	0	0	8	28	64	27	27	30	2	2	1
CIE1034a3		213	141	158	81	361	239	206	219	284	8	10	92	41	771	80	8	11	1	5	0	0	6	28	64	23	32	44	0	3	4
CIE1034a4		253	187	179	86	397	280	202	249	319	7	10	97	26	781	80	9	9	2	9	0	3	14	7	55	26	37	44	3	3	3
CM209		198	138	163	78	400	249	202	226	280	9	9	82	23	807	80	10	8	3	7	0	2	12	25	53	28	44	56	20	13	0
CM160a	bl	198	113	174	71	406	261	248	239	303	8	10	95	28	789	80	8	11	4	8	0	3	15	84	67	43	47	53	6	1	13
CM160a	bcl	181	128	177	80	396	264	177	232	293	8	8	93	44	792	80	10	9	5	8	0	1	14	98	77	29	36	37	1	0	9
CM160a	bc	166	106	174	77	400	258	174	239	290	10	8	100	41	824	80	8	8	4	6	1	3	14	89	68	35	45	61	4	0	8
CM160a	bcr	174	103	164	71	400	258	174	239	290	8	8	106	39	824	80	10	11	3	10	0	2	15	101	62	29	39	45	1	0	10
CM160a	br	194	129	171	77	406	264	187	239	284	7	5	92	39	842	80	8	12	1	6	2	4	13	66	52	12	24	37	0	0	9
CM160a	tl	179	100	148	65	374	281	177	213	277	7	8	98	30	769	80	8	10	1	7	0	2	10	128	60	24	36	53	0	0	12
CM160a	tcl	174	113	168	71	406	277	180	239	290	10	9	93	34	824	80	8	6	6	7	1	1	15	82	66	33	40	49	1	0	6
CM160a	tcr	174	113	174	74	464	284	174	239	303	8	8	93	29	789	80	6	8	5	5	2	1	13	109	54	34	40	53	1	0	12
CM160a	tr	168	116	171	80	348	245	174	239	303	11	9	97	38	789	75	9	11	4	7	2	4	17	122	58	29	38	49	0	2	12
CM171a	tr	129	86	129	71	294	226	152	187	232	7	5	92	46	806	80	9	9	6	3	1	1	11	47	62	18	29	33	0	0	2
CM171a	tcr	185	124	168	80	338	253	177	242	306	8	10	98	38	791	70	8	11	6	10	0	1	17	78	67	34	42	46	4	4	6
CM171a	tcl	194	113	168	74	374	255	161	245	306	7	10	90	30	801	80	11	10	1	8	0	1	10	93	58	34	34	44	3	10	6
CM171a	tl	181	128	161	77	396	252	180	235	284	7	8	93	41	827	80	10	10	6	8	0	1	15	66	63	30	37	46	12	7	3
CM171b	tl	133	82	129	65	303	226	142	174	232	5	7	84	34	750	70	9	12	5	3	1	1	10	80	57	22	31	35	2	2	5
CM171b	tcl	161	94	155	77	354	245	168	206	264	7	6	90	30	780	80	8	10	5	7	0	2	14	100	43	21	30	36	5	3	11
CM171b	tc	168	111	148	77	354	258	183	213	264	7	10	82	46	807	80	8	11	3	6	0	1	10	62	57	25	28	41	14	7	10
CM171b	tcr	164	124	148	77	354	252	187	324	284	10	9	95	41	789	80	9	8	3	3	2	2	10	73	48	15	34	36	2	0	6
CM171b	tr	145	93	142	71	368	258	174	206	271	5	7	72	23	760	80	9	8	3	6	0	2	11	46	54	19	28	35	0	1	4
CM171b	br	145	95	142	71	357	226	168	200	252	10	6	92	48	794	75	9	9	3	7	0	1	11	53	55	29	43	47	0	0	3
CM220a	tl	167	108	161	77	342	239	174	194	264	7	8	89	28	735	75	9	8	2	6	0	3	11	64	46	19	30	45	2	1	4
CM220a	tc	191	132	168	90	381	290	200	213	258	9	10	82	41	826	80	10	7	2	9	0	3	14	150	72	26	43	53	4	1	2
CM220a	tr	191	127	161	84	381	271	200	219	284	13	10	89	30	771	80	9	6	1	8	0	4	13	152	52	24	32	42	8	1	6
CM220a	bl	209	134	174	84	406	290	194	219	290	8	10	106	34	755	80	9	10	2	8	2	5	17	99	65	22	44	51	7	3	10
CM220a	br	205	139	174	90	387	258	187	232	297	10	11	85	39	781	80	10	8	4	6	0	2	12	121	71	18	40	54	11	1	2
CM220b	tl	195	134	161	77	374	264	174	206	277	9	10	89	34	744	80	8	7	5	5	2	3	15	98	61	21	39	44	3	2	9
CM220b	tc	241	157	187	77	413	277	200	232	303	13	11	97	41	766	75	10	14	2	8	2	1	13	170	72	30	47	65	6	1	8
CM220b	tr	275	201	200	90	393	297	232	271	329	9	10	97	48	824	80	10	10	6	8	0	1	15	136	133	33	58	59	24	4	5

Appendix 3.3 continued

CM220b	bl	230	132	161	77	342	277	187	219	284	10	10	93	36	771	80	9	9	3	6	1	3	13	95	62	30	35	50	9	1	5
CM220b	br	219	144	168	84	374	271	180	219	284	11	10	93	44	771	75	8	6	5	5	2	3	15	126	60	24	38	41	6	1	7
1085		511	348	155	77	413	310	200	239	290	7	5	97	39	824	80	9	11	2	4	1	0	7	29	45	15	26	28	0	1	1
1099		503	401	161	71	426	271	181	245	323	8	8	90	34	759	80	9	10	0	5	1	0	6	13	58	20	16	23	2	3	1
CM208a	tl	182	124	160	86	412	241	194	210	268	10	11	73	31	784	80	10	8	2	6	0	1	9	23	48	22	40	53	11	3	0
CM208a	tc	167	110	156	70	366	232	187	226	264	9	7	80	18	856	75	8	9	3	5	1	2	11	21	80	23	39	50	13	4	4
CM208a	tr	185	122	156	86	397	241	171	220	272	9	7	74	27	809	80	9	7	5	8	0	0	13	39	65	27	47	54	13	1	0
CM208a	bl	240	162	166	93	397	280	202	241	288	7	11	106	22	837	80	10	9	3	6	2	0	11	44	58	34	52	63	25	5	1
CM208a	bc	233	164	163	78	424	268	198	249	296	9	10	80	26	841	80	9	10	4	7	2	1	14	63	88	34	44	55	25	13	4
CM208a	br	274	181	171	78	397	264	187	241	288	6	11	73	47	837	80	8	10	5	5	1	1	12	41	68	32	48	61	11	9	5
CM208b	tl	236	136	179	86	389	296	202	249	311	7	8	82	31	801	80	10	10	5	6	0	3	14	48	59	31	46	57	23	9	4
CM208b	bl	174	104	156	78	373	257	179	187	257	7	6	78	15	728	80	8	9	3	6	0	3	12	14	48	28	31	46	13	5	2
CM208b	bc	195	126	187	93	397	288	163	257	324	7	7	92	39	793	80	9	11	5	5	3	3	16	108	69	33	46	51	1	0	2
CM208b	br	176	95	156	74	334	264	171	202	249	8	8	76	23	811	80	8	10	3	6	1	3	13	20	58	22	34	38	1	1	0
BM62-3 1		272	181	181	97	387	290	200	271	316	7	10	103	48	858	80	10	15	0	7	0	3	10	49	50	30	46	50	2	3	3
BM62-3 2		303	216	187	97	387	303	200	297	342	7	11	107	43	868	80	9	16	1	6	0	5	12	68	74	31	38	64	3	0	5
BM62-3 3		316	239	194	97	432	284	194	290	348	7	8	103	36	833	80	9	15	1	7	0	2	10	76	70	16	21	46	5	4	2
BM62-3 4	r	281	210	168	90	355	290	174	277	323	7	8	98	38	858	80	9	14	1	8	0	1	10	57	77	31	49	58	4	1	3
BM62-3 4	cr	248	197	181	90	387	284	206	264	310	8	10	103	43	852	80	9	14	3	8	0	1	12	78	63	28	31	43	6	4	3
BM62-3 5	l	295	231	181	90	413	277	206	264	368	7	7	97	39	717	80	8	16	1	7	0	1	9	133	70	46	61	75	0	0	0
BM62-3 6	c	297	203	181	90	387	271	174	284	316	5	8	95	48	899	80	9	14	1	7	0	1	9	59	68	38	44	59	0	0	4
BM62-3 5	c	323	219	194	97	419	284	206	277	323	8	8	107	48	858	80	9	15	2	9	0	1	12	69	57	35	43	55	7	2	4
BM62-3 7	c	323	250	194	97	419	290	194	290	328	8	8	108	34	884	80	9	9	8	9	2	2	21	94	84	39	52	73	1	2	3
BM62-3 4	cl	310	210	174	84	426	290	174	284	335	7	8	92	33	848	80	9	15	3	8	0	1	12	70	83	48	55	64	1	6	2
BM40/180	l	336	248	200	123	416	335	200	290	348	8	10	107	54	833	80	9	20	2	9	1	1	13	94	100	51	56	84	4	8	4
BM40/180	c	307	254	181	103	393	323	187	281	329	8	8	116	46	854	80	10	16	1	11	0	2	14	79	80	47	60	77	8	8	3
BM40/180	r	300	235	174	97	387	303	200	271	310	7	8	107	44	874	80	9	14	5	7	1	2	15	47	61	31	50	62	0	0	1
BM62-3 4	l	258	226	187	110	406	277	174	271	297	8	8	110	46	912	80	10	11	2	8	1	0	11	80	74	42	45	58	9	3	2
BM62-3 6	l	271	216	168	77	393	271	174	252	284	8	8	100	33	887	80	9	11	1	9	1	0	11	72	58	35	51	63	4	8	5
BM68/27		500	393	174	103	426	284	206	258	323	10	7	98	64	799	80	8	14	5	7	1	2	15	57	101	36	54	50	24	18	7
30.5.69	r	219	152	174	90	393	271	181	232	310	13	8	103	49	748	80	8	13	5	8	1	3	17	69	75	48	58	56	12	8	2
CM215a	l	201	144	174	90	419	290	174	245	303	10	8	92	44	809	80	9	12	5	8	0	2	15	94	57	28	46	60	4	11	11
CM215a	c	190	129	174	77	387	290	200	239	290	10	10	97	61	824	80	8	12	3	7	1	0	11	49	62	35	47	56	9	4	4
CM215a	r	216	160	174	84	381	252	194	232	303	11	10	108	39	766	80	10	10	4	6	1	0	11	69	68	58	69	66	10	10	3
CM215b	l	198	150	168	77	374	310	187	252	310	8	10	106	39	813	80	9	12	2	7	1	3	13	93	74	32	42	56	4	8	8
CM215b	c	199	141	148	84	354	264	180	219	271	10	8	95	41	808	80	8	8	4	7	0	3	14	68	60	33	35	49	10	3	4
CM215b	r	167	120	161	77	381	297	168	206	284	7	7	98	31	725	70	7	8	5	6	0	2	13	30	72	24	39	50	10	9	0
CM215c	l	243	186	180	97	419	323	187	258	323	8	8	112	59	799	80	10	9	7	6	0	3	16	169	79	27	59	49	9	6	7
CM215c	c	238	186	180	84	400	297	180	245	310	11	10	118	43	790	80	10	10	3	9	0	2	14	67	73	29	47	58	2	10	6
CM215c	r	259	179	180	97	393	310	194	277	329	11	10	106	39	842	70	10	10	5	6	0	1	12	74	114	51	59	75	2	2	9
CM215e	tl	177	127	161	90	374	258	187	213	264	7	8	85	38	807	80	8	9	5	8	0	6	19	92	70	26	40	51	4	0	4
CM195a	l	164	88	155	77	310	281	187	194	232	6	8	79	35	836	75	10	9	1	8	0	5	14	66	43	17	25	32	2	1	3
CM195a	r	194	93	135	77	342	297	187	200	245	9	8	90	33	816	75	9	11	5	4	2	4	15	98	47	25	35	37	6	4	5
CM195a	c	157	100	142	71	342	255	174	194	252	5	7	89	30	770	60	9	10	2	7	1	3	13	99	37	21	30	36	0	0	5
Killington		284	195	161	84	348	264	187	232	297	8	8	113	49	781	80	7	13	3	8	2	1	14	86	48	28	39	45	5	12	3
CM196a	tl	252	142	168	77	387	303	187	239	277	10	8	77	34	863	80	9	13	3	7	1	3	14	88	45	16	23	36	2	2	10
CM196a	tc	199	135	158	77	374	284	187	239	277	10	6	102	30	863	80	9	8	6	5	1	3	15	77	57	19	31	33	2	0	2
CM196a	tr	194	123	168	77	361	277	180	226	264	7	10	98	28	856	80	9	9	6	6	1	4	17	70	51	17	23	35	1	1	6
CM196a	bl	213	133	187	84	368	284	213	239	284	8	8	93	28	842	80	11	13	1	7	1	4	13	89	56	16	27	36	2	3	11
CM196a	br	218	132	180	84	393	310	194	245	290	8	11	95	36	845	70	9	9	0	8	1	4	13	104	55	24	36	43	4	4	6
CM196b	l	194	107	174	77	377	297	177	226	258	5	10	87	25	876	80	9	10	2	8	0	5	15	69	38	20	23	32	2	2	8
CM196b	c	180	110	161	71	342	316	245	219	271	5	8	79	28	808	80	11	9	2	7	0	2	11	72	40	17	20	34	4	8	8
CM196b	r	168	97	174	77	368	258	194	219	258	5	7	89	25	849	75	9	7	4	7	2	3	16	69	51	16	26	37	5	1	3
BM45/121b	tl	319	218	168	84	410	245	200	265	310	7	8	108	33	858	80	9	13	0	5	0	2	7	32	57	18	24	31	2	2	5
BM45/121b	tr	271	213	184	81	440	277	200	252	316	7	7	102	36	797	80	8	12	0	7	0	1	8	46	58	13	21	30	1	0	6
BM45/121b	bl	355	248	181	84	440	271	226	271	368	7	10	98	44	736	80	11	13	0	9	0	0	9	37	68	23	33	41	1	1	6
BM45/121b	br	308	246	184	90	430	264	200	264	323	5	10	107	48	817	80	9	15	1	8	0	0	9	40	68	19	30	31	2	2	0

Appendix 3.3 continued

BM45/121c	tl	246	203	158	74	360	239	174	264	335	5	7	93	46	788	80	8	12	0	7	0	0	7	21	28	8	12	18	3	2	0
BM45/121c	tr	307	240	189	77	450	274	213	258	310	10	7	105	44	832	80	10	10	2	6	0	1	9	38	63	23	33	34	1	0	6
CM36a		250	148	187	105	445	323	229	274	326	11	10	108	56	840	80	9	17	0	8	0	0	8	50	88	21	25	32	4	10	2
CM36b		247	175	164	81	361	258	187	226	297	8	15	105	41	761	80	9	15	0	8	0	0	8	48	51	23	22	31	0	9	6
CM36c		395	292	206	106	484	290	194	303	368	11	8	100	38	824	80	12	12	2	7	0	2	11	79	108	34	46	44	5	20	8
CM36d		426	292	200	97	471	335	245	310	393	10	13	108	49	789	80	12	16	2	7	0	7	16	90	81	37	47	45	3	31	1
CM36e		406	279	194	100	452	303	219	284	342	11	11	105	51	830	80	10	17	0	8	0	1	9	49	94	28	38	46	5	11	4
CM36f		335	208	181	97	426	303	206	271	323	8	13	98	38	839	80	11	11	6	6	4	2	18	69	82	28	34	42	3	8	9
CM36g		390	248	184	100	426	297	216	255	310	12	11	105	49	823	80	11	13	7	7	1	2	17	59	82	28	33	40	1	8	5
CM36h		406	242	164	84	393	277	255	232	297	8	8	100	28	781	80	9	14	2	7	0	1	10	45	57	19	17	24	1	7	9
CM36j		221	139	174	94	426	309	206	258	323	7	10	93	44	799	80	11	10	7	10	0	4	21	78	89	24	33	30	0	4	5
CM36k		455	310	200	103	500	323	242	297	374	11	13	116	49	794	80	8	15	7	6	1	3	17	23	95	36	51	53	3	10	6
G. Monroe	t	423	300	206	84	535	316	223	348	393	11	8	103	56	885	80	10	15	0	7	0	2	9	46	124	14	29	39	22	7	6
G. Monroe	bl	403	290	213	97	542	355	232	381	406	8	8	105	56	938	80	12	18	1	8	0	1	10	50	92	23	25	41	31	4	8
G. Monroe	br	361	232	181	97	452	297	206	323	368	10	7	87	49	878	65	8	18	1	8	0	0	9	30	71	21	32	41	3	0	12
CM85a	tl	408	254	190	87	452	320	197	290	348	10	8	110	49	833	80	11	10	5	6	1	2	14	48	98	33	37	43	2	2	10
CM85a	tr	429	248	187	93	452	290	194	290	364	12	12	108	50	797	80	10	11	6	6	1	4	17	49	88	32	45	44	2	0	7
CM85a	bl	439	267	190	93	477	277	194	303	354	12	6	101	37	856	80	13	10	5	9	2	3	19	66	98	30	42	47	4	10	10
CM85a	br	419	252	187	90	455	293	197	303	361	15	9	113	41	839	80	11	12	3	7	1	4	15	43	95	32	51	51	2	2	9
CM85b	tl	372	229	190	90	443	303	194	280	342	10	7	110	49	819	80	9	7	4	8	0	2	14	37	84	26	41	42	2	0	4
CM85b	tc	424	235	194	93	455	288	206	276	330	11	9	110	31	836	80	11	9	7	7	0	4	18	29	78	27	34	37	0	0	12
CM85b	tr	424	219	187	90	455	303	218	280	370	11	9	101	37	757	80	11	12	3	7	4	2	16	35	99	35	42	47	1	2	6
CM85b	bl	364	210	181	101	432	296	202	280	350	12	11	98	30	800	80	10	10	6	8	0	2	16	34	76	24	26	39	1	0	4
CM85b	bc	330	195	182	101	436	288	210	276	342	9	6	110	42	807	80	9	11	7	5	3	3	18	33	76	23	27	34	3	2	11
CM85b	br	405	210	198	97	513	334	241	319	381	7	9	107	47	837	80	11	9	5	7	1	0	13	67	86	31	46	47	1	1	6

Appendix 3.4 Eight-character morphological data, recorded from field-collected specimens

Key: Ref. = reference number to collection data given in Appendix 3.1; Pos. = position of specimen on the slide where l = left, c = centre, r = right, t = top, and b = bottom. The numbers for the morphological characters are those listed in Section 3.2.5. Abbreviations for host-plant groups or species are those given for Figs. 3.2, 3.6 and 3.7.

Ref.	Pos.	Host	Character 1	2	3	4	5	6	7	8
UNITED KINGDOM										
CM34a		AG	10	13	6	11	2	53	9	11
BM81/539a	l	AG	7	11	6	9	7	65	12	3
BM81/539a	r	AG	7	13	7	9	2	72	8	11
BM81/539b		AG	10	12	3	7	6	81	20	10
BM81/539c		AG	7	13	5	9	7	42	14	7
CM307d	l	AG	9	14	10	8	1	40	10	1
CM307d	r	AG	7	11	8	10	0	36	12	0
CM307a	r	AG	6	9	6	9	2	25	1	0
CM307c	tl	AG	5	12	6	9	2	50	5	0
CM307c	bl	AG	7	14	8	10	0	31	0	0
720SC		AG	7	12	6	9	5	33	1	2
CM39a		BP	11	18	6	8	1	50	9	2
CM39b		BP	10	27	9	9	0	80	6	13
CM39c		BP	8	21	9	7	1	58	2	1
CM39d		BP	10	21	10	9	3	62	5	27
CM39e		BP	11	16	6	8	1	70	16	17
CM39f		BP	14	31	5	9	2	72	23	28
CM39g		BP	14	22	9	9	4	66	12	37
CM39i		BP	13	19	8	9	2	63	13	6
CM39j		BP	10	20	9	9	1	59	2	7
CM39l		BP	11	28	7	9	4	59	8	5
CM14		BP	8	12	8	9	5	55	5	5
9342		BP	8	12	6	10	2	66	5	4
BM81/539		BP	6	20	7	9	0	68	65	44
Laing a		BP	5	18	8	9	9	61	22	8
Laing b		BP	8	24	11	8	4	39	30	50
CM222a	tl	BP	7	12	10	9	0	42	0	1
CM222a	tr	BP	11	16	10	9	0	73	13	12
CM222a	bl	BP	10	15	7	9	0	53	19	35
CM222a	br	BP	8	18	10	9	1	77	9	10
CM222b	tl	BP	8	13	10	9	3	53	10	11
CM222b	bl	BP	8	18	9	9	0	72	12	28
CM222b	br	BP	10	12	10	9	1	57	9	19
CM222c	l	BP	8	12	9	9	1	45	8	25
CM222c	r	BP	8	14	7	8	0	62	5	4
CM39h		BP	11	22	9	7	2	86	11	10
CM39k		BP	11	16	9	8	6	84	2	3
CM39m		BP	12	18	13	8	3	68	3	7
CM39n		BP	12	19	8	8	1	65	9	28
CM39o		BP	9	17	10	8	2	83	4	1
CM38b		CM	10	13	6	9	5	42	6	7
CM38c		CM	10	16	7	8	2	51	1	8
CM38h		CM	10	11	5	9	8	41	3	1
CM38j		CM	8	10	7	8	6	40	5	9
CM38k		CM	7	13	6	9	7	43	2	15
CM40a		CM	10	15	9	8	8	38	4	8
CM40b		CM	10	11	4	9	1	44	6	14
CM40d	l	CM	8	14	6	9	6	42	6	3
CM40d	c	CM	5	13	7	8	7	38	1	2
CM40d	r	CM	4	13	7	9	1	41	5	6
CM30a		CM	11	19	7	9	12	47	0	5
CM32a		CM	8	14	6	11	2	47	2	6
CM32b		CM	11	16	7	9	7	38	0	5
CM30b		CM	10	17	8	7	2	41	1	2
.6.1899	tr	CM	7	11	7	9	0	25	9	12
.6.1899	cr	CM	7	11	5	8	0	27	4	19
.6.1899	tl	CM	5	15	5	8	0	25	1	5
.6.1899	cl	CM	5	13	5	8	0	37	3	8
.6.1899	br	CM	5	12	7	9	0	30	0	0
CM10a		CM	8	16	6	10	5	43	11	9
CM10b		CM	8	12	6	7	4	50	1	2
CM201a	tl	CM	7	12	8	10	3	57	5	9
CM201a	tcr	CM	9	11	7	9	2	53	0	1
CM201a	tr	CM	9	9	6	9	4	68	3	6
CM201a	br	CM	7	9	7	8	1	56	0	4
CM201b	tcr	CM	7	12	8	8	1	49	2	9
CM201b	bl	CM	9	13	4	9	4	38	0	0
CM201b	bcl	CM	11	13	6	8	6	68	8	2
CM201c	tcr	CM	7	13	6	9	2	45	2	9
CM264	tl	CM	7	15	6	7	2	45	0	4
CM264	tr	CM	7	13	8	9	3	42	1	2
CM264	bl	CM	7	13	9	10	3	47	0	1
CM264	br	CM	10	12	9	9	1	35	0	0
CM313		CM	7	16	6	8	9	53	0	11
CM110a		CM	10	13	7	10	7	51	3	3
CM110b		CM	11	14	7	8	2	35	27	5
CM110c		CM	10	14	8	9	3	57	15	28
CM264a		CM	9	11	4	8	4	53	4	12
CM40e	l	CM	9	11	7	9	2	42	3	10
CM40e	r	CM	9	14	7	7	5	56	3	4
CM38n	r	CM	9	14	8	8	2	36	6	14
CM38m	l	CM	7	13	8	8	5	46	1	4
CM38m	r	CM	8	13	7	8	3	51	1	3
CM380		CM	8	15	8	8	2	40	4	16
CM40c	tr	CM	10	14	8	9	5	41	5	4
CM40c	bl	CM	10	14	7	10	5	54	11	15
CM40c	br	CM	10	12	8	9	0	41	0	4
CM40g	l	CM	10	14	10	9	8	53	2	12
CM40g	c	CM	9	14	5	9	5	39	3	6
CM40g	r	CM	9	14	10	8	4	62	17	26
BM45.121a	tl	EU	7	19	3	6	8	27	2	3
BM45.121a	br	EU	7	16	9	8	11	32	4	5

Appendix 3.3 continued

CM112q		PP	11	19	8	9	5	75	3	6
CM112o		PP	9	21	6	9	5	56	3	2
CM112n		PP	13	23	5	10	7	69	4	9
CM114g	l	PP	11	18	9	11	6	56	3	23
CM114g	r	PP	8	16	8	9	2	50	6	6
CM114f	l	PP	11	14	7	11	6	54	13	13
CM114f	r	PP	8	15	10	11	10	58	2	2
BM81/539		PP	9	13	6	9	3	31	2	3
CM212a	c	PP	10	17	8	9	5	47	3	4
CM212b	tl	PP	9	14	9	10	4	53	0	3
CM212b	tr	PP	9	15	6	9	6	58	6	9
CM212b	bl	PP	9	14	7	8	0	60	3	4
CM212b	br	PP	10	18	9	11	3	70	4	2
25/76		PP	6	12	7	8	1	30	0	0
CM230a	tl	PC	10	14	7	9	4	52	12	17
Cm230a	tc	PC	11	10	10	9	2	67	15	14
CM230a	tr	PC	7	16	5	9	0	56	3	8
CM230a	bl	PC	9	15	8	9	4	46	4	7
CM230a	bcl	PC	13	17	8	10	6	67	12	12
CM230a	tcr	PC	11	16	8	8	2	54	3	3
CM230a	br	PC	7	13	9	9	1	75	4	19
CM230b	tl	PC	9	17	9	8	3	63	11	21
CM230b	tc	PC	11	13	8	9	5	70	5	26
CM230b	tr	PC	9	11	9	8	9	53	5	16
CM228a	l	PC	8	11	7	9	1	58	13	14
CM228a	r	PC	9	13	5	9	7	44	0	2
CM42b		PC	7	10	7	9	0	40	3	0
CM42d		PC	9	10	5	10	0	44	8	1
CM230b	bl	PC	9	14	8	11	0	72	10	14
CM230b	bcl	PC	10	12	6	11	4	57	2	9
CM230b	bcr	PC	11	14	6	9	5	57	9	6
CM230b	br	PC	9	11	8	8	5	52	6	5
CM180a	tl	PC	9	15	8	9	3	56	1	0
CM180a	tr	PC	8	16	6	10	5	44	0	2
CM180a	br	PC	8	12	7	10	2	42	2	1
BM48/978	l	PC	10	16	10	10	3	40	4	4
BM48/978	r	PC	9	16	11	9	7	41	7	4
CM180d		PC	7	12	7	8	0	35	0	0
CM180e	r	PC	7	12	9	9	3	30	1	2
CM180e	l	PC	11	13	7	8	1	31	1	1
CIEA16688	bc	RN	11	9	6	8	5	51	1	0
CIEA16688	tc	RN	8	12	9	9	8	36	2	0
CIEA16688	t	RN	7	13	8	9	4	53	1	0
CM207a	tl	RN	7	13	10	9	3	66	29	6
CM207a	tr	RN	11	14	10	10	6	72	14	18
CM207a	bl	RN	10	16	9	9	4	58	8	6
CM207a	br	RN	7	10	5	9	5	64	13	12
CM207b	tl	RN	7	15	5	8	2	56	16	7
CM207b	tr	RN	10	12	7	8	5	59	20	8
CM207b	bl	RN	9	14	10	9	6	51	13	5
CM207b	br	RN	9	15	9	9	5	58	12	20
CM207f	l	RN	5	13	8	8	0	44	5	2
CM207f	r	RN	7	9	5	8	1	38	0	1
CM218b	bl	RN	11	10	8	9	5	59	9	4
CM218b	br	RN	11	15	8	9	13	62	8	0

CM218b	tl	RN	11	12	8	9	0	83	36	8
CM218b	tc	RN	9	11	6	9	5	43	9	2
CM218b	tr	RN	11	14	10	9	12	65	5	1
CM218c	tl	RN	11	11	6	9	5	61	17	5
CM218c	tc	RN	11	16	10	7	7	62	16	2
CM218c	tr	RN	9	11	10	10	8	46	2	2
CM218c	bl	RN	11	12	8	10	7	50	8	0
CM218c	br	RN	9	10	7	9	8	47	3	0
CIE16688	b	RN	9	12	7	9	6	51	4	0
CM218a	tl	RN	12	12	8	9	0	81	34	3
CM218a	tc	RN	10	11	6	9	5	46	8	3
CM218a	tr	RN	8	14	8	9	12	64	6	1
CM218a	bl	RN	11	10	8	8	5	53	9	6
CM218a	br	RN	8	15	8	9	12	58	7	0
CM207c	tl	RN	8	15	9	9	3	62	18	12
CM207c	tr	RN	9	12	9	10	3	58	9	2
CM207c	bl	RN	10	15	10	8	6	68	11	3
CM207c	br	RN	9	13	9	10	5	67	16	11
CM207d	tl	RN	10	14	7	8	4	53	11	6
CM207d	tc	RN	11	14	6	9	2	65	9	19
CM207d	tr	RN	9	8	2	9	0	47	8	5
CM207d	bl	RN	8	10	7	8	4	62	19	10
CM207d	br	RN	12	14	5	8	6	70	13	3
CM207e	l	RN	8	13	8	10	3	52	12	4
CM207e	c	RN	7	14	6	10	9	66	10	8
CM207e	r	RN	7	14	7	9	7	57	6	9
CM37a		RS	8	13	9	9	9	48	4	7
CM37d		RS	8	13	8	9	12	48	3	4
CM41a	l	RS	7	13	8	12	0	44	2	0
CM41a	c	RS	10	11	6	9	5	43	3	3
CM41a	r	RS	11	13	5	9	5	39	2	1
CM41b	l	RS	8	11	7	9	8	32	2	2
CM41b	r	RS	7	9	5	8	3	45	2	4
CM41c	r	RS	7	13	8	9	3	32	0	3
CM120	l	RS	7	14	8	9	7	31	5	7
CM120	r	RS	7	11	11	8	5	30	1	2
CM118d		RS	7	12	7	12	7	41	4	2
CM118b		RS	8	14	9	9	5	40	0	0
CM118a		RS	7	14	4	9	3	42	2	0
CIE1034a1		RS	7	11	6	9	1	44	0	0
CIE1034a2		RS	10	18	5	10	1	30	2	2
CIE1034a3		RS	8	12	5	8	4	44	0	3
CIE1034a4		RS	7	11	9	9	3	44	3	3
CM209		RS	9	11	7	10	0	56	20	13
CM160a	bl	RS	8	15	8	8	13	53	6	1
CM160a	bcl	RS	8	14	8	10	9	37	1	0
CM160a	bc	RS	10	12	7	8	8	61	4	0
CM160a	bcr	RS	8	14	10	10	10	45	1	0
CM160a	br	RS	7	13	8	8	9	37	0	0
CM160a	tl	RS	7	11	7	8	12	53	0	0
CM160a	tcl	RS	10	12	8	8	6	49	1	0
CM160a	tcr	RS	8	13	7	6	12	53	1	0
7CM160a	tr	RS	11	15	9	9	12	49	0	2
CM171a	tr	RS	7	15	4	9	2	33	0	0
CM171a	tcr	RS	8	17	10	8	6	46	4	4

Appendix 3.3 continued

CM171a	tcl	RS	7	11	8	11	6	44	3	10
CM171a	tl	RS	7	16	8	10	3	46	12	7
CM171b	tl	RS	5	17	4	9	5	35	2	2
CM171b	tcl	RS	7	15	7	8	11	36	5	3
CM171b	tc	RS	7	14	6	8	10	41	14	7
CM171b	tcr	RS	10	11	5	9	6	36	2	0
CM171b	tr	RS	5	11	6	9	4	35	0	1
CM171b	br	RS	10	12	7	9	3	47	0	0
CM220a	tl	RL	7	10	6	9	4	45	2	1
CM220a	tc	RL	9	9	9	10	2	53	4	1
CM220a	tr	RL	13	7	8	9	6	42	8	1
CM220a	bl	RL	8	12	10	9	10	51	7	3
CM220a	br	RL	10	12	6	10	2	54	11	1
CM220b	tl	RL	9	12	7	8	9	44	3	2
CM220b	tc	RL	13	16	10	10	8	65	6	1
CM220b	tr	RL	9	16	8	10	5	59	24	4
CM220b	bl	RL	10	12	7	9	5	50	9	1
CM220b	br	RL	11	11	7	8	7	41	6	1
1085		RU	7	13	5	9	1	28	0	1
1099		RU	8	10	6	9	1	23	2	3
CM208a	tl	RU	10	10	6	10	0	53	11	3
CM208a	tc	RU	9	12	6	8	4	50	13	4
CM208a	tr	RU	9	12	8	9	0	54	13	1
CM208a	bl	RU	7	12	8	10	1	63	25	5
CM208a	bc	RU	9	14	9	9	4	55	25	13
CM208a	br	RU	6	15	6	8	5	61	11	9
CM208b	tl	RU	7	15	6	10	4	57	23	9
CM208b	tc	RU	7	12	6	8	2	46	13	5
CM208b	tr	RU	7	16	8	9	2	51	1	0
CM208b	bl	RU	8	13	7	8	0	38	1	1
CM208b	bc	RU	7	14	7	9	1	52	3	0
CM208b	br	RU	6	14	6	8	0	42	1	1
BM62-3 1		SA	7	15	7	10	3	50	2	3
BM62-3 2		SA	7	17	6	9	5	64	3	0
BM62-3 3		SA	7	16	7	9	2	46	5	4
BM62-3 4	r	SA	7	15	8	9	3	58	4	1
BM62-3 4	cr	SA	8	17	8	9	3	43	6	4
BM62-3 5	l	SA	7	17	7	8	0	75	0	0
BM62-3 6	c	SA	5	15	7	9	4	59	0	0
BM62-3 5	c	SA	8	17	9	9	4	55	7	2
BM62-3 7	c	SA	8	17	11	9	3	73	1	2
BM62-3 4	cl	SA	7	18	8	9	2	64	1	6
BM40/180	l	SA	8	22	10	9	4	84	4	8
BM40/180	c	SA	8	17	11	10	3	77	8	8
BM40/180	r	SA	7	19	8	9	1	62	0	0
BM62-3 4	l	SA	8	13	9	10	2	58	9	3
BM62-3 6	l	SA	8	12	10	9	5	63	4	8
BM68/27		SA	10	19	8	8	7	50	24	18
30.5.69	r	SA	13	18	9	8	2	56	12	8
CM215a	l	SA	10	17	8	9	11	60	4	11
CM215a	c	SA	10	15	8	8	4	56	9	4
CM215a	r	SA	11	14	7	10	3	66	10	10
CM215b	l	SA	8	14	8	9	8	56	4	8
CM215b	c	SA	10	12	7	8	4	49	10	3
CM215b	r	SA	7	13	6	7	0	50	10	9

CM215c	l	SA	8	16	6	10	7	49	9	6
CM215c	c	SA	11	13	9	10	6	58	2	10
CM215c	r	SA	11	15	6	10	9	75	2	2
CM215e	tl	SA	7	14	8	8	4	51	4	0
BM62-3 5	r	SA	7	16	10	8	5	74	4	2
BM62-3 6	r	SA	5	12	11	9	3	60	8	5
BM62-3 7	l	SA	7	13	10	9	2	56	1	1
BM62-3 7	r	SA	6	17	10	9	2	65	0	0
BM62-3 8	l	SA	8	14	10	9	0	58	0	3
BM62-3 8	c	SA	6	15	10	8	1	60	0	0
BM62-3 8	r	SA	9	9	7	7	1	77	3	0
BM62-3 9	l	SA	9	18	12	9	4	50	0	0
BM62-3 9	c	SA	6	15	10	9	2	92	7	2
BM62-3 9	r	SA	9	18	8	9	2	62	3	8
CM215d	l	SA	9	14	10	8	5	55	15	11
CM215d	c	SA	9	13	7	8	6	50	6	13
CM215d	r	SA	6	14	7	9	6	47	6	5
CM195a	l	SO	6	10	8	10	3	32	2	1
CM195a	r	SO	9	16	6	9	5	37	6	4
CM195a	c	SO	5	12	8	9	5	36	0	0
Killington		SO	8	16	10	7	3	45	5	12
CM196a	tc	SO	10	16	8	9	10	36	2	2
CM196a	tl	SO	10	14	6	9	2	33	2	0
CM196a	tr	SO	7	15	7	9	6	35	1	1
CM196a	bl	SO	8	14	8	11	11	36	2	3
CM196a	br	SO	8	9	9	9	6	43	4	4
CM196b	l	SO	5	12	8	9	8	32	2	2
CM196b	c	SO	5	11	7	11	8	34	4	8
CM196b	r	SO	5	11	9	9	3	37	5	1
BM45/121b	tl	SX	7	13	5	9	5	31	2	2
BM45/121b	tr	SX	7	12	7	8	6	30	1	0
BM45/121b	bl	SX	7	13	9	11	6	41	1	1
BM45/121b	br	SX	5	16	8	9	0	31	2	2
BM45/121c	tl	SX	5	12	7	8	0	18	3	2
BM45/121c	tr	SX	10	12	6	10	6	34	1	0
CM36a		VV	11	17	8	9	9	32	4	10
CM36b		VV	8	15	8	9	6	31	0	9
CM36c		VV	11	14	7	12	8	44	5	20
CM36d		VV	10	18	7	12	4	45	3	31
CM36e		VV	11	17	8	10	4	46	5	11
CM36f		VV	8	17	10	11	9	42	3	8
CM36g		VV	12	20	8	11	5	40	1	8
CM36h		VV	8	16	7	9	9	24	1	7
CM36j		VV	7	17	10	11	5	30	0	4
CM36k		VV	11	22	7	8	6	53	3	10
G. Monroe	t	VV	11	15	7	10	6	39	22	7
G. Monroe	bl	VV	8	19	8	12	8	41	31	4
G. Monroe	br	VV	10	19	8	8	12	41	3	0
CM214a	l	VV	10	15	7	11	10	43	2	2
CM214a	cl	VV	12	17	7	10	7	44	2	0
CM214a	cr	VV	12	15	11	13	10	47	4	10
CM214a	r	VV	15	15	8	11	9	51	2	2
CM214b	l	VV	10	11	8	9	4	42	2	0
CM214b	cl	VV	11	16	7	11	12	37	0	0
CM214b	cr	VV	11	15	11	11	6	47	1	2

Appendix 3.3 continued

CM214b	r	VV	12	16	8	10	4	39	1	0
CM214c	l	VV	9	18	8	9	11	34	3	2
CM214c	cl	VV	7	14	8	11	6	47	1	1
CM38l		VV	10	15	7	9	8	37	2	25
CM38m		VV	10	20	8	10	11	50	3	25
CM38n		VV	12	18	9	10	5	51	2	14
CM38o		VV	10	19	10	11	4	64	5	32
CM38p		VV	11	18	7	10	8	54	3	6
CM38q		VV	12	19	10	10	10	50	8	20
CM38r		VV	11	18	10	10	6	65	4	39
CM38s		VV	14	17	9	9	9	53	2	16
CM38t		VV	11	19	8	10	4	44	3	18
EUROPE										
213.60		AG	12	24	6	9	8	83	4	0
124.73	tl	BP	11	21	10	8	6	60	0	0
124.73	br	BP	11	22	9	8	5	68	0	0
21.63a		BP	7	18	7	9	9	12	1	0
21.63b		BP	6	21	8	9	10	7	0	0
21.63c		BP	12	22	9	9	9	20	0	0
21.63d		BP	6	21	8	9	5	8	0	0
21.63e		BP	12	22	8	10	9	77	0	1
21.63f		BP	8	26	5	8	5	6	0	0
21.63		BP	11	10	4	9	6	42	0	6
58.70a	l	BP	10	15	8	9	5	65	1	0
58.70a	r	BP	9	21	7	8	8	52	2	2
58.70b		BP	10	22	9	8	6	74	0	1
250.71		BP	10	23	5	9	5	65	0	1
38.72	t	BP	11	26	6	11	7	80	4	0
38.72	bl	BP	12	28	6	7	4	22	0	0
38.72	br	BP	11	28	10	8	2	36	0	0
3.6.58Droz		BP	12	18	9	13	5	38	2	0
9.6.58Droz		BP	11	10	4	7	1	42	0	0
14.5.60Dro		BP	12	14	4	6	1	41	1	0
8.7.60Droz		BP	10	12	4	7	4	45	3	0
6.67	r	CY	8	19	4	8	2	30	6	0
6.67	c	CY	10	22	4	11	4	35	1	6
6.67	l	CY	7	21	4	10	4	27	2	4
139.65a		PO	10	20	11	8	9	111	15	0
139.65a	l	PO	10	17	11	9	8	119	31	0
139.65a	r	PO	10	20	8	8	6	113	5	0
3.64		PO	14	17	9	10	5	81	1	0
57.57a		PO	16	18	9	7	2	68	26	0
57.57b		PO	14	20	7	9	6	65	17	0
38/74a		PO	12	13	3	7	6	37	2	0
38/74b		PO	11	11	4	7	5	35	0	0
38/74c		PO	9	11	5	6	5	49	3	0
38/74d		PO	10	8	3	7	5	23	2	0
NEW ZEALAND										
2188/1981	b	VV	7	15	9	9	4	43	3	2

CM38u		VV	10	20	10	11	5	52	2	25
CM214c	r	VV	12	12	7	11	6	41	0	3
CM214c	cr	VV	13	15	13	12	2	47	1	1
CM214d	l	VV	11	16	9	12	6	34	1	1
CM214d	cl	VV	11	14	10	12	8	39	0	2
CM214d	cr	VV	11	17	8	10	10	28	0	0
CM214d	r	VV	9	16	7	11	6	36	0	0
CM214e	tl	VV	8	15	8	8	11	24	1	0
CM214e	tr	VV	8	12	9	10	8	26	0	0
CM214e	bl	VV	11	18	10	11	10	33	2	4
CM214e	bcl	VV	11	14	8	10	8	32	0	2
38/74e		PO	14	11	6	7	6	37	0	0
38/74f		PO	10	15	5	6	3	38	0	0
Droz7		RN	6	11	3	7	3	33	5	0
Droz8		RN	10	10	3	7	3	34	0	5
Droz9	t	RN	7	10	4	7	0	38	1	2
Droz9	b	RN	8	10	5	6	0	32	0	2
Droz10	t	RN	8	10	4	6	0	42	0	1
Droz10	bl	RN	9	9	3	6	1	36	4	1
Droz10	br	RN	9	10	4	6	1	34	3	0
Droz11		RN	8	8	4	7	2	42	2	0
H104	r	RN	10	17	10	6	5	66	8	8
BM62.3a		RN	13	19	11	12	0	25	0	0
BM62.3b		RN	15	20	13	12	0	22	0	0
BM62.3c		RN	13	17	8	12	0	20	0	0
BM62.3d		RN	13	18	7	13	0	26	3	1
BM62.3e		RN	13	21	6	7	0	23	0	0
N91	l	RN	7	20	9	7	5	74	2	26
N91	r	RN	6	18	10	9	6	43	4	2
862a		SA	15	18	8	9	0	27	3	5
862b		SA	16	16	7	10	0	20	0	2
862c		SA	12	15	8	9	0	13	0	0
N88		SP	15	20	6	11	10	65	14	18
30.685		SP	15	18	10	8	13	43	1	11
30.68	l	SP	11	22	10	9	2	55	3	17
17.65	tl	VV	11	17	9	12	5	90	25	0
17.65	tc	VV	12	19	9	12	6	74	13	0
17.65	tr	VV	14	15	10	10	3	46	25	0
17.65	bl	VV	9	20	11	8	2	51	12	0
17.65	bc	VV	16	15	9	9	2	48	26	0
17.65	br	VV	15	19	9	11	6	75	12	1
1441c	r	VV	16	15	9	9	3	46	26	0
1441c	l	VV	16	19	9	11	6	75	12	1
1438c	l	VV	11	17	9	12	5	90	25	0
2188/1981	t	VV	8	11	6	10	7	30	2	2

Appendix 3.3 continued

NORTH AMERICA			
19.3.56	l	BP	11 15 7 9 0 44 9 0
19.3.56	r	BP	10 12 7 10 0 51 7 0
B3C1425.1	l	CM	11 13 7 8 1 31 0 4
B3C1425.1	m	CM	12 14 8 10 4 38 0 1
B3C1425.1	r	CM	11 12 8 9 7 29 0 6
B3C1425.2	l	CM	8 10 8 8 7 47 1 12
B3C1425.2	cl	CM	11 11 9 10 3 41 5 9
B3C1425.2	cr	CM	11 14 9 11 3 38 1 21
B3C1425.2	r	CM	11 11 9 9 3 41 1 16
B3C1425.3	cl	CM	10 9 11 10 6 27 0 5
B3C1425.3	cr	CM	11 12 9 9 5 36 2 9
D23.481	c	CM	11 10 6 9 0 20 1 4
D23.481	cl	CM	11 14 8 10 8 41 1 9
D23.481	ccr	CM	10 11 6 9 3 28 0 1
BM60/1	r	PP	11 18 12.11 4 58 0 1
CAN	c	PP	12 13 10 10 7 37 4 3
CAN	l	PP	10 19 10 9 3 57 2 1
195680c	l	PP	9 11 8 10 6 59 0 0
195680c	r	PP	9 10 9 9 2 58 1 0
62E7.38		PU	10 10 4 10 11 44 1 9

1.4.59			
A1	r	PY	8 17 8 9 2 29 0 3
A1	l	PY	11 16 12 10 2 46 0 28
A2	r	PY	11 17 9 8 4 43 0 19
A2	l	PY	7 14 5 9 4 22 0 1
A3	l	PY	8 17 6 11 8 30 0 3
A3	r	PY	10 14 8 10 9 34 1 15
A4	l	PY	8 11 7 9 0 23 0 8
A4	r	PY	8 14 7 7 2 20 0 8
A5	l	PY	11 16 7 10 2 26 4 20
A5	r	PY	11 16 12 9 2 38 0 33
A6	l	PY	11 12 7 9 5 30 1 9
A6	r	PY	10 10 9 9 2 34 1 11
A7	l	PY	11 16 7 10 8 38 0 31
A7	r	PY	11 17 11 10 2 24 1 12
A8	r	PY	13 15 12 12 7 45 1 21
5726	l	RS	9 15 7 12 9 58 1 1
5726	c	RS	9 15 10 11 9 52 2 2
5726	r	RS	10 10 8 11 4 50 2 3
87/162		SA	10 8 6 8 0 26 0 14

Appendix 4.1
Morphological data recorded from parasitised and unparasitised Pulvinaria vitis reared on Ribes nigrum

Key: Ref. = slide reference number; Pos. = position of specimen on slide where t = top, b = bottom, l = left, r = right and c = centre.

Numbers for the morphological characters are those used in Section 4.2.3

Ref.	Pos.	Morphological character 1	2	3	4	5	6	7	8	9	10	11	12	13
Unparasitised														
HT1a	tl	165	78	372	222	287	10	14	6	9	6	39	2	9
HT1a	tc	163	83	346	222	280	11	14	8	8	4	47	10	7
HT1a	tr	160	87	365	222	280	9	10	8	9	7	44	8	14
HT1a	bl	159	78	378	235	300	9	13	5	9	8	35	14	20
HT1a	bc	176	93	463	267	333	12	7	7	9	7	54	14	19
HT1a	br	160	78	333	228	293	11	14	8	8	5	40	5	11
HT1b	tl	176	88	430	254	295	12	11	7	9	3	64	12	14
HT1b	tc	183	98	443	270	339	11	7	8	9	4	58	9	13
HT1b	tr	170	93	430	228	293	9	13	5	8	5	28	0	4
HT1b	bl	190	104	485	287	352	12	17	9	9	2	79	26	38
HT1b	br	183	98	443	261	326	10	11	7	9	8	52	15	34
RSyl	tr	170	85	365	248	313	10	11	8	9	3	56	9	17
RSyl	bl	170	91	378	248	300	8	12	7	8	7	46	10	12
RSyl	br	170	104	430	261	316	11	11	5	10	5	49	6	9
HT2.1	br	156	78	362	222	274	8	12	8	9	5	43	21	30
HT2.3	bl	160	88	385	228	287	10	13	7	8	6	48	14	10
HT2.2	tr	192	98	443	274	323	11	11	6	9	5	54	25	30
HT2.2	tl	170	85	424	235	287	10	12	8	11	5	6	25	30
HT2.2	br	156	78	350	222	274	9	11	4	10	8	35	12	12
Parasitised														
RSyl	tl	176	91	450	241	300	8	14	4	9	4	53	7	13
RSyl	tc	140	78	365	202	261	8	12	6	8	8	47	7	14
HT2.1	tl	193	98	404	267	319	13	19	12	5	4	61	12	9
HT2.1	tc	145	76	339	215	267	11	10	6	8	0	37	9	4
HT2.1	tr	176	95	404	254	302	10	13	9	8	6	49	9	7
HT2.1	bl	165	91	372	235	300	8	12	8	7	6	47	5	4
HT2.1	bc	170	85	372	254	313	10	17	6	8	4	66	27	13
HT2.3	tl	156	85	380	222	274	9	12	7	7	4	29	9	8
HT2.3	tr	150	78	376	215	261	8	8	6	9	1	54	10	13
HT2.3	br	156	78	350	218	267	9	15	8	8	1	48	20	9
HT2.2	bl	160	85	359	222	278	8	13	6	9	9	32	13	17

Appendix 5.1
Morphological data recorded from Pulvinaria vitis reared in the host transfer experiments

Key: Trans. = transfer between hosts; Ref. = slide reference number; Pos. = position of specimen on slide where l = left, c = centre, r = right, t = top and b = bottom.

Abbreviations for host plant species are those given in Section 5.2.2 and the numbers for the morphological characters are those used in Section 3.2.5

Ref.	Pos.	Trans.	Character 1	2	3	4	5	6	7	8
237a	tl	BP bp	12	21	10	9	9	60	5	17
237a	tc	BP bp	11	23	13	10	7	57	4	17
237a	tr	BP bp	14	26	9	11	6	58	5	29
237a	bl	BP bp	11	21	10	10	6	58	5	14
237a	br	BP bp	12	21	13	10	9	51	3	3
274a	tl	BP bp	11	13	7	9	4	61	8	4
274a	tc	BP bp	8	18	6	8	3	64	6	9
274a	tr	BP bp	9	18	8	10	7	62	6	9
274a	bl	BP bp	9	16	8	9	2	83	15	11
274a	tr	BP bp	10	14	9	10	2	75	23	5
274b	tl	BP bp	7	16	9	10	6	56	10	7
274b	tr	BP bp	7	16	9	11	9	55	1	3
274b	bl	BP bp	8	17	8	10	5	54	16	4
274a	bc	BP bp	8	13	7	9	4	54	17	3
274a	br	BP bp	11	19	10	11	4	64	4	1
HT2	tc	BP bp	12	22	10	9	5	49	10	4
HT2	tr	BP bp	10	26	12	9	5	48	0	1
238	tl	BP cm	8	12	7	8	7	54	3	3
238	tcl	BP cm	9	18	10	8	9	75	7	11
238	tc	BP cm	8	13	11	9	9	50	0	1
238	tcr	BP cm	9	14	9	10	6	56	0	1
238	tr	BP cm	10	14	9	8	11	55	0	3
238	bl	BP cm	8	10	8	9	1	43	1	2
238	bcl	BP cm	7	13	10	9	9	48	0	1
238	bc	BP cm	8	12	6	10	10	38	0	0
238	bcr	BP cm	10	12	7	8	9	57	1	3
238	br	BP cm	10	14	7	8	7	47	1	1
HT2		BP cm	9	23	8	9	7	63	24	5
154	l	BP rn	12	21	11	10	4	56	10	5
154	l	BP rn	10	24	4	9	3	47	1	0
154	r	BP rn	10	24	9	9	3	52	0	0
157a	tc	BP rn	10	19	11	10	5	52	2	11
157a	bc	BP rn	8	20	8	9	2	61	4	6
157a	b	BP rn	9	22	11	8	2	32	0	3
155a	tl	BP rn	11	20	9	9	4	62	4	8
155a	tr	BP rn	11	24	12	7	3	65	0	4
155a	bl	BP rn	8	19	11	10	7	72	1	2
155a	br	BP rn	8	20	11	9	5	76	7	20
236		BP rn	12	18	8	9	3	66	8	11
155b	tl	BP rn	8	25	11	8	5	82	11	28
155b	tr	BP rn	9	21	9	9	7	66	4	13
155b	bl	BP rn	10	17	13	9	3	54	1	6
155b	br	BP rn	9	20	11	9	5	58	5	3
157a		BP rn	11	18	10	9	4	65	3	3

Ref.	Pos.	Trans.	Character 1	2	3	4	5	6	7	8
275	tl	BP sa	9	18	10	9	8	85	19	10
275	tc	BP sa	9	19	8	8	4	45	3	5
275	bl	BP sa	8	15	6	10	8	50	10	22
HT2A4	tl	BP sa	11	23	13	9	10	93	8	19
HT2A4	tc	BP sa	15	16	8	8	7	76	3	2
HT2A4	tr	BP sa	9	14	11	10	4	77	3	1
HT2A4	bl	BP sa	11	19	8	9	3	84	6	4
HT2A4	bc	BP sa	12	18	10	9	3	81	4	11
HT2A4	br	BP sa	13	20	10	9	3	80	6	2
HT2B4	tl	BP sa	12	23	13	9	8	66	5	4
HT2B4	tr	BP sa	11	21	6	8	7	77	6	5
HT2T4	bl	BP sa	10	18	10	9	8	62	10	5
HT2T4	br	BP sa	11	18	11	9	8	78	3	5
240a	tl	BP sa	11	14	9	10	10	60	36	32
240a	tc	BP sa	12	20	9	10	9	60	21	50
240a	tr	BP sa	12	20	10	10	5	52	11	16
240a	bl	BP sa	11	23	12	9	4	60	6	17
240a	br	BP sa	12	17	10	11	9	57	24	60
165a	tl	BP vv	15	29	4	9	6	37	4	17
165a	tr	BP vv	13	32	10	11	6	78	13	39
165a	bl	BP vv	12	29	10	9	7	76	11	44
165a	br	BP vv	15	25	9	9	2	74	3	20
165b	tl	BP vv	11	28	9	7	1	61	8	64
165b	tr	BP vv	11	30	11	11	9	66	10	31
165b	bl	BP vv	15	28	10	10	7	58	2	15
165b	bc	BP vv	14	29	12	9	3	71	10	22
165b	br	BP vv	12	26	8	9	2	54	4	12
239		BP vv	12	32	10	8	4	69	10	11
148a		BP vv	12	28	11	10	8	78	20	51
HT2		CM bp	9	16	6	7	0	48	7	5
38z		CM cm	6	15	9	9	11	45	1	9
38		CM cm	10	15	9	9	1	32	2	5
38x		CM cm	10	17	6	8	5	42	1	11
38w		CM cm	8	14	6	9	3	40	0	2
38v	l	CM cm	9	14	6	8	7	45	5	0
38s		CM cm	8	13	6	9	4	36	3	8
38r		CM cm	10	13	7	9	5	47	7	5
38q	l	CM cm	8	12	7	7	6	44	1	2
38q	r	CM cm	6	16	7	7	2	28	1	2
HT1a	tl	CM rn	10	14	6	9	6	39	2	9
HT1a	tc	CM rn	11	14	8	8	4	47	10	7
HT1a	tr	CM rn	9	10	8	9	7	44	8	14
HT1a	bl	CM rn	9	13	5	9	8	35	14	20
HT1a	bc	CM rn	12	7	7	9	7	54	14	19

Appendix 5.1 continued

HT1a	br	CM	rn	11	14	8	8	5	40	5	11
HT1b	tl	CM	rn	12	11	7	9	3	64	12	14
HT1b	tc	CM	rn	11	7	8	9	4	58	9	13
HT1b	tr	CM	rn	9	13	5	8	5	28	0	4
HT1b	bl	CM	rn	12	17	9	9	2	79	26	38
HT1b	br	CM	rn	10	11	7	9	8	52	15	34
RSyl	tl	CM	rn	8	14	4	9	4	53	7	13
RSyl	tc	CM	rn	8	12	6	8	8	47	7	14
RSyl	tr	CM	rn	10	11	8	9	3	56	9	17
RSyl	bl	CM	rn	8	12	7	8	7	46	10	12
RSyl	br	CM	rn	11	11	5	10	5	49	6	9
HT2.1	tl	CM	rn	13	19	12	5	4	61	12	9
HT2.1	tc	CM	rn	11	10	6	8	0	37	9	4
HT2.1	tr	CM	rn	10	13	9	8	6	49	9	7
HT2.1	bl	CM	rn	8	12	8	7	6	47	5	4
HT2.1	bc	CM	rn	10	17	6	8	4	66	27	13
HT2.1	br	CM	rn	8	12	8	9	5	43	21	30
HT2.3	tl	CM	rn	9	12	7	7	4	29	9	8
HT2.3	tr	CM	rn	8	8	6	9	1	54	10	13
HT2.3	bl	CM	rn	10	13	7	8	6	48	14	10
HT2.3	br	CM	rn	9	15	8	8	1	48	20	9
HT2.2	tl	CM	rn	11	11	6	9	5	54	25	30
HT2.2	tr	CM	rn	10	12	8	11	5	6	25	30
HT2.2	bl	CM	rn	8	13	6	9	9	32	13	17
HT2.2	br	CM	rn	9	11	4	10	8	35	12	12
HT1.1		CM	sa	10	14	7	10	5	56	25	11
HT2.1	tr	CM	sa	11	9	7	9	9	55	16	23
HT2.1	br	CM	sa	12	14	8	9	6	42	20	15
166	tl	PP	ag	8	17	7	8	8	46	1	15
166	tc	PP	ag	10	16	5	8	10	55	1	7
166	tr	PP	ag	9	14	10	10	8	45	7	2
166	bl	PP	ag	9	14	11	10	8	51	3	20
166	br	PP	ag	9	16	8	10	10	42	3	22
266	l	PP	ag	9	11	8	9	12	39	1	6
266	r	PP	ag	9	12	9	9	10	52	5	6
159a	tr	PP	bp	8	12	8	8	7	43	1	3
159a	bl	PP	bp	8	11	10	9	5	46	8	3
159a	bc	PP	bp	9	17	8	9	5	54	4	5
159a	br	PP	bp	8	19	8	9	4	44	2	0
159b	bl	PP	bp	10	17	8	9	5	44	2	3
159b	bcl	PP	bp	8	19	5	9	4	50	3	2
159b	bcr	PP	bp	9	16	8	8	7	47	5	5
159b	br	PP	bp	9	14	8	9	1	47	2	7
159b	tl	PP	bp	11	17	8	8	6	47	4	1
159b	tcl	PP	bp	10	12	8	11	7	40	2	2
159b	tcr	PP	bp	10	12	9	9	10	43	0	0
159b	tr	PP	bp	11	14	7	10	8	42	3	0
151	bl	PP	bp	9	13	9	10	8	47	0	6
151	br	PP	bp	9	17	5	11	7	41	0	0
167a	tl	PP	cm	9	13	8	10	6	64	0	0
167a	tc	PP	cm	10	13	10	10	10	45	0	0
167a	tr	PP	cm	9	15	11	10	9	55	0	0
167a	bl	PP	cm	9	14	7	10	11	51	0	0
167a	br	PP	cm	9	15	10	9	10	43	1	1
167b	tl	PP	cm	8	15	9	10	12	47	3	0
167b	tc	PP	cm	11	15	11	9	9	52	1	0

167b	tr	PP	cm	12	13	5	9	10	40	0	0
167b	bl	PP	cm	9	14	7	10	9	50	1	0
167b	br	PP	cm	9	10	8	9	10	51	1	5
156	l	PP	cm	10	15	9	9	10	53	0	0
156	r	PP	cm	8	16	10	9	9	49	0	0
168a	l	PP	pp	10	16	9	10	3	68	7	8
168a	c	PP	pp	11	14	11	9	5	73	14	10
168a	r	PP	pp	12	9	8	11	2	78	10	3
168b	l	PP	pp	11	11	9	9	8	51	8	4
168b	c	PP	pp	12	15	10	8	5	58	4	2
168b	r	PP	pp	12	16	7	9	10	64	9	2
242	l	PP	pp	12	16	9	10	7	59	13	10
242	r	PP	pp	10	17	7	9	10	61	7	5
HT1		PP	pp	11	15	8	8	8	55	3	3
170a	tl	PP	rn	10	13	5	9	3	47	1	4
170a	tc	PP	rn	10	10	5	9	5	36	0	3
170a	tr	PP	rn	10	17	9	9	2	45	1	2
170a	bl	PP	rn	9	13	6	9	10	45	0	1
170a	br	PP	rn	12	18	7	9	8	65	0	2
170b	tr	PP	rn	10	17	8	9	6	62	2	2
170b	bl	PP	rn	10	15	9	10	3	52	0	4
170b	bcr	PP	rn	7	14	8	9	6	36	1	4
170a	br	PP	rn	12	13	8	10	6	51	1	3
243	r	PP	rn	11	14	4	8	6	51	9	12
243	l	PP	rn	10	19	6	8	8	62	25	27
153	tl	PP	sa	10	9	4	9	4	65	3	4
153	tc	PP	sa	11	16	8	10	7	63	10	12
153	tr	PP	sa	12	14	10	8	10	63	12	4
153	bl	PP	sa	10	18	10	10	5	62	13	16
153	br	PP	sa	10	16	8	11	12	64	18	15
267	tl	PP	sa	10	16	8	9	8	52	10	10
267	tr	PP	sa	11	14	7	9	6	60	15	12
267	bl	PP	sa	10	15	9	9	6	43	0	2
267	bc	PP	sa	9	12	6	9	5	47	4	7
267	br	PP	sa	9	13	7	8	11	45	2	5
169	tl	PP	sa	9	14	8	9	5	75	17	22
169	tc	PP	sa	9	16	7	9	7	74	13	15
169	tr	PP	sa	10	18	10	10	6	57	3	12
169	bl	PP	sa	9	15	9	10	5	54	18	20
169	br	PP	sa	8	17	8	9	5	74	5	7
244	l	PP	sa	8	16	10	9	6	43	4	10
244	r	PP	sa	9	14	7	10	11	41	10	8
137a		PP	vv	9	18	7	10	8	50	5	14
137b		PP	vv	11	16	6	10	3	38	4	12
137c	l	PP	vv	12	14	8	12	6	55	3	24
137c	r	PP	vv	10	16	7	9	8	53	2	0
139a		PP	vv	12	14	11	9	11	48	8	14
139b	l	PP	vv	12	14	11	10	10	42	3	12
139b	r	PP	vv	9	17	9	10	11	59	13	10
139c	l	PP	vv	14	18	9	10	14	41	6	3
139c	r	PP	vv	10	16	10	10	9	48	6	8
139d	r	PP	vv	13	17	10	9	12	35	4	7
139d	l	PP	vv	9	21	10	11	7	48	9	12
142a	tl	PP	vv	10	16	8	8	11	60	12	7
142a	tr	PP	vv	10	19	10	12	9	43	12	20
142a	bl	PP	vv	12	21	10	9	9	40	12	6

Appendix 5.1 continued.

142a	br	PP	vv	11	12	9	11	9	50	4 6
142b	tl	PP	vv	11	18	8	12	10	60	3 21
142b	tr	PP	vv	10	16	9	11	11	55	13 17
142b	bl	PP	vv	9	17	11	10	10	46	8 13
142b	br	PP	vv	9	16	10	12	8	42	3 20
161a	tl	PP	vv	11	14	9	11	9	56	10 3
161a	tr	PP	vv	10	17	10	11	6	40	6 5
161a	b	PP	vv	12	14	10	10	6	38	6 20
161b	l	PP	vv	11	18	8	12	12	46	6 12
161b	r	PP	vv	15	16	10	10	9	42	6 0
149	tl	RS	cm	10	13	7	9	10	47	0 0
152a	tl	RS	cm	9	14	11	9	6	46	0 2
152a	tr	RS	cm	9	15	9	8	7	39	0 11
152a	bl	RS	cm	8	11	11	8	6	37	0 0
152a	br	RS	cm	9	15	11	8	6	46	2 0
152b	l	RS	cm	9	14	11	9	5	45	0 0
152b	c	RS	cm	10	12	12	9	8	59	0 0
152b	r	RS	cm	8	13	13	8	8	41	0 2
HT1	l	RS	cm	8	11	8	9	9	51	1 0
HT1	c	RS	cm	8	13	10	8	4	36	1 3
HT1	r	RS	cm	9	11	9	8	7	47	0 2
246	l	RS	cm	8	12	10	9	7	37	1 14
246	cl	RS	cm	9	14	10	8	6	44	0 2
246	c	RS	cm	12	14	11	9	11	44	1 8
246	cr	RS	cm	8	15	11	9	6	49	0 11
246	r	RS	cm	9	12	8	8	4	35	1 0
HT1a	tl	RS	rn	7	14	10	8	8	44	2 5
HT1a	tc	RS	rn	8	11	9	8	9	39	1 16
HT1a	tr	RS	rn	8	12	9	9	9	37	0 10
HT1a	bl	RS	rn	8	12	7	8	9	48	2 5
HT1a	bc	RS	rn	10	11	10	8	5	43	3 3
HT1a	br	RS	rn	7	11	5	8	4	45	7 6
HT1b	tl	RS	rn	8	11	7	9	7	33	2 0
HT1b	tr	RS	rn	9	11	8	9	7	43	2 5
HT1b	bl	RS	rn	9	12	7	8	5	37	4 0
HT1b	bc	RS	rn	8	12	7	8	5	46	3 3
HT1b	br	RS	rn	7	9	7	7	6	40	2 4
247a	tl	RS	rn	7	10	9	8	6	48	1 8
247a	tc	RS	rn	9	12	6	9	6	52	1 7
247a	tr	RS	rn	9	14	7	9	6	48	5 20
247a	bl	RS	rn	10	11	9	8	6	53	4 7
247a	bcl	RS	rn	11	12	7	9	8	49	6 6
247a	bcr	RS	rn	10	13	11	9	4	41	4 7
247a	br	RS	rn	10	11	9	9	7	48	10 22
247b	tl	RS	rn	10	14	8	9	4	47	3 1
247b	tr	RS	rn	11	11	8	9	5	56	5 10
247b	bl	RS	rn	9	13	10	8	2	42	1 1
247b	bc	RS	rn	9	14	8	8	4	51	7 21
247b	br	RS	rn	8	13	8	8	6	49	4 4
282a	tl	RS	sa	10	13	8	9	4	53	1 4
282a	tr	RS	sa	9	12	10	9	7	47	5 6
282a	bl	RS	sa	8	13	9	9	11	56	5 1
282a	bc	RS	sa	8	13	8	8	8	51	4 3
282a	br	RS	sa	8	17	9	7	4	48	7 13
282b	tl	RS	sa	10	12	8	8	6	43	6 1
282b	tr	RS	sa	8	12	9	8	6	68	4 11

282b	bl	RS	sa	10	11	8	8	5	52	3 10
282b	bc	RS	sa	9	14	10	9	8	54	3 5
282b	br	RS	sa	9	16	10	10	10	56	3 8
245a	tl	RS	sa	9	14	8	9	7	62	7 12
245a	tr	RS	sa	10	13	8	10	8	45	3 6
245a	bl	RS	sa	9	14	8	8	6	46	5 9
245a	bc	RS	sa	9	15	6	7	7	56	4 2
245a	br	RS	sa	11	15	10	8	9	58	1 5
245b	tl	RS	sa	9	11	8	9	7	71	3 9
245b	tr	RS	sa	8	15	6	8	5	54	2 2
245b	bl	RS	sa	10	14	9	10	9	77	14 36
245b	bc	RS	sa	9	12	8	9	4	38	6 12
245b	br	RS	sa	10	12	7	10	7	9	8 24
164	-	RS	vv	10	14	9	8	8	54	4 5
VV1.1	tl	VV	bp	11	11	10	9	2	46	1 2
VV1.1	tc	VV	bp	11	19	8	11	6	43	6 9
VV1.1	tr	VV	bp	11	14	9	9	3	47	3 1
VV1.1	cl	VV	bp	9	14	9	10	9	45	1 1
VV1.1	ccl	VV	bp	9	15	8	10	10	43	4 5
VV1.1	ccr	VV	bp	12	19	7	9	9	56	3 7
VV1.1	cr	VV	bp	13	16	9	10	4	50	7 4
VV1.1	bl	VV	bp	10	14	9	9	6	49	2 2
VV1.1	bc	VV	bp	10	10	4	9	2	40	2 5
VV1.1	br	VV	bp	10	12	10	9	7	41	3 0
263a	tl	VV	bp	9	12	7	9	4	46	2 0
263a	tr	VV	bp	11	14	7	10	7	50	0 1
263a	bl	VV	bp	10	12	8	10	8	52	4 2
263a	br	VV	bp	9	13	8	8	7	45	5 1
263b	tl	VV	bp	12	14	6	10	3	38	4 3
263b	tr	VV	bp	9	15	7	9	10	42	7 4
263b	bl	VV	bp	11	16	7	9	5	47	10 1
263b	br	VV	bp	10	13	7	9	4	38	8 8
VV2.a	tl	VV	cm	8	9	8	9	6	21	0 2
VV2.a	tc	VV	cm	9	10	8	9	6	18	0 1
VV2.a	tr	VV	cm	9	10	7	9	6	20	0 0
VV2.a	br	VV	cm	8	12	9	9	10	28	2 1
VV2.a	bl	VV	cm	8	15	8	10	10	25	1 2
VV2.b	tl	VV	cm	9	15	8	9	12	32	2 1
VV2.b	tc	VV	cm	9	11	7	9	4	16	0 0
VV2.b	tr	VV	cm	9	10	6	8	4	17	0 4
VV2.b	bl	VV	cm	8	11	8	6	5	18	0 1
VV2.b	bcl	VV	cm	9	11	8	9	4	17	0 0
VV2.b	bcr	VV	cm	7	11	7	8	4	16	0 0
VV2.b	br	VV	cm	9	11	6	8	6	16	0 0
VV1.1	l	VV	rn	8	15	8	9	6	46	22 27
VV1.1	c	VV	rn	9	15	5	9	6	71	13 13
VV1.1	r	VV	rn	8	12	6	9	2	54	3 10
VV1.a	tl	VV	sa	7	14	9	12	4	59	3 2
VV1.a	tr	VV	sa	9	14	7	11	1	61	6 6
VV1.a	bl	VV	sa	10	17	11	10	7	56	11 26
VV1.a	bc	VV	sa	9	17	9	9	1	52	0 0
VV1.a	br	VV	sa	9	17	10	9	6	54	0 2
VV1.b	tl	VV	sa	9	16	8	9	9	50	0 0
VV1.b	tc	VV	sa	10	16	11	11	9	50	0 0
VV1.b	tr	VV	sa	7	18	8	10	9	59	1 2
VV1.b	bl	VV	sa	11	18	9	10	4	74	4 3

Appendix 5.1 continued

VV1.b	br	VV	sa	10	17	9	9	8	65	1	4
254c	l	VV	sa	10	17	7	9	7	77	6	10
254c	c	VV	sa	10	18	10	9	10	57	4	11
254c	r	VV	sa	11	14	8	9	9	80	6	8
254d	tl	VV	sa	10	15	8	10	4	51	7	6
254d	tr	VV	sa	12	14	6	10	5	76	6	17
254d	bl	VV	sa	11	15	8	9	7	54	7	4
254d	br	VV	sa	9	16	10	9	4	65	1	2
254e	tl	VV	sa	12	13	7	9	7	68	5	4
254e	tr	VV	sa	10	15	9	10	10	67	2	6
254e	bl	VV	sa	10	19	10	10	8	79	11	4
254e	br	VV	sa	12	18	10	11	6	52	7	6
146a		VV	vv	8	16	10	12	7	50	3	7
146a	tl	VV	vv	12	14	9	9	12	57	0	2
146a	tr	VV	vv	14	18	12	12	7	54	3	8
146a	bl	VV	vv	11	12	8	9	8	50	4	8
146a	br	VV	vv	15	16	10	12	10	79	9	1
146b	tr	VV	vv	10	20	9	9	10	57	0	3
146b	bl	VV	vv	10	15	9	9	8	56	5	5
146b	br	VV	vv	10	11	11	11	9	59	9	7
162a	l	VV	vv	15	12	7	10	4	41	1	2
162a	r	VV	vv	9	16	8	9	9	64	7	11
162b	tl	VV	vv	9	19	10	12	8	58	2	1
162b	tr	VV	vv	9	17	10	13	8	61	3	8
162b	bl	VV	vv	10	16	10	9	11	41	2	2
162b	br	VV	vv	10	15	9	12	10	64	5	0
HT1.1	tl	PC	cm	10	14	8	10	4	31	0	0
HT1.1	tcl	PC	cm	5	13	8	9	1	39	0	1
HT1.1	tc	PC	cm	5	16	8	11	8	28	0	0
HT1.1	tcr	PC	cm	5	9	7	9	6	30	0	1
HT1.1	tr	PC	cm	6	10	9	11	4	29	0	0
HT1.1	cl	PC	cm	6	13	8	12	6	35	1	0
HT1.1	ccl	PC	cm	7	10	8	10	6	34	0	2
HT1.1	cc	PC	cm	7	12	7	8	1	20	0	1
HT1.1	ccr	PC	cm	7	14	9	9	7	48	0	0
HT1.1	cr	PC	cm	7	16	6	11	3	44	0	0
HT1.1	bl	PC	cm	8	13	8	11	7	35	0	1
HT1.1	bcl	PC	cm	8	14	9	11	6	42	0	1
HT1.1	bcr	PC	cm	8	16	10	10	5	47	0	1
HT1.1	br	PC	cm	9	15	10	10	8	47	0	0
HT1	l	SA	rn	8	13	6	9	0	44	38	36
HT1	cr	SA	rn	8	13	7	9	1	37	9	12

Appendix 10.1 Morphological description of Pulvinaria vitis (L.) in Britain

Appearance of live adult female varies considerably with age, host-plant species and position on host. Teneral female pale beige, reddish-brown or dark-brown, occasionally with a raised pale-yellow longitudinal mid-dorsal band. Often mottled and sometimes with black stripes radiating from the mid-dorsum to the margin. Body becoming greatly convex, heavily sclerotised, uniform dark-brown and wrinkled at maturity. Ovisac white, large, strongly convex, attaining a length of 10.0mm, width 5.5mm and height 5mm.

Slide-mounted adult female ovate; length 1.1-8.5mm, width 0.9-6.9mm. Anal cleft length about 1/7 body length.

Dorsum. Derm heavily sclerotised in post-reproductive specimens and with clear circular or oval areas; many characters are not visible in such specimens. Anal opercula long, slender, triangular; length 110-213µm, width 55-123µm. Posterior margin longest, angles rounded. Each operculum bears 3-4 apical and 3 ventral ridge setae. Fringe setae numbering 2 pairs. Anal ring almost rectangular, length 64-72µm, width 60-68µm with 2 rows of translucent wax pores. Anal ring setae consist of 3 long, robust pairs, 1 shorter, slender pair and a fifth very fine pair that is only visible if the anal ring is everted. Small submarginal tubercles number 0-14. Preopercula pores circular or ovate, varying considerably in number, 7-170. Simple disc pores and minute filamentous ducts numerous, scattered. Setae straight, acute, scattered except for two longitudinal rows on mid-dorsum; length 4.0-15µm.

Margin. Setae spine-like, slender, straight or slightly curved at the tips, acute; length around anal flaps 42-84µm; length laterally 28-42µm. Setae arranged in a staggered alignment. Spiracular setae in groups of 3, conical, acute. Medial seta length 57-133µm, lateral setae length 15-72µm.

Venter. Antennae 8 segmented, rarely 6 or 7, length 257-542µm. Setae number on segments: I,3; II,2; III,0; IV,1; V,3; VI,1; VII,2; VIII,9-10. Eyes visible. Mouthparts well developed, total length 200-358µm, width 135-284µm. Legs well developed, normal. Hind leg femur + trochanter length 161-381µm, tibia + tarsus length 210-400µm. Tibiotarsal articulatory sclerosis present. Tarsal digitules long and slender with small apical swelling, length 63-82µm; claw digitules short, stout with broad apical club, length 43-57µm. Minute claw denticle sometimes present. Posterior spiracles length 100-120µm, width

80-82µm. Spiracular pores mostly quinquelocular, forming bands 2-4 pores wide in spiracular furrows, number 25-168, there being more in the posterior furrow than the anterior furrow. Multilocular pores with 7-13 loculi, most with 10 loculi, in vulvular area and in transverse rows on preceding abdominal segments. Microducts distributed mainly in a narrow ventral marginal band. Two distinct kinds of tubular duct; one with a slender knobbed filament and one with a thick flowery tipped filament. The thick-filamented tubular ducts occur mainly in 2 forms; one with the filament not much longer than the duct and one with the filament much longer than the duct. Tubular ducts distributed in a wide submarginal band from the antennal bases to the anal cleft; thick-filamented tubular ducts also sparsely distributed over the mid region. Body setae straight, tapering to acute points, scattered except for two longitudinal rows on the abdomen. One or more rows of longer submarginal setae. A pair of stout submedian setae on the last 3 abdominal segments. Interantennal setae number 4-5 pairs.

Mean body size and correspondingly the enumerated characters, vary with host-plant species. For example, characters recorded from specimens collected from birch, peach and grapevine are usually larger than the same characters recorded from specimens collected from hawthorn and currant.

Material examined is listed in Appendix 3.1.

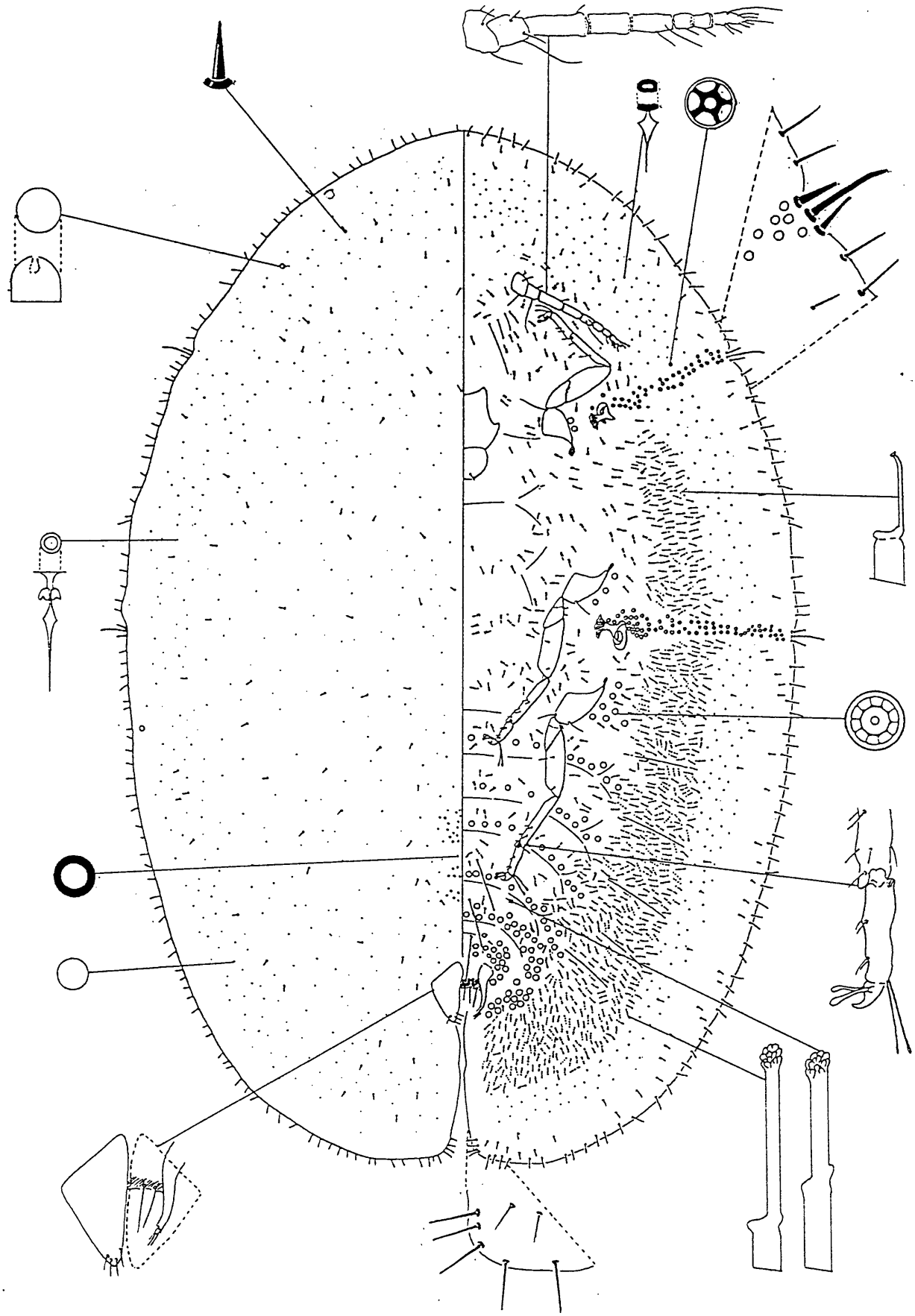

Fig. 10.1 Pulvinaria vitis (Linnaeus)