# CHAPTER THREE

## ORIGINS AND BIOGEOGRAPHY OF MACARONESIAN LEGUME-FEEDING PSYLLIDS


### Abstract

This study presents the first phylogenetic analysis of a phytophagous insect group from Macaronesia. Molecular, morphological and ecological data are used to investigate the origins of the 23 species in the Macaronesian islands by reassessing the classification of the five Palaearctic legume-feeding psyllid genera in the Arytaininae. Molecular phylogenies generated from two mitochondrial regions: part of the small ribosomal subunit rRNA (12S), and a second region incorporating part of cytochrome oxidase I (COI) and cytochrome oxidase II (COII), are analyzed independently and in combination with the morphological data generated from adult and nymphal characters. These analyses confirm the paraphyly of the Arytaininae in relation to the Psyllinae, as well as the paraphyly of the two largest arytainine genera (*Arytainilla* and *Livilla*) – both of which are represented by anomalous taxa in the Canary Islands. The phylogenetic results suggest there have been five colonizations of the Canary Islands by arytainine psyllids, one or two colonizations of Madeira, and probably one or two back colonizations of the continent. Optimization of the host plant genera onto the psyllid phylogeny suggests that preadaptation is important in determining host plant selection when new regions or islands are colonized. The psyllid phylogeny together with ecological and biogeographic data provides evidence, in at least one case, for sympatric speciation on the same host plant, while the majority of cases involve allopatric speciation via host shifts and inter-island colonization. A surprising number of closely related psyllids in the Canary Islands are found on the same host plant and there is evidence that the sharing of host resources is facilitated by geographical segregation, ecological specialization and divergence in the timing of development. Human activity and habitat alteration are implicated in both the reduction and expansion of host plant distributions, as well as the promotion of host plant hybridization, all of which may affect psyllid host preferences and distributions. Habitat and host population fragmentation may reach a critical host plant abundance, below which a host specific psyllid fauna can not survive.

## 3.1 Introduction

Ocean islands have discrete boundaries and vary in their size and isolation from other islands and from the mainland. These elements have been greatly advantageous in the study of evolution (MacArthur & Wilson, 1967). In addition to the unique physical characteristics of islands, there is a spectacular diversity of species which is often in sharp contrast with the mainland, of which the most famous examples are Darwin's Galapagos finches (Grant, 1986), and the Hawaiian *Drosophila* with more than 1000 island species (Kaneshiro, Gillespie & Carson, 1995). Recent work using a combination of approaches – ecological, morphological and molecular – has addressed the mechanistic questions raised by the evolutionary fecundity of islands (Wagner & Funk, 1995; Givnish & Sytsma, 1997; Grant, 1998).

There are common patterns in biotas from different island systems, yet processes common to all of these – dispersal, colonization, isolation and adaptation – are also present in mainland areas. However, a primary difference between oceanic islands and continents is that islands provide a 'terra nullus' or virtually unoccupied territory, with low levels of immigration and establishment. The natural filters to immigration – isolation, surrounding ocean, small size of islands – limits the number of colonists. For those that do become successfully established, the low level of immigration is likely to reduce the number of competitors and/or predators, and allow the successful colonists to undergo an 'ecological release', which may take the form of an adaptive radiation into a variety of niches.

The age and size of islands are important determinants of species diversity: smaller and younger islands are likely to have accumulated fewer immigrants than larger, older islands; but as islands age they are increasingly eroded, resulting in loss of habitat and extinction (MacArthur & Wilson, 1967; Carson & Clague, 1995). An important structural aspect of island size is altitude which, together with the prevailing climatic zone, determines the number and quality of habitat types (high islands accommodate more habitat types than low islands). Thus, a high altitude, large sized island, that is middle-aged would be expected to have the greatest species diversity (e.g. the island of Tenerife in the Canaries, Fig 1 & Table 1). The Hawaiian archipelago has been the location for the most comprehensive research to date on the colonization and speciation processes of a progressively older island chain (Wagner & Funk, 1995), but recently research has also focussed on the flora and fauna of the archipelagos of Macaronesia (Francisco-Ortega, Jansen & Santos-Guerra, 1996; Mes & 'T Hart, 1996; Pinto *et al*., 1997; Brunton & Hurst, 1998; Emerson, Oromí & Hewitt, 2000b; Juan *et al*., 2000).

Macaronesia was originally circumscribed, not as a geographical or political region, but as a unique phytogeographical concept (Sunding, 1979). Macaronesia includes five Atlantic Ocean archipelagos (north to south: Azores, Madeiras, Salvagens, Canaries, and Cape Verdes) which lie off the west coast of North Africa and southern Europe, between 15° and 40°N latitude. The geological ages of individual islands range from 1-30 Myr. The centrally positioned Canary Islands (27°-29°N) have proven particularly rewarding for evolutionary studies because they provide the most extreme ranges of altitude, habitat types, size, and age of islands (Fig. 1 & Table 1).

Research in both Pacific and Atlantic archipelagos has mainly focussed on plant and animal groups independently. Some studies have addressed plant-pollinator systems or the endozoochorous dispersal of plants (Percy & Cronk, 1997; Barrett, 1998; Givnish, 1998), and there has been research into the plant substrate-mediated radiation in Hawaiian *Drosophila* (Kambysellis & Craddock, 1997). But only rarely has research focussed on herbivorous insects and their host plants, such as studies by Asquith (1995) and Roderick (1997) of Hawaiian Hemiptera.

This is the first Macaronesian study to examine island evolution in a highly host specific group of phytophagous insects. Related continental groups are included to determine the origin of the island species and to provide a comparison of species diversity between island and continental regions. The insects – psyllids – are a group of small, sap-sucking Hemiptera, and (in this study) the host plants – brooms – are shrubby papilionoid legumes (Genisteae). Psyllids (Psylloidea, also known as 'jumping plant lice') feed on a wide variety of dicotyledonous and a few monocotyledonous plants, but are generally less well known than the other hemipteroid groups of the Sternorrhyncha: aphids (Apidoidea), scales (Coccoidea) and whiteflies (Aleyrodoidea), due to the relatively low occurrence of psyllid pests. Habitat and host specialization in psyllids makes them an ideal group for investigating evolutionary patterns which are associated with habitat or host shifts, and geographic isolation. The psyllid group selected for this study (the Genisteae-feeding Arytainininae) combines continental species that are locally restricted or widespread across Europe and North Africa, with taxa that are isolated on two of the central Macaronesian archipelagos (Canary Islands and Madeira) (Fig.1). Among the island taxa, species may be endemic to a single island or more widespread on islands of different geological ages.

### **3.1.1** *The arytainine psyllids*

The psyllids that feed on papilionoid legumes in the Genisteae are all in the Arytainininae, a subfamily of the Psyllidae. The delimitation of the subfamily Arytainininae is unclear,

particularly in relation to the subfamily Psyllinae (Heslop-Harrison, 1951, 1961; Loginova, 1976b, 1977; Hodkinson & Hollis, 1987). There are thirteen genera, distributed in both the Old and New World, that are considered to be in the Arytaininae; nine of these genera feed on host plants in the subfamily Papilionoideae (Leguminosae), and a further four, somewhat anomalous North American genera, feed on host plants in the families Rosaceae (two genera) and Rhamnaceae (two genera). Five of the genera are Palaearctic, all of them papilionoid legume-feeders, and four of these genera are confined to the west Palaearctic region and feed exclusively on host plants in the Genisteae. The Genisteae-feeding group will be the focus of this study. Diversification of the Genisteae in the Mediterranean basin, where these shrubs often form a dominant part of the vegetation, is likely to have been important in promoting the diversification of the associated psyllid group. There are around 96 arytainine species confined to this region and 91 of these feed on genistoid legumes. Represented in this study are all five Palaearctic arytainine psyllid genera – *Arytaina*, *Arytainilla*, *Livilla*, *Pseudacanthopsylla* (west Palaearctic, on genistoid legume hosts) and *Cyamophila* (Mediterranean to central Asia, on non-genistoid legumes). The monophyly of this Palaearctic group within the Arytaininae, is investigated in this study.

### **3.1.2** *The host plants*

The Genisteae (broom, gorse and relatives) is a monophyletic tribe of papilionoid legumes which has been the subject of a number of morphological revisions (Gibbs, 1967, 1974; Gibbs & Dingwall, 1972; Polhill, 1976; Bisby, 1981) and a recent molecular investigation (Käss and Wink, 1997). The lupins (*Lupinus*) are a large (c. 200 species), clearly delimited monophyletic group on which there are no recorded psyllid species. The remaining members of the Genisteae (c. 260 species in 20 genera) can be separated into three groups, a *Genista* group, a *Cytisus* group, and a number of generic ‘outliers’, and each of these groups has members that are psyllid hosts. However, about half of these genistoid genera do not have a psyllid fauna, which implies that there are constraints on intergeneric host switching.

The objectives of this study were: a) to test the monophyly of the Palaearctic psyllid genera in the subfamily Arytaininae, and in particular, the Genisteae-feeding subgroup; b) to investigate the taxonomic and biogeographic origin of the Macaronesian species; c) to investigate psyllid speciation using biogeographic patterns and host associations in the Macaronesian islands.

## 3.2 Materials and methods

### **3.2.1** *Ingroup selection*

The ingroup includes representatives of all Arytaininae genera that feed on legumes in the Palaearctic region (*Arytaina*, *Arytainilla*, *Cyamophila*, *Livilla* and *Pseudacanthopsylla*). All known species from the Macaronesian region were comprehensively sampled, and continental groups which were identified as possible sister groups to the island species were selectively sampled (Table 2, with additional details given in the Taxonomic Appendix). Multiple individuals are included where species occur on different islands/continental regions and/or different host plants. *Pseudacanthopsylla* is represented by a single species (= *Psylla improvisa*, see Taxonomic Appendix), and is a morphologically atypical arytainine genus that feeds on hosts in the Genisteae. *Pseudacanthopsylla* and *Cyamophila* (Arytaininae, but not Genisteae-feeding) are therefore included to test both the monophyletic origin of Genisteae-feeding and the monophyly of the Arytaininae. In addition, two species of *Cacopsylla* (Psyllinae) were included (but see discussion under outgroup selection).

### **3.2.2** *Outgroup selection*

Two subfamilies, Psyllinae and Acizziinae, were initially selected as possible outgroups for the subfamily Arytaininae, based on morphological affinity (Psyllinae) and host affinity (Acizziinae). Members of Acizziinae feed on mimosoid legumes in the tribes Acacieae and Ingae, while members of Psyllinae feed on a wide variety of predominantly non-leguminous plant families. Trial phylogentic analyses which included more distantly related taxa (e.g. *Psyllopsis*, *Livia* and *Trioza* spp., as well as *Schizaphis* (Aphididae)) using 12S rRNA data, indicated that the most suitable outgroup (based on topology and bootstrap values) for the Arytaininae was Acizziinae (*Acizzia*). These preliminary analyses affiliated the Psyllinae species with *Cyamophila* (Arytaininae) suggesting that the Psyllinae would be an unsuitable outgroup for the Arytaininae. Two species of Acizziinae were selected as the outgroup: one species native to North Africa and the Middle East (*Acizzia hollisi*) and one Australian species (*Acizzia uncatoides*). The two Psyllinae species (*Cacopsylla alaterni*, *C. mali*) were included to analyse the monophyly of the Arytaininae, but not defined as an outgroup.

**3.2.3** *DNA sampling and extraction*

Eighty-four individuals representing 62 taxa (61 species and one subspecies) are included in this study, and 17 of these species are undescribed, having been recently collected from Macaronesian and adjacent continental areas (these species are described and illustrated in Chapter 2). Based on the assessment of substitution rates within different cytochrome oxidase (CO) regions by Lunt *et al*. (1996) and Zhang & Hewitt (1997), faster evolving CO regions were selected to provide resolution between recently diverged species and intraspecific taxa (73 individuals from 50 species were sampled). The small subunit rRNA region (12S) was selected as a slower evolving region to resolve intergeneric and interspecific groups (68 individuals from 61 species were sampled). Fifty of the 61 species included are sampled for both molecular regions, but there are 11 continental species only sampled for 12S (10 *Livilla* spp. and *Arytainilla gredi*) (Table 2). Samples were collected during field work from 1997-2000 in the Canary Islands, Madeira and adjacent continental areas (except where indicated in Table 2). Insects were collected into 100% ethanol in the field and stored at -20°C. One to three insects (abdomens and wings removed and retained as vouchers) were either ground in 50µl of 80% SDS lysis buffer and 20% Proteinase K (10mg/ml), or alternatively whole insects were bisected and placed, with Proteinase K, in the buffer provided in the QIAGEN DNeasy Tissue Kit (in which case, the whole insect was retained as a voucher after incubation). Specimens were incubated for 24 hr at 55°C. The remainder of the extraction was performed with either the Geneclean II kit (Bio 101) in the first protocol, or the QIAGEN DNeasy Tissue Kit in the second. In both cases extracts were resuspended in 35µl of sterile water and stored at -20°C; 1µl of this solution was used for each 25µl PCR reaction. Specimens collected by the author were processed within three years, however, dry mounted specimens and alcohol preserved material up to 20 years old (supplied by Daniel Burckhardt and Ian Hodkinson) amplified successfully for the shorter 12S region (probably aided by highly conserved primers) but did not, or only poorly amplified for the longer CO region.

**3.2.4** *PCR and sequencing*

Amplification of the small ribosomal subunit (12S rRNA) fragment was accomplished using primers 12Sai and 12Sbi (equivalent to SR-N-14588 and SR-J-14233 respectively, of Simon *et al*., 1994). A single region incorporating the 3' end of COI, the tRNA leucine and the 5' end of COII was amplified for some taxa using primers UEA9 (Lunt *et al*., 1996) and ‘Marilyn’ (equivalent to C2-N-3389 of Simon *et al*., 1994). These CO primers amplified approximately 68% of the taxa. Additional primers were designed to amplify the remaining

taxa for the CO regions – DP1: 5'–GTTAGTAGTGGGTTATTAAGTTCRTC–3' (positioned in COII, and used as an antisense primer to UEA9, amplified approximately 30% of the taxa); DP2: 5'–CGATAATTTTAATTGTTAGTAGYGG–3' (also positioned in COII as an antisense primer to UEA9, amplified *Pseudacanthopsylla*); UEA9-MOD: 5'–GGTATGCCTCGTCGTTATTCTAAYTAYC–3' (positioned in COI, and used as a sense primer to 'Marilyn', amplified approximately 10% of the taxa). Appendix 1 gives the primer combinations used for DNA amplification from each species.

Each PCR cycle comprised denaturation at 94°C for one minute, followed by 92°C for 30 seconds (41 cycles), annealing at 45°C for 40 seconds and an extension of 65°C for 90 seconds, with a final extension of 72°C for 10 minutes. Amplified PCR products were either run on agarose gels and purified with a QIAGEN QIAquick Gel Extraction Kit or were purified with a QIAGEN QIAquick PCR Purification Kit, in both cases resuspension was in 30µl of $H_2O$ for direct sequencing using an automated Perkin-Elmer ABI 377 sequencer with ABI Prism Dye Terminator Cycle Ready Reaction Kit. All the sequences referred to here will be deposited in GenBank database.

### **3.2.5** *Alignment and sequence analyses*

Sequences were aligned manually using the program Se-Al (version 1.0a1; Rambaut, 1998). The alignment of the CO regions was unambiguous, with the codon position assignment determined by comparison to other insect sequences from GenBank. The 12S fragment was generally easy to align, but three regions from 3-11bp in length were ambiguous (positions 152-154, 254-264 and 300-305 of the aligned matrix) and these were excluded from all analyses. Secondary structures of the tRNA and 12S genes were referred to for improved alignment. Sequence characteristics are given in Table 3. Appendices 2-3 give the aligned matrices, and the matrices and trees (Figs 3-5) are available from TreeBASE (http://www.herbaria.harvard.edu/treebase/).

### **3.2.6** *Morphological sampling and treatment*

Forty-six species were sampled for the morphological analysis. The Macaronesian taxa were comprehensively sampled and selected taxa from continental sister groups were included. The only groups present in the molecular data but not represented in the morphological phylogeny are the non-Genisteae-feeding genera (*Cacopsylla* and *Cyamophila*). Morphological characters were obtained from alcohol, slide and capillary mounted material. Sixty-seven characters were scored for phylogenetic analysis, of which 43 were derived from adults and 24 were derived from 5$^{th}$ instar nymphs. Seventeen (39%)

of the adult characters and five (21%) of the nymphal characters are based on continuous morphometric data. These were partitioned for discrete character states determined by eye from graph plotted data, which incurred minimal polymorphism. The data were compiled using the program NDE (Nexus Data Editor; Page, 2000). Appendices 4-5 give the list of characters/character states and the data matrix, which are also available from TreeBASE.

### **3.2.7** *Phylogenetic analyses*

Phylogenetic analyses were performed with PAUP* (version 4.0b3; Swofford, 1999). The following heuristic search parameters were employed for the parsimony analyses: 100 random stepwise addition replicates with tree bisection-reconnection (TBR) branch swapping, other options included saving multiple trees (MULTREES) and collapsing zero-length branches (COLLAPSE). For the analysis of the morphological data, all morphometric characters were treated as ordered and multiple states as polymorphisms, in order to preserve information on the relative similarity between taxa and overlapping states as a result of the somewhat arbitrary data partitions. Bootstrap analyses (2000 replicates) were performed using simple addition sequence of taxa with TBR branch swapping. Congruence between the different types of data (12S, CO and morphology) were tested using the partition homogeneity test implemented in PAUP*. Parameters and assumptions used in the maximum likelihood (ML) searches were selected using program Modeltest (Posada & Crandall, 1998) based on the Akaike Information Criterion (AIC). The model selected for the 12S data was HKY85 with invariable sites and gamma distribution. The model selected for the CO data was general time-reversible with invariable sites and gamma distribution. ML heuristic search parameters included simple addition sequence of taxa with TBR branch swapping, MULTREES and COLLAPSE. Assumptions of monophyly (e.g. of the genera *Livilla* and *Arytainilla*), and particular biogeographic assumptions (i.e. continental versus island groups) in the 'Macaronesian clade', were tested using constraint trees and the nonparametric (Templeton's Wilcoxon signed-rank) test implemented in PAUP*.

### **3.2.8** *Mapping host plant relationships*

Host plant relationships were mapped, as an unordered character, onto the combined molecular phylogeny of the Genisteae-feeding psyllids using MacClade 3.07 (Maddison & Maddison, 1992) with accelerated transformation (ACCTRAN) optimization. One taxon (*Livilla pseudoretamae*), for which the host plant is unknown, was excluded.

## 3.3 Results

### **3.3.1** *Molecular evolution of the 12S small subunit rRNA*

The total aligned length of the 12S matrix is 342bp (of the 322 included sites, 168 were variable, of which 134 were parsimony informative), with A+T content 74-80% (further sequence details are given in Table 3). The equally weighted maximum parsimony (MP) search found 750 trees on five islands (length 807, CI 0.32 excluding uninformative characters, RI 0.614).

The 12S region alone provides poor resolution at the base of the phylogeny, and this may be compounded by the short segment of sequence (c. 321bp) and large number of samples (68). The 12S and CO data both show saturation of transitions relative to transversions (Fig. 2). However, the 12S region is evolving more slowly, relative to the CO region, and periods of rapid speciation are likely to be the cause of the poor resolution. Extremely short branch lengths at the base of the phylogeny and at the base of several clades using maximum likelihood (ML) and neighbour-joining (NJ) analyses (not shown) suggest there has been rapid speciation during the evolution of the Arytaininae.

Three major clades have bootstrap support greater than 75% (*Arytaina* – AR, the 'Macaronesian clade' – M, and the 'core *Livilla*' group – L; see Fig. 3A). The 12S data are equivocal on the distinction between the Arytaininae and Psyllinae. The placement of the morphologically atypical genus *Pseudacanthopsylla* is the most variable between the different islands of MP trees: consensus trees for two of the five islands place this taxon within the Genisteae-feeding Arytaininae (i.e. a single evolution of the Genisteae-feeding habit), while consensus trees for the other three islands place *Pseudacanthopsylla* basal to the remaining ingroup taxa, with the Psyllinae nested within the Arytaininae (i.e. multiple evolution of the Genisteae-feeding habit). The MP 12S tree in Figure 3A shows the topology most similar to that recovered after weighting the 12S data using the mean rescaled consistency index in PAUP*. Excluding *Pseudacanthopsylla* from the analyses in order to assess whether the remaining Genisteae-feeding genera were a well supported monophyletic group resulted in a strict MP consensus with the Genisteae-feeding Arytaininae monophyletic (but with low bootstrap support: 66%) and sister to the Psyllinae. The application of distance and likelihood methods does not resolve this conflict. NJ analysis using the HKY85 model (see methods) supports the inclusion of *Pseudacanthopsylla* in a monophyletic Genisteae-feeding clade with NJ bootstrap > 80%. However, the ML analysis using the same model of nucleotide substitution places *Pseudacanthopsylla* basal to the remaining Arytaininae and Psyllinae.

### **3.3.2** *Molecular evolution of the COI-tRNA-COII region*

The combined COI-tRNA-COII matrix provides 639 characters when aligned (352 variable sites, of which 297 are parsimony informative), with A+T content 67-83% (further sequence details are given in Table 3). There are no gaps in COI or COII and sequence length differences are due to differences in the length of readable sequence obtained with various primer combinations. The tRNA leucine is 65-71bp in length, providing 79 characters when aligned (positions 263-341 of the aligned COI-tRNA-COII matrix, see Appendix 3). A MP search found a single island of six trees (length 2280, CI 0.248 excluding uninformative characters, RI 0.613), one of which is shown in Figure 3B.

When compared to the 12S data, the combined CO regions (including the tRNA leucine) provide improved resolution and bootstrap support in parts of the phylogeny. Interspecific genetic divergence is generally 30-50% higher in the CO than in the 12S region: 'Macaronesian clade' – CO: 2-13%, 12S: 0-9%; *Arytainilla sensu stricto* – CO: 6-14%, 12S: 3-11%; *Arytaina* – CO: 2-15%, 12S: 2-10%. As with the 12S data, the CO regions also show saturation, but there is a linear relation between transitions and transversions at low levels of divergence (Fig. 2), suggesting that the CO data is more appropriate for resolving groups that have recently speciated. However, there is variation in the grouping of taxa in the 'Macaronesian clade' using different analyses (MP, NJ and ML) possibly as a result of short branch lengths at the base of the clade. As with the 12S data, the low accumulation of substitutions in several parts of the CO phylogeny suggests periods of rapid speciation. *Pseudacanthopsylla* is placed in all analyses (MP, NJ, ML) within the Genisteae-feeding Arytaininae.

### **3.3.3** *Morphological evolution*

MP analysis found a single island of 18 most parsimonious trees (length 554, CI 0.484 excluding a single uninformative character, RI 0.612). The number of morphologically discrete characters that were applicable to all taxa was, to a certain extent, limited by a combination of many closely related taxa with little interspecific variation, and populations of widespread taxa isolated on islands or fragmented on the continent exhibiting considerable intraspecific morphological variation. In general, nymphal morphology was more plastic and incured greater polymorphism than adult characters. Few of the nodes in the morphological phylogeny are well supported (Fig. 3C), but excluding polymorphic characters, or the subset of morphometric (continuous) characters, resulted in a loss of resolution. (Appendix 6 shows the unambiguous character state changes mapped onto the morphological phylogeny.) Nevertheless, the morphological phylogeny recovers many of

the groups present in molecular analysis – the 'Macaronesian clade' (M), *Arytaina* (AR) and *Arytainilla sensu stricto* (A), as well as similar paraphyletic groupings of *Livilla sensu lato*.

### **3.3.4** *Molecules and morphology combined*

The partition homogeneity test (ILD test of Farris *et al*., 1994) indicated that the molecular data sets (12S and CO) were compatible ($P = 0.99$), but that the molecular and morphological data were significantly incongruent ($P = 0.01$) with one another. A combined molecular MP analysis (12S and CO), including all taxa sampled for one or both regions, found eight trees (length 3112, CI 0.265 excluding uninformative characters, RI 0.609), one of which is shown in Figure 4. Recent criticism of the ILD test suggests that the inclusion of small data sets with a relatively high degree of noise (e.g. the morphological data in this analysis) may give significant ILD test results even in the absence of systematic incongruence (Dolphin *et al*., 2000). To investigate the presence of underlying similarities in the molecular (12S and CO) and morphological phylogenies, consensus trees (Strict and Adams) of the independent phylogenies were constructed (Fig. 5A & B). The lack of resolution in the strict consensus tree shows there is conflict in the MP topologies, but the Adams consensus is generally a more appropriate method for assessing underlying topological similarities, and it indicates considerable shared structure among the trees (Fig. 5B). Combining all three data sets in a total evidence MP analysis resulted in eight trees (length 2858, CI 0.342 excluding uninformative characters, RI 0.504), with the strict consensus showing clear delimitation and higher bootstrap support for several groups (e.g. *Livilla*, *Arytainilla sensu stricto*, and the Macaronesian *equitans* group) (Fig. 5C).

### **3.3.5** *The arytainine psyllids in the Canary Islands and Madeira*

The Macaronesian members of Arytaininae are from the Canary Islands and Madeira (Loginova, 1976a; Hodkinson, 1990). All the species from this region are endemic, and belong to three of the Genisteae-feeding genera (*Arytaina*, *Arytainilla* and *Livilla*). There are six genera of Genisteae on these islands, five of which host between one and 14 psyllid species. On the five central and western Canary Islands there are 21 arytainine species (four to 11 species per island). There are no native members of the host plant group on the dry, eastern islands of Fuerteventrua and Lanzarote. The island of Madeira (equivalent in area to La Palma, which has nine Genisteae species and seven arytainine psyllids) has only

two arytainine psyllids and two native legumes in the Genisteae – the only Macaronesian island with a one-to-one legume to psyllid ratio (Fig. 1 & Table 1).

After recent sampling in the Macaronesian region, it is clear that a substantial radiation has resulted in a group which includes the two Madeiran taxa, 16 Canary Island species and three continental taxa referred to as the 'Macaronesian clade' due to the probable origination of this group in Macaronesia. This clade is by far the most speciose psyllid group in the Macaronesian region with no more than three species found in any other generic groups. All of the 'Macaronesian clade' species are presently in *Arytainilla* (Loginova, 1972, 1976a) but the evidence presented here distinguishes this clade as both morphologically and genetically distinct from the core *Arytainilla* (defined by the type species, *A. delarbrei*). This latter group, referred to as *Arytainilla sensu stricto*, is in fact only represented by a single species in the Canary Islands. Two additional genera present in the Canary Islands are *Livilla*, also represented by a single species (*L. monospermae*), and *Arytaina* (*sensu* Loginova, 1976a) represented by three species (*Ar. devia*, *Ar. nubivaga* and *Ar.* sp.14). Diversity and speciation within each genus in the Canary Islands appears mainly to be restricted by diversification in the host genus. The largest number of psyllids (13 species in the 'Macaronesian clade') occur on the most species rich host genus, *Teline* (ten species); and at the other end of the scale a single legume species representing the genus *Retama* is host to the one Canarian species of *Livilla*.

### **3.3.6** *Intergeneric host switching*

The number of host switches that have occurred between plant genera in the 'Macaronesian clade' is inferred by mapping the host genera onto the psyllid phylogeny (Fig. 6). The optimization of the host genera on the psyllid tree suggests that intergeneric host switches are less common than intrageneric host switching. The switches to *Adenocarpus*, *Chamaecytisus* and *Genista*, all appear to have been made by psyllids that were originally *Teline*-feeding (Fig. 6). There appears to have been a single host switch to *Adenocarpus* (indicated by a white bar, Fig. 6) which resulted in the evolution of two *Adenocarpus*-feeding species (*A. nigralineata* and *A. proboscidea*); and there appears to have been at least two, possibly three, host switches to *Chamaecytisus* (indicated by a black bar, Fig. 6). The three host switches to *Chamaecytisus* are required to explain the evolution of *A dividens* (one switch) and to explain the bi-generic host preference of *A. modica* (two switches). The samples of *A. modica* from *Chamaecytisus* and *Teline* hosts on two islands (La Palma and El Hierro) are segregated geographically (based on CO data) rather than by host affiliation. Therefore, it appears that *A. modica* made at least one host

switch between *Chamaecytisus* and *Teline* on each island. *A. modica* is the only Macaronesian species found on more than one host genus and is a recently derived species on young islands (1-2 Myr). Further intraspecific sampling within islands, is required to determine whether members feeding on each plant genus have formed host races. Switching between *Genista* and *Teline* in the Madeira/continental subclade was equivocal, but when resolved using ACCTRAN optimization, shows a single switch to *Genista* (indicated by a black triangle, Fig. 6), followed by a switch back to *Teline* (indicated by a white triangle, Fig. 6).

### **3.3.7** *Continental versus island divergence*

The CO regions have been shown to evolve sufficiently rapidly to provide information on the phylogeographic patterns within species of Canarian beetles (Juan *et al*., 1998; Emerson, Oromí & Hewitt, 2000a). Sequences of individuals from different populations in continental and Macaronesian taxa provided a comparative measure of intraspecific divergence. Three continental species (*Ar. adenocarpi*, *Ar. genistae* and *A. spartiophila*) are widespread occurring on several closely related host species. Individuals of these taxa were sampled from Morocco, Spain, Portugal and Scotland (Table 2 and Figs 3 & 4). Among the island species individuals were sampled from populations that either occurred on different islands and/or different host plants. Intraspecific genetic divergence (CO) was greatest within four Canary Island species that occurred on different islands but on the same host plant (*L. monospermae* from three islands, 4–5%; *Ar. devia* from four islands, 1–10%; *A. dividens* from three islands, 1–4%; and *A.* sp.10 from two islands, 5%). Divergence within the widespread continental species which were sampled from different hosts was less than 3%, and in each case divergence between Moroccan and European samples was double or more than double, the divergence within Europe (e.g. *Ar. genistae* from Portugal and Scotland showed 0.8% sequence divergence, but both samples showed 2.8% divergence from the Moroccan individual) (Figs 3 & 5). The same pattern in *Ar. adenocarpi* and *A. spartiophila* suggests that the Moroccan region may have provided glacial refugia, with rapid northward migration during inter-glacial periods, as has been shown for other invertebrates and mammals (Hewitt, 1996, 1999).

### **3.3.8** *Widespread taxa*

In the Canary Islands, nine of the 21 species (43%) occur on more than one island (*L. monospermae*, *Ar. devia*, *Ar*. sp.14, *A. dividens*, *A. diluta*, *A. modica*, *A. nigralineata*, *A. proboscidea* and *A.* sp.10). All but one of these are specific to a single host plant. About

half of these species show low intraspecific molecular CO divergence (max 0.02-2%), suggesting recent inter-island dispersal; but four species (each specific to a single host) show considerably greater divergence (max 4-10%) (Figs 3 & 5). Specificity to a single host may be contributing to a lack of speciation (morphological stasis) in these taxa, particularly where host habitat and ecology are relatively uniform between islands. In other examples, where colonization of a new island is unaccompanied by a host switch but has resulted in speciation, the intraspecific host variation – distinct subspecies – may have promoted speciation. However, two of the widespread species occur on one of the most variable hosts, *Chamaecytisus proliferus* (3 subspecies, 4 varieties); and one these species (*Ar. devia*) was found to have almost as much intraspecific divergence (CO: 1-10%) as interspecific divergence found within the entire 'Macaronesian clade' (CO: 2-13%).

### **3.3.9** *Colonization patterns*

The 21 species of arytainine psyllid in the Canary Islands are probably derived from five independent colonizations of these islands (indicated by *, Fig. 6). Only one of these colonizations has resulted in a significant radiation (16 species in the 'Macaronesian clade'). Four of the colonizations have resulted in little or no radiation and are represented by one or two species in *Livilla*, *Arytainilla s.s.* and *Arytaina*. There has been one (ACCTRAN optimization), possibly two colonizations of Madeira (Fig. 6 shows the ACCTRAN optimization). The two Madeiran species (*A. incuba* and *A. umbonata*) form part of a group that includes the three continental species (*A. cognata*, *A. hakani* and *A.* sp.13) in the 'Macaronesian clade', and the derived position of this subclade within the 'Macaronesian clade' suggests one, or even two (with ACCTRAN optimization) possible back colonizations of the continent (i.e. one colonization of the continent from the Canary Islands and one from Madeira, Fig. 6 shows the ACCTRAN optimization). These continental recolonizations, however, may also be considered equivocal because the additional tree length (10 steps) required to exclude the Madeiran and continental species while constraining the Canary Island members of the 'Macaronesian clade' to be monophyletic, is not significant (Table 4).

The continental sister groups of the five Canary Island species not in the 'Macaronesian clade' (e.g. *Arytaina*, *Arytainilla s.s.* and *Livilla* spp.) are well resolved, and the continental sister taxa are all widespread and occur in the Iberian Peninsula and NW Africa. A comparison of host associations in the continental and island sister groups is used to assess the role of preadaptation to particular host plants in colonizing psyllids. In Figure 6, where the legume host genera of the island and continental sister taxa are mapped

onto the psyllid tree, four of the five psyllid colonizations of the Canary Islands (indicated by *, Fig. 6) would also have required colonization of a novel host genus. The only example of an island psyllid occurring on the same host genus as its continental sister taxon, is *Livilla monospermae* on the host genus *Retama* (Fig. 6). However, the switches between host genera during island/continental colonization events, are all between host genera within the same host groups (e.g. the *Genista* group or the *Cytisus* group) within the Genisteae (Käss & Wink, 1997). Figure 7 presents these generic host groups mapped onto the psyllid phylogeny. Following dispersal, therefore, a psyllid may search among available hosts for recognizable features, and select the host as closely related to the original host as it can find, implying an important role for preadaptation to the selection process of particular types of available hosts.

## 3.4 Discussion

### **3.4.1** *Phylogeny of the Arytaininae*

The molecular analyses (Figs 3 & 4) confirm the reservations expressed by Hodkinson & Hollis (1987) concerning the monophyly of the Arytaininae. The current classification represents a paraphyletic group with respect to the Psyllinae (represented by *Cacopsylla*). Amongst the papilionoid legume-feeders, the genus *Cyamophila* whose members feed on the tribes Galegeae, Loteae, Phaseoleae, Soporeae and Loteae, are distinct from members feeding on the tribe Genisteae. *Cyamophila* appears to be more closely related to the subfamily Psyllinae whose members feed on a wide variety of hosts including species in the Rosaceae, Rhamnaceae, Salicaceae, and the Caesalpinioideae (Leguminosae). There is some evidence for a monophyletic Genisteae-feeding group (combined molecular analysis: MP bootstrap 59%, NJ bootstrap 92%, Fig. 4), but the inclusion of *Pseudacanthopsylla* in this group remains equivocal. Whether *Pseudacanthopsylla* unequivocally belongs to a monophyletic Genisteae-feeding group, may only be tested satisfactorily with additional sampling of Afro-tropical genera thought to be related to this genus (Hodkinson & Hollis, 1987).

The ‘Macaronsian clade’ (‘M’ in Figs 3-5) is a strongly supported monophyletic group, though the origin of this clade from within the Arytaininae remains ambiguous. The combined molecular analysis (12S and CO) suggests that it is nested within a paraphyletic *Livilla sensu lato*, and sister to the ‘core *Livilla*’ group (‘L’ in Figs 3-5). The three Macaronesian *Arytaina* species (*Ar. devia*, *Ar. nubivaga* and *Ar.* sp.14) do not appear to be a monophyletic group, but further intrageneric sampling is needed to confirm this. The

single Macaronesian species of *Arytainilla s.s.* (*A.* sp.1) is strongly supported as grouping with the type species of this group (*A. delarbrei*), and the single Macaronesian species of *Livilla* (*L. monospermae*) is closely related to a continental outlying *Livilla* species (*L. retamae*) (Fig. 4). Further conclusions on the taxonomic implications of the phylogenetic analyses are given in the Taxonomic Appendix. Notably, there is good resolution and support for some major groups ('Macaronesain clade' – M, *Arytainilla s.s* – A, *Arytaina* – AR and the 'core *Livilla*' – L group), but among these groups, the relationships remain unsupported.

**3.4.2** *Origins and colonization in Macaronesian Ayrtaininae*

Is there evidence to determine the sequence of colonization or to identify which of the Canary Islands was first colonized? The three basal species in the 'Macaronesian clade' (*A.* sp.5, *A. diluta* and *A. prognata*) occur on Gran Canaria (the geologically oldest and closest island to the African continent of the central islands). It is probable, therefore, that this island was the first island colonized, and the location of early diversification of the 'Macaronesian clade'.

Within the genus *Arytaina*, species or populations (*Ar*. sp.14 and *Ar. devia*) from geologically younger islands have 5$^{th}$ instar nymphs with fewer nymphal sectasetae on the abdomen. Optimization of this character on the psyllid phylogeny suggests that loss of sectasetae is derived in these taxa, which would reflect colonizations (east to west) from older to younger islands. However, a more extensive phylogeographic approach than the limited intraspecific sampling here, is required to test this interpretation, and would allow a comparison of the 'stepping stone' pattern of colonization found in other island invertebrate groups (Roderick & Gillespie, 1998; Juan *et al*., 1998; Emerson *et al*., 2000a). One common pattern that is evident in three species from different genera (*Ar. devia*, *A. dividens* and *L. monospermae*), is the close relationship between individuals sampled from the two closest islands, Tenerife and La Gomera (27 km apart). Further phylogeographic data for these taxa should be able to differentiate between a pattern of recent colonization (with markedly structured populations on each island) versus a pattern of gene flow between these geographically close islands, with distant islands more effectively isolated.

**3.4.3** *Ecological and geographical patterns*

Host plant preference, ecology and geographical distribution in sister taxa, determined over three years of field surveys, are used to investigate the diversification of psyllids in Macaronesia. By combining field data with the psyllid phylogeny, three patterns of sister

taxon relationships were inferred for the island Arytaininae. 1) the majority of sister taxa are ecologically and geographically allopatric, occurring both on different host plants and different islands or different regions within an island; 2) there are four cases of sister taxa occurring on the same host but different islands; 3) and there is only one case where sister taxa occur both on the same host and the same island (*A. diluta* and *A. prognata*). The latter may represent a case of sympatric speciation, however, there is evidence for a micro-allopatric adaptive shift, with each species restricted to either northern mesic habitats or dry southern habitats of the host. Geographical allopatry is the primary division when sister taxa occur on separate islands but on the same host, and furthermore, exhibit a shared preference for ecologically specialized subspecies of the host (*T. stenopetala*): sister taxa *A.* sp.10 and *A.* sp.11 are more common on the host's xerically adapted subspecies on La Palma (ssp. *sericea*) and La Gomera (ssp. *pauciovulata*); while *A. modica* and *A.* sp.12 are more common on the host's mesically adapted subspecies from the same islands (ssp. *stenopetala* and ssp. *microphylla*). These patterns suggest that, independently, ecological or geographical divergence may be sufficient to promote psyllid speciation, but both of these mechanisms are apparent in the majority of sister taxon relationships. The prevailing pattern that emerges is one of varying amounts of ecological and geographical shifts similar to other island invertebrate groups (Roderick & Gillespie, 1998).

A common feature of the Canarian Arytaininae is the presence of multiple (2-6) psyllid species on the same host (Table 5). The sharing of host resources may be facilitated by geographical segregation, ecological specialization and divergence in the timing of development. On Tenerife, *T. canariensis* is a widespread legume on which four psyllid species occur, but only one psyllid is found throughout the host range (*A. pileolata*) while the other species (*A. diluta*, *A.* sp.7 and *A.* sp.8) occur in particular regions or habitats. A similar pattern is found on Gran Canaria, where three psyllid species occur on *T. microphylla*, one widespread (*A. equitans*) and two localized species (*A. diluta* and *A. prognata*). As a widespread legume occurs in several types of habitat, a widespread psyllid species may be considered an ecological generalist, whereas psyllids restricted to local habitats types may be ecological specialists. Interestingly, the phylogeny suggests that, on Tenerife, the localized species are more derived, but this is reversed on Gran Canaria where the widespread species is more derived. In addition, both widespread species develop later in the season than the ecologically localized species, and this temporal division is also found in two widespread species which both occur throughout the host range (*Ar. devia* develops later than *A. dividens* on *Chamaecytisus proliferus*). These

patterns suggest a degree of competitive displacement and/or exclusion is operating to partition the use of plant resources by psyllid species.

An example which supports a role for competitive exclusion is found in the two *Adenocarpus*-feeding species (*A. nigralineata* and *A. proboscidea*). On Tenerife both the psyllids are present and each psyllid is specific to one of two hosts (the hosts are divided altitudinally and ecologically). But on La Palma only one psyllid species is present, and this psyllid occurs throughout both host ranges. Oviposition in *Adenocarpus*-feeding species is on the flowers and inflorescence, and exclusivity on Tenerife may be driven by adaptation to phenological differences (*Adenocarpus foliolosus* flowers earlier than *A. viscosus*). The effects of phenology and altitudinal clines are therefore more likely to influence patterns of host specialization when the presence of another psyllid requires a competetive advantage. A study of willow psyllids found similar variation in resource exploitation along an altitudinal gradient (Hill, Hamer & Hodkinson, 1998).

**3.4.4** *Anthropogenic effects on host populations and host hybridization*

The Genisteae species that were found to host psyllids were all estimated to have an abundance equal to, or greater than 2000 individuals. The rarest legume on which a psyllid was found is *T. osyroides* ssp. *osyroides* with a single population of c. 2000-3000 individuals in the Barranco de Masca. The Genisteae species which do not host psyllids (up to one third of the legumes (17-33%) per island) are all rare species with less than 2000 individuals, and in many cases these are rare because of human activity. Thus, there is a possibility that there were psyllids on these hosts in the past but these psyllids are now extinct. There may be a critical host plant abundance, below which a psyllid species may be unable to maintain a viable population. Fragmentation of host populations may also be critical: an uncommon legume (*T. rosmarinifolia*) occurs in several small, fragmented populations, and a rare psyllid species (*A.* sp.5) was found on only one of these populations. In contrast, cultivation of some native host plants for animal fodder (*Chamaecytisus proliferus* and *Teline stenopetala*) and the adaptation of other native hosts to disturbed or grazed landscapes (*Adenocarpus viscosus*) appears to have increased the abundance of psyllid species associated with these hosts.

Another effect of human disturbance is the breakdown of ecological barriers between plant species, resulting in hybridization (Lems, 1958; Francisco-Ortega *et al*., 2000). Several psyllid species occur on multiple hosts (*A. diluta*, *A.equitans*, *A. pileolata*, *A. proboscidea* and *A*. sp.8) and hybrids have also been recorded between these hosts (Table 5). On Tenerife, hybrids between three host species (*T. canariensis*, *T. stenopetala*

and *T. osyroides*) occur around the Ladera de Güímar (Arco Aguilar, pers. comm.) – an area settled by the aboriginal Guanches; and the most polyphagous psyllid species in the Canary Islands (*A. pileolata* in the 'Macaronesian clade') is found on these three hosts. As hybrids are more likely to occur between closely related species, it is not clear, without mapping host and psyllid genotypes across hybrid zones, whether the host shifts occurred because of phylogenetic compatibility, or whether hybridization could have promoted the shift via a 'hybrid bridge' effect (Floate & Whitham, 1993; Roderick, 1997).

## 3.5 Taxonomic Appendix

### **3.5.1** *Arytainilla*

There are currently 34 species in this genus, but the genus is clearly paraphyletic (Table 4). The majority of the species (21) belong to the 'Macaronesian clade' discussed below. The remaining species can be divided into a small group which includes the type species (*Arytainilla sensu stricto*: nine species, of which six species are included in this study), and four residual and heterogeneous species that are morphologically atypical (*A. ima*, *A. sulci*, *A. gredi* and *A*. sp.4: *Arytainilla sensu lato*). *Arytainilla s.s.* is characterized by unusually large female genitalia and a massive ovipositor. This group (labelled 'A' in Figs 3-5) is supported as monophyletic in the CO phylogeny (bootstrap MP: 78%, Fig. 3B; NJ: 89%) and the combined morphological and molecular analysis (bootstrap MP: 87%, Fig. 5C) but appears paraphyletic in respect to the genus *Arytaina* in the 12S analysis using NJ and ML methods, and is unresolved in the MP 12S phylogeny (Fig. 3A). Hence the absence of support for *Arytainilla s.s.* in the MP combined molecular analysis (Fig. 4). There is a single species of *Arytainilla s.s.* in the Canary Islands (*A.* sp.1), which groups with the type species (*A. delarbrei*, MP bootstrap > 80% in the combined molecular (Fig. 4) and total evidence (Fig. 5C) phylogenies). The nymphal morphology of this group is extremely variable. *A. sulci* is unique within the Arytaininae in the tergal plates of the 5$^{th}$ instar nymph which are barely reduced. The combined molecular phylogeny (12S and CO, Fig. 4) suggests that *A. gredi* and *A*. sp.4 have closer affinities to *Livilla* than to *Arytainilla*, and that *A. sulci* is sister to *Arytaina*. *A. ima* belongs to a small, well supported group (MP bootstrap > 80% in both combined analyses, Figs 4 & 5) of outlying *Livilla* species that feed on the genus *Adenocarpus* (Fig. 7). Members of *Arytainilla s.s.* predominantly feed on host plants in, or allied to the genus *Cytisus* (*Cytisus* group) (Fig. 7).

**3.5.2** The 'Macaronesian clade' of *Arytainilla*

This clade of 21 species is not exclusively Macaronesian, but appears to have evolved and diversified in Macaronesia. The clade (labelled 'M' in Figs 3-5) is unambiguously resolved in all molecular analyses and in the morphological analysis. Support for the monophyly of the clade is strong in the independent 12S and CO phylogenies (bootstrap MP: 79-99%,Fig. 3A & B; NJ: 91-100%); and although the clade lacks bootstrap support in the morphological phylogeny (Fig. 3C), the combined morphological and molecular data provide 99% MP bootstrap (Fig. 5C). Members of the 'Macaronesian clade' predominantly feed on host plants in, or allied to the genus *Genista* (*Genista* group) (Fig. 7).

**3.5.3** *Arytaina*

There are 14 species in *Arytaina* of which five have been included in this study: three Macaronesian taxa which are endemic to the Canary Islands (*Ar. devia*, *Ar. nubivaga* and *Ar*. sp.14), and two continental species (*Ar. adenocarpi* and the genus type species, *Ar. genistae*). Although sampling is limited, the strong support for this group (labelled 'AR' in Figs 3-5), suggests that *Arytaina* is the only unambiguously monophyletic genus in the Genisteae-feeding Arytaininae (Figs 3-5). The Macaronesian taxa, although originally placed in this genus (Loginova, 1976a), were later removed and placed in *Arytainilla* based mainly on the peculiar morphology of the male genitalia (Hodkinson & Hollis, 1987). Nevertheless, there is strong support from both the morphological (MP booststrap 74%, Fig. 3C) and molecular (MP booststrap 89-100%, Fig. 3A & B) phylogenies for reverting to the original classification. Members of *Arytaina* predominantly feed on host plants in, or allied to the genus *Cytisus* (*Cytisus* group) (Fig. 7).

**3.5.4** *Livilla*

This is the largest Genisteae-feeding genus and, like *Arytainilla*, it is clearly paraphyletic as indicated by the additional tree length required when the genus is constrained to be monophyletic (Table 4). There are 39 species, 19 of which are included in this study. The monophyly of a core group of *Livilla* species (labelled 'L' in Figs 3-5) is well supported (combined 12S and CO: MP bootstrap 81%, Fig. 4). Neither the single Macaronesian species (*L. monospermae*), nor the type-group as defined by Hodkinson & Hollis (1987) (represented in this study by *L. vicina*) is included in this 'core *Livilla*' group. However, constraining the 'core *Livilla'* species to be monophyletic with the inclusion of three of the outlying species (*L. nervosa*, *L. monospermae* and *L. retamae*) but not including *L. vicina* or the *Adenocarpus*-feeding species (*L.* sp.15, sp.16 and sp.17) does not require a

significant increase in tree length (Table 4); and this group is recovered in the total evidence phylogeny (but with low bootstrap support, MP: 57%, Figs 5C). Members of this genus typically feed on hosts plants in, or allied to the genus *Genista* (*Genista* group, Fig. 7).

**3.5.5** *Pseudacanthopsylla improvisa* **comb. nov.**

*Psylla improvisa* Loginova, 1972: 30

This genus, previously monotypic (a single species in the Middle East and NE Africa, *P.retamae*), is represented in this study by a second species (*Psylla impovisa*) from NW Africa, which is transferred to *Pseudacanthopsylla* based on the following generic features: unpatterned, parallel-sided forewing with well developed pterostigma, short robust genal cones, metatarsal spur absent, and a unique 'hedgehog' nymphal morphology with near entire coverage of sectasetae (Fig. 6B). *P. improvisa* differs from the type species (*P. retamae*, Samy, 1972: 455) in the narrower, more acutely rounded forewing apex, more slender genal cones, and the distinctive shape of the male and female genitalia (illustrated by Loginova, 1972). Both the species occur on the same widespread host plant (*Retama raetam*). The conflicting placement of this peculiar genus is discussed in the main body of the paper under the separate molecular analyses.

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