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The Evolution of Ectoparasitism in the Genus *Lucilia* (Diptera: Calliphoridae)

JAMIE STEVENS* and RICHARD WALL

School of Biological Sciences, The University of Bristol, Woodland Road, Bristol BS8 1UG, U.K.

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Abstract—Stevens J. & Wall R. 1997. The evolution of ectoparasitism in the genus *Lucilia* (Diptera: Calliphoridae). *International Journal for Parasitology* 27: 51–59. To consider the evolutionary origin of the ectoparasitic habit in the blowfly genus *Lucilia* (Diptera: Calliphoridae), phylogenetic analyses of mitochondrial DNA sequence data were performed for 10 species, including all the common *Lucilia* agents of myiasis, collected from Africa, Australasia, North America and Europe. Complementary genetic distance and parsimony analyses are used to consider inter and intraspecific relationships within the genus with reference to previous morphological work. The results support the hypothesis of independent multiple evolution of the ectoparasitic habit in *Lucilia sericata*, *Lucilia cuprina* and the *Lucilia caesar*/*Lucilia illustris* group and suggest that it has coevolved in relatively recent history along with the domestication and husbandry of sheep. The geographic differences in pathogenic importance of various species of *Lucilia* also suggest that there is a strong climatic influence determining which species has dominated. *Lucilia cuprina* has become the predominant pathogenic species in sub-tropical and warm temperate habitats (e.g., Australia and South Africa), *L. sericata* in cool temperate habitats (e.g., Europe and New Zealand) and *L. caesar* and *L. illustris* become more common in sheep myiasis in more northerly Palaearctic regions. Copyright © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: Lucilia; blowfly; phylogeny; mitochondrial DNA (mtDNA); myiasis; ectoparasitism; evolution.

INTRODUCTION

Myiasis is the infestation of the living tissues of animals with dipterous larvae. In the family Calliphoridae at least 80 species have been recorded as agents of myiasis (Zumpt, 1965). These species can be divided generally into 3 functional groups based on their larval feeding habits: (1) saprophages normally living in decaying organic matter and animal carcasses, which cannot initiate a myiasis but which may secondarily invade existing infestations; (2) facultative ectoparasites, normally adopting an ectoparasitic habit and which are capable of initiating myiases but which occasionally live as facultative saprophages; and (3) primary, obligate parasites feeding only on the tissues of living vertebrates, usually mammals and birds (Hall & Wall, 1995).

*To whom correspondence should be addressed. Tel: +44 117 928 9000: Fax: +44 117 925 7374; E-mail: j.r.stevens@bristol.ac.uk.

It has been proposed that this functional division may reflect the evolution of the parasitic habit in the calliphorid ectoparasites. Generalised free-living saprophagous feeders, which may occasionally act as agents of myiasis in wounded, dying or otherwise clinically predisposed animals, may have formed the ancestral origins of the parasitic habit. These then gave rise to facultative ectoparasites, attracted to skin soiled by faeces, bacterial infection and suppurating wounds, which behave as primary myiasis agents rather than saprophages. From this intermediate stage, obligate parasitism developed (Zumpt, 1965; Erzinclioglu, 1989). In support of this general view, within each of the calliphorid genera, species displaying a range of stages in their dependency on ectoparasitism can be identified. For example, the genus *Chrysomya* contains the obligate ectoparasite *Chrysomya bezziana* Vill. and the secondary facultative ectoparasites *Chrysomya rufifaces* (Macq.), *Chrysomya megacephela* (F.) and *Chrysomya albiceps* (Weid.). The genus *Cochliomyia* contains the obligate

ectoparasite *Cochliomyia hominivorax* Coquerel and the secondary facultative ectoparasite *Cochliomyia macellaria* (Fabr.). The aim of the work described in this paper was to investigate the evolution of the myasis habit in the calliphorid genus *Lucilia* through examination of the phylogenetic relationships between species and, in particular, the mono or polyphyletic origins of ectoparasitism in this genus.

A number of features make species of *Lucilia* useful subjects for such a study. The genus is a small, relatively homogeneous group of at least 27 species, all of which bear a very close resemblance to each other (Aubertin, 1933; Stevens & Wall, 1996). The larvae of most species are saprophages. However, 2 species, *Lucilia sericata* (Mg.) and *Lucilia cuprina* (Wied.), commonly act as primary facultative ectoparasites, and the species *Lucilia caesar* (L.) and *Lucilia illustris* (Mg.) and more occasionally *Lucilia ampullacea* Vill. may be found in myiases, usually as secondary facultative ectoparasites. All these species of *Lucilia* are most commonly found in cutaneous myiasis of sheep, although they may also infest a range of other wild and domestic animals (Hall & Wall, 1995). Another species, *Lucilia bufonivora* Mon., is a specialised, obligate agent of myiasis in toads (Zumpt, 1965). These species are predominantly Palaearctic and Oriental in distribution (Aubertin, 1933), but some have also spread worldwide, particularly, in the case of *L. cuprina* and *L. sericata*, with the movement of the domestic sheep, *Ovis aries* (Waterhouse & Paramonov, 1950; Norris, 1990).

MATERIALS AND METHODS

Fly collection. Specimens of *Lucilia* species were caught at a range of sites in Africa, Australasia, Europe and North America using sticky targets baited with liver and sodium sulphide solution (Wardhaugh, Read & Neave, 1984; Wall *et al.*, 1992b) or hand nets. Traps were checked at least twice daily, allowing flies for molecular characterisation to be collected alive and undesiccated. After collection, flies were placed in 100% ethanol and stored at 4°C prior to analysis. *Lucilia* were identified to species using the morphological characters described by Aubertin (1933) and Holloway (1991), including analysis of male genitalia. Specimens of 10 *Lucilia* species were collected: *L. ampullacea* Vill., *L. caesar*, *Lucilia cluvia* (Walk.), *L. cuprina*, *L. illustris*, *Lucilia mexicana* Macq., *Lucilia richardsi* Coll., *L. sericata*, *L. silvarum* and *Lucilia thatuna* Snn. (Table 1). In addition, samples of a closely related species, *Hemipyrellia fernandica* (Macq.), obtained from infested drying fish in Tanzania and *Calliphora vicina* (L.), from a laboratory colony maintained at the University of Bristol, were also included in the analysis as outgroups. *Hemipyrellia fernandica* is an Afrotropical

Table 1—Specimen details

Species	Site and year of collection	No. of specimens
Lucilia ampullacea	Langford, Bristol, U.K., 1994	(2)
Lucilia caesar	Langford, Bristol, U.K., 1994	(2)
Lucilia cluvia	New Orleans, LA, U.S.A., 1994	(2)
Lucilia cuprina	Canberra, A.C.T., Australia, 1995	(2)
	Serpentine, Perth, W.A., Australia, 1995	(1)
	Townsville, Queensland, Australia, 1994	(2)
	Blenheim, South Island, New Zealand, 1994	(1)
	Dorie, South Island, New Zealand, 1994	(1)
	Dakar, Senegal, 1994	(1)
	Nairobi, Kenya, 1994	(1)
	Tororo, Uganda, 1994	(1)
Lucilia illustris	Langford, Bristol, U.K., 1994	(1)
Lucilia mexicana	San Francisco, CA, U.S.A., 1994	(2)
Lucilia richardsi	Usk, Gwent, U.K., 1995	(2)
Lucilia sericata	Glendalough, Perth, W.A., Australia, 1995	(1)
	Hilerod, Sjelland, Denmark, 1994	(1)
	Dorie, South Island, New Zealand, 1994	(1)
	Rotorua, North Island, New Zealand, 1994	(1)
	Sacramento, CA, U.S.A., 1994	(2)
	Uckfield, East Sussex, U.K., 1994	(1)
	Wrington, Bristol, U.K., 1994	(1)
	University of Bristol colony, U.K., 1994	(1)
	Harare, Zimbabwe, 1994	(1)
Lucilia silvarum	Sacramento, CA, U.S.A., 1994	(3)
Lucilia thatuna	San Francisco, CA, U.S.A., 1994	(1)
Hemipyrellia fernandica	Tanzania, 1994	(1)
Calliphora vicina	University of Bristol colony, 1995	(1)

species which acts as an occasional agent of myiasis (Zumpt & Ledger, 1967). Morphologically, *Hemipyrellia* are extremely similar to species of *Lucilia*, being differentiated by fine, erect hairs on the supraspiracular convexity, which are longer than those of species of *Lucilia*. At various times the 8 species of *Hemipyrellia* have been included with the *Lucilia* (Zumpt, 1956).

DNA extraction. Initial attempts to extract DNA from dried, preserved specimens of *L. cuprina*, *L. sericata*, *Lucilia eximia* (Wied.) and *Lucilia graphita* Snn. did not yield DNA of suitable quality for reliable PCR amplification. In consequence, only species for which recently caught specimens were available were included in the study. See Post, Flook & Millest (1993) and Stevens & Wall (1995) for details of DNA extraction techniques and preservation methods. DNA was extracted from all fly specimens as total nucleic acid by the cetyl trimethyl ammonium bromide (CTAB) method according to the protocol described by Stevens & Wall (1995). To avoid contaminating samples with DNA from eggs, ingested protein or gut parasites, only the head, legs and flight muscles of male flies were used as sources of DNA. Details of all flies included in this study are presented in Table 1.

Mitochondrial DNA sequence analysis. Based on the degree of variation detected in a previous population level study of *L. cuprina* and *L. sericata* (Stevens & Wall, 1997), the 12S rRNA gene was targeted as a conservative mtDNA marker (Simon *et al.*, 1994) suitable for an interspecific study. The fragment was amplified using a pair of universal primers (29-mer TIN8X 5′-XCTATCAAGGTAACCCTT TTTAT-CAGGCA-3′ and 20-mer SRJ14612 5′-AGGGTATCTAA-TCCTAGTTT-3′; Simon *et al.*, 1994). PCR reaction components per 50 μl reaction were as follows: 50 ng template DNA, 0.2 μM primer TIN8X, 0.2 μM primer SRJ14612, 1.0 U SuperTaq *Taq* polymerase, dNTPs 0.2 mM, 1.5 mM $MgCl_2$, 1 × reaction buffer. The protocol for PCR reactions consisted of 3 min at 94 °C; 1 min at 94 °C, 1 min at 51 °C, 1 min 30 s at 72 °C for 30 cycles; 5 min at 72 °C (Stevens & Wall, 1997). For each fly DNA to be sequenced, PCR amplifications (× 12) were performed in parallel and then pooled. Any amplification errors, which could be carried through to the sequencing stage, were thus diluted 12-fold, such that they would be negligible in the aliquot of DNA sequenced. Solid-phase sequencing was performed as described by Hultmann *et al.* (1989) using streptavidin magnetic beads (Dynabeads, Dynal A.S., Norway). Labelling reactions were performed with ^{35}S by the T7 DNA polymerase dideoxy base-specific termination method (Sanger, Nicklen & Coulson, 1977) using a T7 sequencing kit (Pharmacia Biotech, U.S.A.). Sequence fragments were then run on acrylamide gel; manual sequencing is preferred for AT-rich material, where sequences of 10 or more identical bases are not uncommon.

Phylogenetic analyses. Sequence data were analysed by 2 phylogenetic methods: parsimony analysis (Eck & Dayhoff, 1966) and a genetic distance measure (Kimura, 1980) using the package PHYLIP 3.5c (Felsenstein, 1993). Parsimony analysis was performed using the program DNAPARS. Distance matrices were produced with the program DNADIST, calculated using the nucleotide substitution model of Kimura (1980). Cluster analysis of genetic distances was performed using the neighbour-joining method of Saitou & Nei (1987) with the program NEIGHBOR. Neighbour-joining is believed to be one of the better performing distance measures currently available (Nei, 1991). For both distance and parsimony analyses a measure of support for the clades identified was provided by constructing a majority-rule consensus tree from 100 bootstrapped data sets, using the programs SEQBOOT and CONSENSE.

RESULTS

Mitochondrial DNA sequences

A number of variations in the 322 bases sequenced in the 12S rRNA gene of individual flies were identified, both between and within *Lucilia* species (Table 2). All *L. sericata* specimens examined were identical, regardless of their geographic origin. For *L. cuprina*, the majority of flies collected had an identical nucleotide sequence; however, 2 different *L. cuprina* sequences were also obtained. *Lucilia cuprina* collected from Senegal differed from the majority-type by 2 single nucleotide insertions. The *L. cuprina* collected from Townsville, Australia differed from the majority type by 1 single nucleotide substitution. The 2 specimens of *L. cluvia* analysed differed from each other by a single nucleotide insertion. One of the 3 specimens of *L. silvarum* analysed differed from the other 2 specimens by a single nucleotide insertion. Specimens of the remaining species possessed sequence types unique to each species.

Genetic distance analysis

The genetic distance analysis showed that genetic variants of single species grouped together in all cases (Fig. 1). Close interspecific relationships were identified between *L. caesar* and *L. illustris*, and between *L. cluvia* and *L. mexicana*. All genetic variants of *L. cuprina* clustered more closely with *L. silvarum* than with any other species, supporting the close relationship between these 2 species indicated by morphological analysis (Stevens & Wall, 1997; see also Fig. 2). Despite the limited number of informative nucleotides (Table 2), support for the above relationships was provided by the bootstrap values of > 50%. The positions of *L. richardsi*, *L. sericata* and *L. thatuna*, however, were unresolved with respect to each other.

Hemipyrellia fernandica was well separated from the species of *Lucilia*, supporting the status of *Hemipyrellia* as a separate genus. All *Lucilia* were also well separated from the outgroup *C. vicina*.

Parsimony analysis

Parsimony analysis was performed on the mtDNA sequence data and a majority rule consensus tree constructed (Fig. 2). The majority rule consensus method groups taxa based on the number of times they cluster together in the trees produced from the selected number of bootstrapped data sets. The percentage of times that a cluster appears can be taken as a rough measure of relative support. Clusters in majority rule trees which occur in less than 100% of trees are less robust

Table 2--Mitochondrial DNA sequence data (322 base pairs) for *Lucilia* sp., *H. fernandica* and *C. vicina*[a]

L. sericata	5′	TCAAG	CTTCAATTAT	TCTAATAAAA	
L. cuprina		.T...	....	....	
L. cuprina--D		.T...	....	....	
L. cuprina—T		.T...	....	....	
L. caesar		.?...	....	.T........	
L. cluvia—1		.?...	....	.T......G.	
L. cluvia—2		.?...	....	.T......G.	
L. mexicana		.....	.........A	.T......G.	
L. illustris		.?...	....	.T........	
L. ampullacea		.?...	....	.T........	
L. richardsi		.?...	....	....	
L. thatuna		.....	....	.......T..	
L. silvarum		.T...	T.........	....	
L. silvarum—1		.T...	T.........	....	
Hemipyrellia fernandica		.?...	.?........	.....A....	
Calliphora vicina		.?...	.T........	.....AT...	

14651					
sericata	AAATTTATAA	ATTTAAAATT	TCACCTAATA	AATTTATTTT	TATTTTATAA
cuprina	..T.......	....	....	....	....
cuprina—D	..T.......	....	....	....	....
cuprina—T	....	....	....	....	....
caesar	....	....	....	....	....
cluvia—1	.T..A.....	....	....	....	.....C....
cluvia—2	.T..A.....	....	....	....	.....C....
mexicana	.T..A.....	....	....	....	.....C....
illustris	....	....	....	....	....
ampullacea	....	....	....	....	....
richardsi	....	....	....	....	....
thatuna	....A.....	....	....	....	....
silvarum	....	....	....	....	....
silvarum—1	....	....	....	....	....
H. fernandica	....A....	....	....	......C...	....
C. vicina	....A.....	....	....T.....	....	.........T

14701					
sericata	ATAAACAATT	TAACTTCAAC	TAAAAAAAA-TT	TATTTGCATT	ATTCGTATAA
cuprina	....	....	.........-..	....	....
cuprina—D	....	....	.........A..	....	....
cuprina—T	....	....	.........-..	....	....
caesar	C.........	....	.........-..	....	....
cluvia—1	TA........	......T...	.........-..	....C.....	....
cluvia—2	TA........	......T...	.........-..	....C.....	....
mexicana	TA........	....	.........-..	....	....
illustris	C.........	....	.........-..	....	....
ampullacea	T.........	....	.........-..	....	....
richardsi	.C........	......T...	.........A..	....	....
thatuna	....	....	.........-..	....	....
silvarum	....	....	.........A..	....	....
silvarum—1	....	....	.........-..	....	....
H. fernandica	.A........	......A...	.........-..	....	....
C. vicina	-.........	......T...	.........-..	....	....

14751					
sericata	CCGCGGCTGC	TGGCACAAAT	TTAGCCAATA	CTCTTTAGTA	TTACTATTTC
cuprina	....	....	....	....	....
cuprina--D	....	....	....	....	....
cuprina—T	....	....	....	....	....
caesar	....	....	....	....	....
cluvia—1	....	....	....	....	....
cluvia—2	....	....	....	....	....
mexicana	....	....	....	....	....
illustris	....	....	....	....	....
ampullacea	....	....	....	....	....
richardsi	....	....	....	....	....
thatuna	....	....	....	....	....
silvarum	....	....	....	....	....
silvarum—1	....	....	....	....	....
H. fernandica	....	....	....	....	....
C. vicina	....	....	.......C..	....	....

Table 2—*continued.*

14801					
sericata	TAAGTTTCCT	TAATTAATAA	TATTAATTAC	TGCGGATAA-A	A-AAAAATT-AT
cuprina	. . . . .	. . . . .	. . . . .	. . . . . -.	. - . . . . .
cuprina—D	. . . . .	. . . . .	. . . . .	. . . . . -.	. A . . . . .
cuprina—T	. . . . .	. . . . .	. . . . .	. . . . . -.	. - . . . . .
caesar	. . . . .	. . . . .	. . . . .	. . . . . T.	. T . . . . .
cluvia—1	. . . . .	. . . . .	. . . . .	. . . . . T.	. A . . . . .
cluvia—2	. . . . .	. . . . .	. . . . .	. . . . . T.	. - . . . . .
mexicana	. . . . .	. . . . .	. . . . .	. . . . . -T	. A . . . . .
illustris	. . . . .	. . . . .	. . . . .	. . . . . -T	. A T . . . . T . .
ampullacea	. . . . .	. . . . .	. . . . .	. . . . . G . . . -G	. A . . . . .
richardsi	. . . . .	. . . . .	. . . . .	. . . . . -.	. - . . . . .
thatuna	. . . . .	. . . . .	. . . . .	. . . . . -.	. A . . . . .
silvarum	. . . . .	. . . . .	. . . . .	. . . . . -.	. A . . . . .
silvarum—1	. . . . .	. . . . .	. . . . .	. . . . . -.	. A . . . . .
H. fernandica	. . . . .	. . . . .	. . . . .	. . . . . -.	. - . . . . .
C. vicina	. . . . .	. . . . .	. . . . .	. . . . . -.	. - T . . TT . . . C
14851					
sericata	TTATTATTAA	AATAAATAAA	TATTCATATA	AAAATTTACA	TATAAATTAA
cuprina	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
cuprina—D	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
cuprina—T	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
caesar	. C . C	. . . . .	. . . . .	. . . . .	. . . . .
cluvia—1	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
cluvia—2	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
mexicana	. . . . .	. . . . .	. . . . . G	. . . . .	. . . . .
illustris	. . . C	. . . . .	. . . . .	. . . . .	. . . . .
ampulacea	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
richardsi	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
thatuna	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
silvarum	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
silvarum—1	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .
H. fernandica	. . . . . T .	. . . . .	. . . . . C . . .	. . . . .	. . . . .
C. vicina	. . . . .	. . . . .	A . . . . . C . . .	. . . . .	. . . . .
14901					
sericata	ACTAATAATA	AATTTACAAG	CAAAATAAAA	CTTTATACAC	TA 3′
cuprina	. . . . .	. . . . .	. . . . .	. . . . .	. .
cuprina—D	. . . . .	. . . . .	. . . . .	. . . . .	. .
cuprina—T	. . . . .	. . . . .	. . . . .	. . . . .	. .
caesar	. . . . .	. . . . .	. . . . .	. . . . . A	. T
cluvia—1	. . . . .	. . . . .	. . . . .	. . . . . A	. T
cluvia—2	. . . . .	. . . . .	. . . . .	. . . . . A	. T
mexicana	. . . . .	. . . . .	. . . . .	. . . . . A	. T
illustris	. . . . .	. . . . .	. . . . .	. . . . . A	. T
ampullacea	. . . . .	. . . . .	. . . . .	. . . . . A	A .
richardsi	. . . . .	. . . . .	. . . . .	. . . . .	. .
thatuna	. . . . .	. . . . .	. . . . .	. . . . .	. .
silvarum	. . . . .	. . . . .	. . . . .	. . . . .	. .
silvarum—1	. . . . .	. . . . .	. . . . .	. . . . .	. .
H. fernandica	. . . . .	. . . . .	. . . . .	. . . . . ?	? ?
C. vicina	. . . . . C	. . . . .	. . . . .	. . . . GA . . . A	. .

[a]Details of specimens analysed are given in Table 1. *L. cuprina*: D = Dakar, Senegal; T = Townsville, Australia. Numbers at the beginning of each data block are for reference only and relate to the sequence identification numbers in the published *Drosophila yakuba* sequence (Clary & Wolstenholme, 1985). Insertions or deletions are noted as "-".

than those identified by a strict consensus method but, nevertheless, can provide a useful insight into underlying relationships. In this study only clusters occurring in at least 50% of trees were included.

In the majority rule consensus tree (Fig. 2), relationships between *L. cuprina*, *L. richardsi*, *L. sericata*, *L. silvarum* and *L. thatuna* are unresolved at the 50% bootstrap support level. However, all *Lucilia* were well separated (91% bootstrap support) from *H. fernandica*. The most parsimonious tree produced from the mtDNA sequence data was compared with a most parsimonious tree from a previous cladistic analysis,

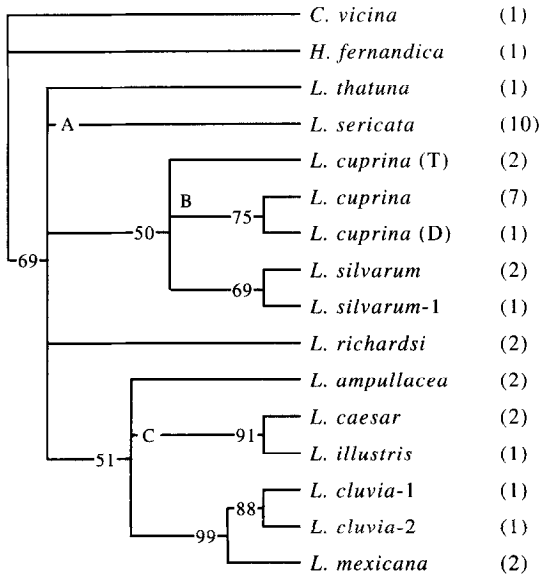

Fig. 1. Majority-rule consensus tree derived from 100 neighbour-joining trees/bootstrapped mtDNA data sets. Genetic distances were calculated using the nucleotide substitution model of Kimura (1980). Bootstrap values >50% are indicated at branch nodes. Numbers in parentheses indicate the number of flies characterised. (D), Dakar, Senegal; (T), Townsville, Australia. Outgroup, *C. vicina*. A, B, C: points at which the myasis habit is required to have evolved (assuming the most parsimonious explanation) based on the distribution of the major myasis species in the tree.

based on morphological characters (Stevens & Wall, 1996). The results of this comparative analysis (Fig. 2) show that the 2 phylogenies derived from mtDNA sequences and morphological data are concordant, providing increased support for the relationships described (Swofford, 1991). However, while *L. caesar* and *L. illustris* group closely in both trees, cladistic relationships for the species which act as agents of myiasis are only fully resolved in the morphologically based tree. This result indicates the limitation of parsimony analysis with a relatively conservative molecular marker. The only conflicting result is the grouping of *L. cluvia* with *L. mexicana*. These 2 species are well separated in the morphological tree, but cluster at the 95% level in the molecular tree. This anomaly could be affected by a range of factors, including the paucity of good characters for these particular species in the morphological analysis (Stevens & Wall, 1996). This problem will undoubtedly have to be addressed in future studies.

DISCUSSION

Within the genus *Lucilia* considerable variation in myiasis behaviour exists both between and within individual species. *Lucilia sericata* is the most important agent of sheep myiasis throughout northern Europe (MacLeod, 1943; Wall, French & Morgan, 1992a). It was first recorded as an ectoparasite in England in the 15th century and, at present, over 80% of sheep farms are affected by blowfly strike and about 750 000 sheep are infested, of which approximately 2% die (French *et al.*, 1992; French, Wall & Morgan, 1995). Mortalities of 20–30% among animals infested by *L. sericata* have been recorded in parts of Europe (Liebisch, Froehner & Elger, 1983; Mashkei, 1990). Although present in Australia, *L. sericata* is largely a synanthropic species and is rarely implicated in myiasis of sheep (Waterhouse & Paramonov, 1950). In contrast, in New Zealand, *L. sericata* was introduced over 100 years ago and soon established itself as the primary myiasis fly (Miller, 1939). In 1976, it was estimated that about 1.7% of sheep were struck each year by *L. sericata* on the North Island of New Zealand and about 0.7% on the South Island, at an annual cost of about $NZ1.7 million (Tenquist & Wright, 1976). In North America, *L. sericata* (syn. *Phaenicia sericata*) is also the most important species of *Lucilia* implicated in sheep myiasis (Williams *et al.*, 1985). Its economic impact, however, remains unquantified.

Lucilia cuprina is absent from most of Europe, although it has been recorded from southern Spain and North Africa (Rognes, 1994). Originally Oriental or Afrotropical in distribution, *Lucilia cuprina* was probably introduced into Australia towards the middle or end of the 19th century (Mackerras & Fuller, 1937; Norris, 1990) and it is now the dominant sheep myiasis species for mainland Australia (Watts *et al.*, 1976; Dalwitz, Roberts & Kitching, 1984) and Tasmania (Ryan, 1954). It is present in 90–99% of flystrike cases. In the early 1980s *L. cuprina* was discovered in New Zealand, probably introduced from Australia, and in northern areas of New Zealand it is now becoming an important primary cause of flystrike in sheep. In southern Africa, although *L. cuprina* had been known to be present since 1830, little sheep strike was recorded until the early decades of the 20th century, following which it became the most important primary myiasis fly (Waterhouse & Paramonov, 1950). Interestingly, although *L. cuprina* (syn. *Phaenicia cuprina* = *Phaenicia pallescens*) is known to be present in the U.S.A., it does not appear to be important in sheep myiasis (Williams *et al.*, 1985).

At the interspecific level, if the myiasis habit in this genus evolved through the commonly proposed route, with saprophagous species, giving rise to occasional facultative ectoparasites and, in turn, to primary facultative ectoparasites, phylogenetic relationships reflecting the behavioural differences between species might have been expected. Hence, a close phylogenetic

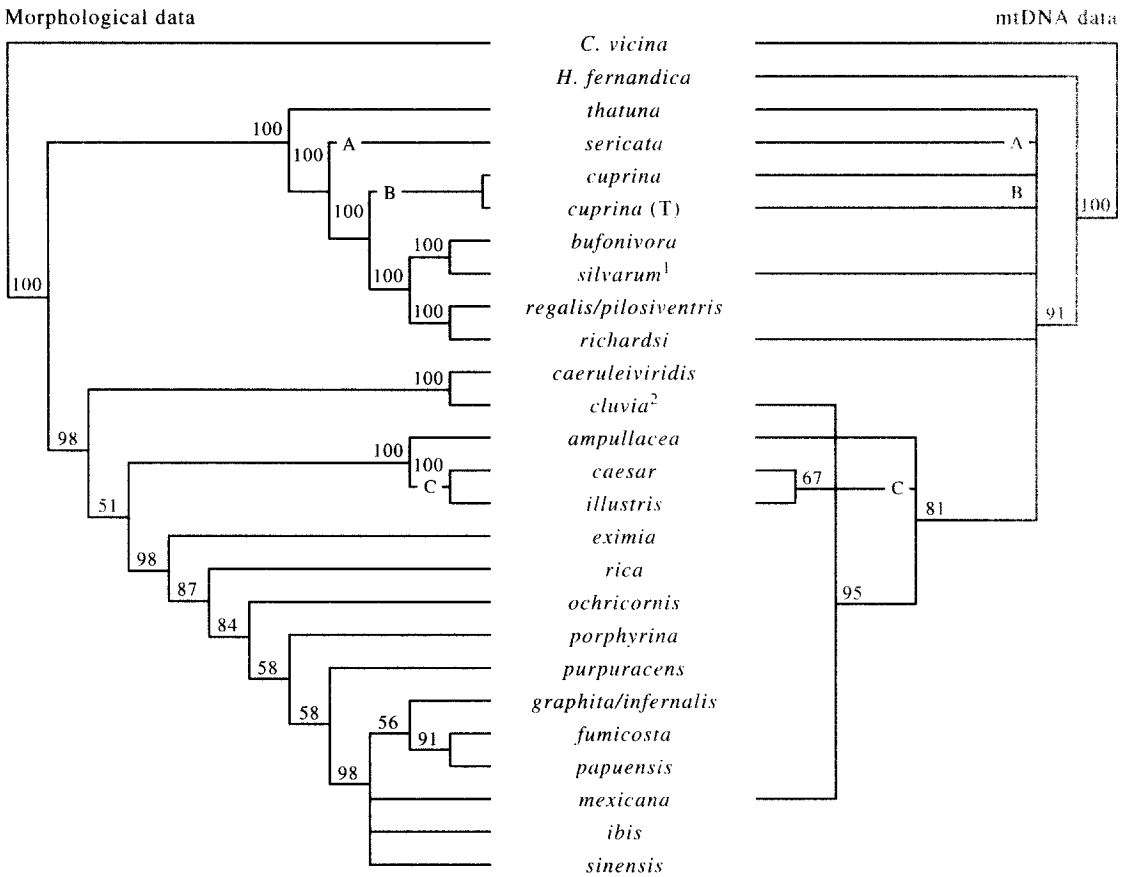

Fig. 2. Majority-rule consensus tree derived from mtDNA sequence data for 10 species of *Lucilia*, *H. fernandica* and *C. vicina* (outgroup) compared with a majority-rule consensus tree derived from morphological data for 25 species of *Lucilia* based on 14 morphological characters coded as 17 binary factors (see Stevens & Wall (1996) for full details). Node values on mtDNA tree are bootstrap values based on 100 mtDNA data sets. For both trees, only values >50% are presented: node values on the morphological tree indicate the percentage occurrence of a particular clade in the 45 most parsimonious trees. (T), *L. cuprina* collected from Townsville, Australia. A, B, C: points at which the myasis habit is required to have evolved (assuming the most parsimonious explanation) based on the distribution of the major myasis species in each tree. [1]Includes both *L. silvarum* mtDNA types; bootstrap value = 50%. [2]Includes both *L. cluvia* mtDNA types; bootstrap value = 86%.

relationship between *L. sericata* and *L. cuprina* might have been anticipated. These 2 species might also have been expected to be more closely related to possible "ancestral" forms, such as the 2 species of secondary facultative myiasis fly *L. caesar* and *L. illustris*, than to species not known to act as myiasis agents. However, the analyses presented show that this is not the case. *Lucilia sericata* appears to be no more closely related to *L. cuprina* than a number of other *Lucilia* species that have never been implicated in strike, such as *L. richardsi*, despite the fact that *L. richardsi* is sympatric and morphologically almost identical to *L. sericata*. Similarly, although *L. caesar* and *L. illustris* are very closely related to each other, they are well separated from *L. sericata* and *L. cuprina*. Hence, there appears to be no evidence for the existence of a progression of the myiasis habit, with species increasing in their dependency on living hosts, within phylogenetic groups. The most parsimonious explanation of the data suggests polyphyletic evolution of the myiasis habit, probably on at least 3 occasions (A, B, C. Figs 1 and 2) by *L. sericata*, *L. cuprina* and the *L. caesar*/*L. illustris* group, respectively. If the highly specialised myiasis of amphibians by *L. bufonivora*, a close relative of *L. silvarum*, is also considered, a fourth independent evolutionary event may need to be invoked.

At the intraspecific level, pronounced genetic variation within species, particularly *L. sericata* and *L. cuprina*, might have been expected, reflecting their known differences in myiasis behaviour in different geographic parts of their range. However, within *L. sericata* no genetic differences were detected in flies from North America, Europe, southern Africa, Australia or New Zealand. Within *L. cuprina*, the majority of flies collected from Australia, New Zealand and Africa were genetically identical and only 3 of the

specimens analysed, 2 collected from Townsville, Australia and 1 from Senegal in West Africa, showed genetic differences from each other and the majority-type *L. cuprina*. Hence, the data do not indicate that there is any clear relationship between genetic variation and the described differences in pathogenicity for either *L. sericata* or *L. cuprina*.

Apart from some specialist investigations (Sperling, Anderson & Hickey, 1994; D. M. Gleeson 1995. The genetic effects following the colonisation of New Zealand by *Lucilia cuprina*. Ph.D. Thesis, Australian National University, Canberra, A.C.T.) few molecular-based characterisation studies have so far been performed and most taxonomic and evolutionary studies of the genus *Lucilia* to date have been based on morphological characters (e.g., Aubertin, 1933; Stevens & Wall, 1996). The limited level of resolution of the relationships between some taxa included in this study indicates the need for more detailed work using a greater number of species, specimens and molecular characters to explore fully the diversity of this important genus. Nevertheless, when viewed in combination with morphological information, the data suggest that, as proposed by Erzinclioglu (1989), the myiasis habit in *L. sericata* and *L. cuprina* probably coevolved in relatively recent history along with the domestication and husbandry of sheep. The process of selection of these animals for a thick woolly fleece which grows all year round created a microhabitat suitable for colonisation by fly larvae. It is notable that the dramatic growth in reported prevalence of flystrike in South Africa and Australia coincides with the import or "improvement" of breeds of Marino sheep with heavier fleeces (Tillyard & Seddon, 1933; Norris, 1990). More primitive hairy breeds of sheep (e.g., Soays) are rarely struck. Species such as *L. sericata* and *L. cuprina*, which are early colonisers of carcasses and which possibly were occasional facultative ectoparasites of diseased or wounded mammals, perhaps had an immediate selective advantage which allowed them to move into this new niche. However, the geographic differences in the behaviour of *L. sericata* and *L. cuprina* also suggest that the myiasis habit probably arose independently in geographically isolated populations after the initiation of sheep husbandry in these areas, the fly species becoming dominant in each area being dependent largely on climate. Hence, *L. cuprina* has become the predominant pathogenic species in sub-tropical and warm temperate habitats (e.g., Australia and South Africa) and *L. sericata* in cool temperate habitats (e.g., Europe and New Zealand). This influence of climate on the development of myiasis in various species of *Lucilia* is further exemplified by the fact that *L. caesar* and *L. illustris* become more common in sheep myiasis only in more northerly Palaearctic regions (Brinkmann, 1976), despite being present throughout the temperate Palaearctic. Given this proposed recent history of myiasis, local adaptation and allopatry would not yet be expected to be reflected in changes in the relatively conservative mtDNA sequence analysed here.

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