Evolution, 60(2), 2006, pp. 315–327

DO ANTS ENHANCE DIVERSIFICATION IN LYCAENID BUTTERFLIES? PHYLOGEOGRAPHIC EVIDENCE FROM A MODEL MYRMECOPHILE, *JALMENUS EVAGORAS*

ROD EASTWOOD,[1,2] NAOMI E. PIERCE,[3,4] R. L. KITCHING,[1,5] AND JANE M. HUGHES[1,6]
[1]*Australian School of Environmental Studies, Griffith University, Nathan 4111, Queensland, Australia*
[2]*E-mail: r.eastwood@griffith.edu.au*
[3]*Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, Massachusetts 02138*
[4]*E-mail: npierce@oeb.harvard.edu*
[5]*E-mail: r.kitching@griffith.edu.au*
[6]*E-mail: jane.hughes@griffith.edu.au*

Abstract.—The ant-tended Australian butterfly, *Jalmenus evagoras*, has been a model system for studying the ecology and evolution of mutualism. A phylogeographic analysis of mitochondrial DNA cytochrome oxidase I sequences from 242 butterflies (615 bp) and 66 attendant ants (585 bp) from 22 populations was carried out to explore the relationship between ant association and butterfly population structure. This analysis revealed 12 closely related butterfly haplotypes in three distinct clades roughly corresponding to three allopatric subpopulations of the butterflies. Minimal genetic diversity and widespread haplotypes within biogeographical regions suggest high levels of matrilineal gene flow. Attendant ants are significantly more diverse than was previously thought, with at least seven well-defined clades corresponding to independent morphological determinations, distributed throughout the range of the butterflies. Nested analysis of molecular variance showed that biogeography, host plant, and ant associate all contribute significantly in explaining variation in butterfly genetic diversity, but these variables are not independent of one another. Major influences appear to come from fragmentation due to large-scale biogeographical barriers, and diversification following a shift in habitat preference. A consequence of such a shift could be codiversification of the butterfly with habitat-adapted ants, resulting in apparent phylogenetic concordance between butterflies and ants. The implications of these results are discussed in terms of possible effects of ant attendance on the diversification of Lycaenidae as a whole.

Key words.—Ant association, biogeography, habitat specialist, *Iridomyrmex*, mutualism, myrmecophily, obligate.

Received July 26, 2005. Accepted November 8, 2005.

The butterfly family Lycaenidae (including the Riodinidae, often considered a subfamily of the Lycaenidae) contains an estimated 30% of all butterfly species (Shields 1989; Ackery et al. 1998) and exhibits a diverse array of life-history strategies (Hinton 1951; Cottrell 1984; Fiedler 1991; Pierce et al. 2002). The early stages of about 75% of all lycaenids associate with ants to varying degrees, ranging from casual facultative coexistence to obligate association where the long-term survival of the butterfly is dependent on the presence of its attendant ants. Attendant ants guard the butterflies against predators and parasites during their vulnerable period of larval growth and pupation. The caterpillars, in return, reward the ants by providing attractive secretions from specialized glands in the cuticle (Malicky 1969, 1970; Maschwitz et al. 1975; Pierce and Nash 1999).

The prevalence of caterpillar-ant associations in the species-rich Lycaenidae is in contrast with other Lepidoptera, where ant association appears only rarely and in disparate lineages (Hinton 1951). This has led to the proposal that ant association may have accelerated diversification in the group (Pierce 1984; Pierce et al. 2002). At least two mechanisms have been proposed: first, the stringent requirements of both suitable host plants and appropriate attendant ant species may have resulted in smaller population sizes and population fragmentation of ant-associated lycaenids (Pierce 1984; Costa et al. 1996). This would increase the likelihood of isolation, leading to allopatric speciation (Mayr 1942). At the population level, a signal of such fragmentation could be detectable as high levels of genetic differentiation and significant levels of isolation by distance among butterfly subpopulations.

A second hypothesis suggests that a shift in ant association, particularly a radical shift, may result in an escape-and-radiation mode of diversification (Pierce et al. 2002). Shifts in attendant ants would be important events for obligately ant-associated lycaenids since most have highly specialized ant associations (Fiedler 1991; Eastwood and Fraser 1999). The conservative nature of these associations is highlighted in phylogenetic studies by a correlation of discrete ant taxa associating with highly myrmecophilous lycaenids at the species and higher clade levels (*Maculinea*, Thomas et al. 1989; Elmes et al. 1994; *Jalmenus*, Pierce and Nash 1999; *Chrysoritis*, Rand et al. 2000; *Acrodipsas*, Eastwood and Hughes 2003a; *Arhopala*, Megens et al. 2005). However, the appearance of butterfly sister taxa associating with different ant species may be explained by at least three processes, only one of which would be causative. These mechanisms would exhibit different phylogeographic patterns that could be detected in a population of obligately ant-associated lycaenids as follows: (1) A shift to an ecologically different ant species or to an attendant ant species from another genus or subfamily could result in ecological diversification with the novel ant. If this were to occur, it should be detectable at the population level as genetic breaks in butterfly subpopulations coinciding with radically different ant associates, particularly if the different associations were to occur in sympatry or parapatry. (2) Butterfly sister species associating with different ant species could be observed coincidentally in a phylogeny if fragmentation of an oligomyrmecophilous butterfly population (associating with several closely related species of ants across its range) coincided with a geographically restricted or locally

preferred ant partner. This could be detected at the population level if an isolated butterfly subpopulation, associating with the local ant, was genetically divergent from the remaining butterfly population. (3) Butterfly sister taxa associating with ant sister taxa (parallel cladogenesis) could occur if a butterfly population tended by a single ant species across its range underwent fragmentation together with its attendant ant. In time, this could result in cospeciation of the two partners. Evidence at the population level would be seen if a fragmented subgroup of butterflies showed genetic differences in parallel with a subgroup of its attendant ant.

An earlier attempt at unraveling the relationships between ants and butterflies at the population level found little evidence in favor of ant-associated effects on population size and structure (Costa et al. 1996). Estimates of Wright's F_{ST} using multilocus allozyme data from 1052 specimens of *Jalmenus evagoras* sampled from 15 geographically separated sites in an area of about 150 km^2 revealed low amounts of genetic structure at the level of geographic subpopulation, and no evidence of population differentiation corresponding to the two species of ant associate common to these sites. However, this study was conducted at a small spatial scale relative to the overall species range of *J. evagoras*, and did not consider the degree of differentiation of the associated ants. Moreover, subsequent behavioral observations showing that *J. evagoras* females preferentially deposit eggs on plants containing their locally predominant attendant ant species, even when the butterflies are reared in the absence of ants (Fraser et al. 2002), further raised the question of whether this behavior might be reflected in the population structure of the butterflies.

The purpose of this study was to apply phylogeographic techniques to populations throughout the range of the obligate myrmecophile, *J. evagoras*, addressing the following questions, some of which may not be mutually exclusive: (1) Are butterfly populations highly structured genetically, as predicted for a species with specialized ecological requirements? (2) Is there evidence that a shift to an ecologically novel attendant-ant, fragmentation coincident with locally divergent ant associates, or species-pair fragmentation could result in the conservatism of ant association observed in phylogenies of obligate lycaenids? (3) Have geological, climatic or other processes shaped the extant distribution of genetic variation in the species?

Materials and Methods

Study System

Jalmenus evagoras is one of 10 species in the genus (Braby 2000), all of which are endemic to Australia. Two subspecies of *J. evagoras* are recognized: *J. evagoras evagoras* is found mainly in the wooded foothills in coastal regions extending from southern and eastern Victoria, through coastal New South Wales and into southeastern Queensland, and inland at Binjour (see Fig. 1). Its conspecific, *J. evagoras eubulus*, is a habitat specialist confined to the brigalow (*Acacia harpophylla*) belt west of the Dividing Range in central and southern Queensland from near Eungella to just south of the Queensland border (DeBaar 1977). The two subspecies are contiguous from near Toowoomba through the Bunya Mountains to Binjour (see Fig. 1) where there may be a stepped cline or intermediate populations (Braby 2000). In general, nominotypical specimens from eastern Queensland are paler than their southern counterparts, and the very pale subspecies *J. e. eubulus* is regarded as the morphological extreme.

Female *J. evagoras* eclose with a large complement of mature eggs (Hill and Pierce 1989) and are often mated upon eclosion, before they have even expanded their wings (Pierce and Nash 1999). They exhibit high levels of site fidelity and preferentially deposit eggs on suitable *Acacia* host plants in or around their natal colony (Elgar and Pierce 1988). Larvae feed gregariously and are recorded as having an obligate mutualistic relationship with *Iridomyrmex* ants (Dolichoderinae), primarily *I. anceps* and *I. rufoniger* (Eastwood and Fraser 1999; Pierce and Nash 1999). Attendant ants protect the caterpillars from predators and parasitoids and act as an important stimulus for female *J. evagoras* to deposit their eggs on the host plant (Pierce and Elgar 1985; Pierce et al. 1987; Pierce and Nash 1999; Fraser et al. 2002).

Specimens

The 242 *J. evagoras* specimens used in this analysis were collected from 22 sites across the range of both subspecies extending from Wallan in southern Victoria to Taroom in central Queensland (Appendix 1). Where possible, at each site, adults, larvae, or pupae were collected from different trees to maximize the potential for sampling genetic diversity. Latitude and longitude coordinates were determined for all sites, and place names shown in Figure 1 are the nearest town to the sampling site. Specimens at all life stages were preserved in 95% ethanol, and wing vouchers stored in glassine envelopes.

Attendant ants were also collected from the same sampling sites and stored in 95% ethanol (Appendix 2). Up to 20 ants were removed from a single tree where *J. evagoras* larvae were being tended, and multiple trees were sampled at several sites. At least two ants from each sample were pointed for morphological identification ($n = 88$) and two from each sample tree were sequenced ($n = 66$). Morphological identifications were conducted by A. Andersen (CSIRO, Darwin, Australia) independently of genetic data. Host-plant data were recorded at most sites as shown in Appendix 1. Morphological vouchers of ants (pointed and wet preserved) are held at CSIRO, and tissue samples of ants and butterflies are stored in the Museum of Comparative Zoology tissue collection, Harvard University, Cambridge, MA.

DNA Characterization

Isolation of DNA from larval or adult tissue followed the CTAB mini-prep format of Doyle and Doyle (1987). The dissected terminal segments of larvae or anterior segments of butterfly abdomens, or a whole worker ant, were ground and incubated overnight at 55°C in 700 μl 2× CTAB buffer and 5 μl 20 mg/ml proteinase K. Homogenates were extracted twice with phenol/chloroform and DNA was precipitated with isopropanol at −20°C. The pellet of DNA was twice washed in 70% ethanol and resuspended in 50 μl H_2O prior to polymerase chain reaction (PCR) amplification of the 3′ end of the cytochrome oxidase I (COI) gene subunit using Folmer

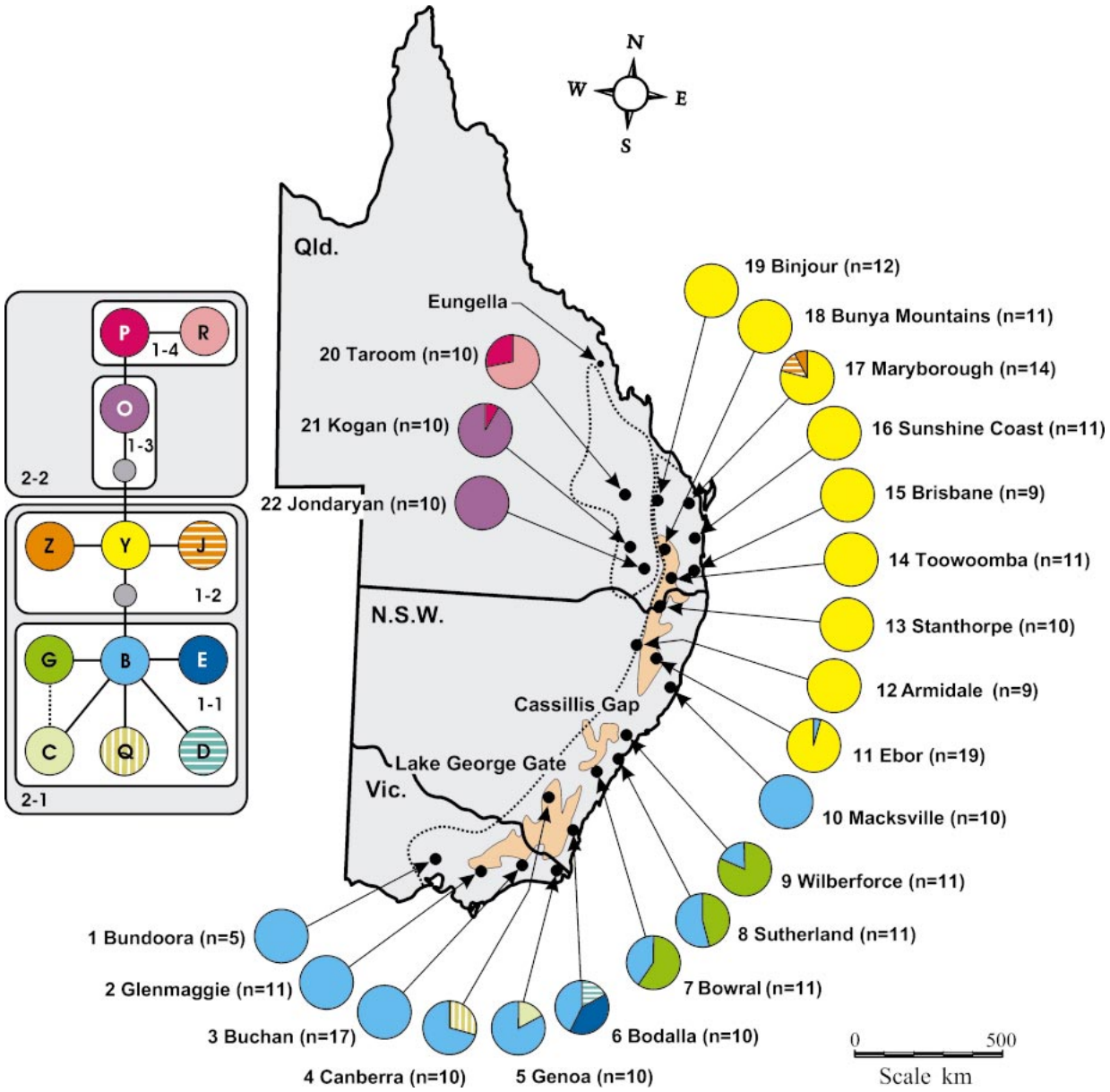

FIG. 1. *Jalmenus evagoras* haplotype distribution based on a 615-bp fragment of the mitochondrial cytochrome oxidase I gene. Numbers preceding the sampling site names correspond to site numbers in Appendixes 1 and 2. Dotted line delineates the subspecies boundaries: *Jalmenus evagoras eubulus* in the northwest, *J. e. evagoras* along the coast; *n* indicates sample size. Buff-colored areas show the 900 m contours on the Great Dividing Range. Diagram at left shows the relationships between haplotypes and the clade nesting arrangement. Haplotypes are separated by single base mutations; gray haplotypes were not sampled.

et al.'s (1994) LCO1490 primer, and Simon et al.'s (1994) Nancy heavy strand primer.

Most PCRs and sequencing were undertaken at Griffith University; at Harvard University the protocols were modified slightly. A typical 25-μl PCR reaction comprised 12.5 μl Qiagen *Taq* PCR Master Mix Kit (Qiagen 2002), 0.5 μl each primer, 1 μl 10:1 diluted template, and 10.5 μl dd H_2O. Amplification of DNA was performed on an Eppendorf (Hamburg, Germany) MasterCycler. Pre-PCR was set at 94°C for 5 min followed by initial cycling at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec repeated for 35 cycles with a 5-min extension at 72°C after the final cycle; holding temperature was set at 4°C. Purification of PCR product followed the QIAquick PCR Purification Kit Protocol (Qiagen 2001) prior to asymmetric re-amplification using 4 μl dye terminator mix (10 μl reaction) (Perkin Elmer, Foster City, CA) and sequencing on an ABI 377 Automated Sequencer (Applied Biosystems, Foster City, CA).

Data Analysis

Sequencher 4.1.1 (Gene Codes Corporation, Ann Arbor, MI) was used for sequence editing and alignment of heavy and light strands. The 242 aligned fragments were converted

to noninterleaved NEXUS format in MacClade (Maddison and Maddison 1992) to generate a haplotype genealogy in TCS version 1.13 (Clement et al. 2000). Nested clade analysis (NCA) was performed with GeoDis version 2.0 (Posada et al. 2000) after allocation of clades according to the schema detailed in GeoDis 2.0 documentation and the criteria of Posada and Crandall (2001). The nesting arrangement captures temporal information in the haplotype genealogy by grouping clades in a hierarchy from tip to interior; that is, youngest to oldest. GeoDis assesses the grouping of haplotypes and clades according to geographic locations, and then quantifies the spread of haplotypes and clades (D_c), and the distances between haplotypes or clades and those clades within which they are nested (D_n) using geographic distance as an explicit variable. An interior-tip distance within nested clades is also measured to contrast old versus young clades (Templeton 2004). Statistical significance of all variables is assessed against a null hypothesis of no geographic association using a randomization procedure. Population structure can then be separated from population history using a dichotomous inference key (Templeton 2004), which contrasts the relationships among interior and tip clades in which significantly large or small distances are recorded. The underlying assumptions are based on coalescent theory; for example, under restricted gene flow, older haplotypes should have a wider distribution than those more recently derived. They should also have more mutational derivatives and be placed internally in the network, whereas recent haplotypes are more likely to be found at the tips (Templeton et al. 1995). Thus, a significantly large interior-clade distance (D_c) in contrast with significantly small tip-clade distances is interpreted as evidence of restricted gene flow. Conversely, tip-clade distances that are significantly larger than interior-clade distances would be interpreted as range expansion or long-distance colonization. Although NCA can distinguish population history from population structure, there is still some doubt whether NCA can correctly infer the combination of factors that best explains the current distribution of genetic diversity in populations (Knowles and Maddison 2002). Where possible, independent assessments of the data were made.

Six butterflies from a population just south of Wallan, Victoria (near La Trobe University campus), with haplotypes identical to those otherwise only found north from Ebor in northern New South Wales (>1000 km away) were excluded from all analyses. This population most likely originated from escapees during experiments conducted there in the mid-1980s. The probability that two random mutations would occur at identical positions involving the same nucleotides in a fragment of 615 bases is sufficiently small that an accidental release seems the most parsimonious explanation for the co-existence of these haplotypes.

We plotted a distribution of pairwise sequence differences (mismatch distribution) for all butterfly haplotypes using Arlequin version 2.0 (Schneider et al. 2000) as an independent test for demographic events in each of the three major subgroups identified in the haplotype network (Fig. 1). Group 1 consisted of *J. e. eubulus* and groups 2 and 3 of *J. e. evagoras* north and south of the Cassillis Gap, respectively. A unimodal mismatch distribution is characteristic of haplotypes drawn from a population that has undergone a recent demographic expansion and is the null hypothesis by which the mismatch distribution is assessed. Significant divergences from this model, that is, multimodality, reflect the highly stochastic shape of gene trees under demographic equilibrium (Slatkin and Hudson 1991; Rogers and Harpending 1992). We calculated Tajima's (1989) *D* and Fu's (1997) *Fs* statistics to test for selective neutrality in these subgroups.

We used a nested analysis of molecular variance (AMOVA; Excoffier et al. 1992) implemented in Arlequin to partition *J. evagoras* genetic variation into components attributable to differences among the specified hierarchical groups (Φ_{CT}), among populations within hierarchical groups (Φ_{SC}), and among sites across the species distribution (Φ_{ST}). Four hierarchies were analyzed to investigate which factors had a significant effect on genetic variability. First, we divided the population into two groups along taxonomic lines (i.e., subspecies, also synonymous with morphology). Second, the population was subdivided according to the attendant ant species (identified a posteriori). Third, we partitioned by locally used host-plant species (Appendix 1). Finally, we identified four biogeographical regions based on two known biogeographical barriers (the Cassillis Gap and Lake George Gate) and by an environmental barrier that separates the arid inland from coastal regions (Waterhouse 1922; Keast 1961; Ford 1987; Nicholls and Austin 2005). Thus, the population was strictly divided latitudinally at the Cassillis Gap and Lake George Gate, and longitudinally between the coast and inland areas. Significance of the AMOVA Φ statistics was tested by 1000 permutations of haplotypes among and within populations under the null hypothesis of panmixia. In all cases, uncorrected sequence divergence was incorporated as the measure of genetic differentiation.

An infinite-sites model was used to estimate the time since butterfly populations were separated by the Cassillis Gap using MDIV (Nielsen and Wakeley 2001). The approach of Nielsen and Wakeley (2001) implements both likelihood and Bayesian methods using Markov chain Monte Carlo simulations to estimate jointly the time to most recent common ancestor (TMRCA), expected nucleotide heterozygosity (θ), divergence time (*T*), and migration rate (*M*). Five initial runs, using different random seeds, were applied to ensure the stability of the posterior estimate, each time sampling 5.0×10^6 trees with a burn-in value of 5.0×10^5. The final run sampled 5.0×10^6 trees from an infinite sites model ($M < 2.0$, $T < 6.0$ in coalescent units). A confidence interval for the population separation time was estimated with the mutation rate for COI set at 1.3%–1.9% absolute sequence divergence per million years (see review of arthropod COI mutation rates in Quek et al. 2004), while *T*, *M*, and θ point estimates were determined from the modal value of their posterior distributions.

Ant-species clades were identified by homologous sequences from the mitochondrial COI gene using an unweighted neighbor-joining algorithm in PAUP* 4.0b10 (Swofford 1998). No attempt was made to define phylogenetic relationships of these ants since too little is known about related species and/or potential outgroups for this small sample. Shattuck (1999) records 79 named species and subspecies of *Iridomyrmex*. The purpose of the analysis was simply to identify taxonomic groups of attendant ants.

TABLE 1. Results of the nested clade analysis of *Jalmenus evagoras* populations and their biological inferences. Significantly large (L) or small (S) values for clade (D_c), nested clade (D_n) and interior- to tip-clade (I-T) distances are indicated in superscript. Chi-squared statistics for the contingency analysis of geographic association are given with corresponding *P*-value. Inference numbers relate to a sequence of questions in the dichotomous key of Templeton (2004) with the final answer (Y or N). Inferences in brackets are interpretations for clades in which the null hypothesis of no geographic association was not rejected by the contingency test.

Clade	χ^2 (*P*)	Nested clades	Position	D_c	D_n	Inference
1–1	167.9 (0.000)	B	I	302.6^{L}	310.2^{L}	1.2.3.4N restricted gene flow with isolation by distance
		C	T	0.0	229.3	
		E	T	0.0^{S}	102.9^{S}	
		Q	T	0.0^{S}	36.8^{S}	
		G	T	45.2^{S}	211.4	
		D	T	0.0	102.9^{S}	
			I-T	272.9^{L}	134.4^{L}	
1–2	20.1 (0.081)	J	T	0.0	242.3	(panmixia)
		Y	I	162.9	162.4	
		Z	T	0.0	242.3	
			I-T	162.9	−79.9	
1–4	1.92 (0.380)	P	I	68.7	54.1	(small sample size)
		R	T	0.0	16.7	
			I-T	68.7	37.4	
2–1	207.2 (0.000)	1–1	T	269.3^{S}	482.1	1.2.11.12N contiguous range expansion
		1–2	I	164.4^{S}	486.4	
			I-T	-104.9^{S}	4.3	
2–2	26.2 (0.000)	1–3	I	43.8^{S}	78.8^{S}	1.2.11.12N contiguous range expansion
		1–4	T	30.3^{S}	138.8^{L}	
			I-T	13.5	-60.0^{S}	
Total	240.0 (0.000)	2–1	—	484.9	485.7	1.19N allopatric fragmentation
		2–2	—	100.8^{S}	493.7	

RESULTS

Twelve closely related butterfly haplotypes (615-bp fragment) were divided into three clades, with each clade separated by a minimum of two base mutations. These groups aligned with three broadly allopatric subpopulations of butterflies as shown in Figure 1. There were 11 variable sites: nine at third- and two at first-codon positions. Mean overall nucleotide frequencies were A = 34.58%, T = 37.54%, G = 13.83%, C = 14.06%, which represents a 72.12% AT bias consistent with the mitochondrial DNA of most insect groups.

The haplotype diagram and nesting arrangement shown in Figure 1 has four one-step clades, two two-step clades, and the total cladogram (seven clades in all). Significant geographic association at the 95% confidence level was identified for four of the six clades analyzed (Table 1). Inability to detect geographic association can be caused by panmixia, insufficient sampling or insufficient resolution in the genetic marker. Of the two clades in which the null hypothesis of no geographic association was not rejected, clade 1-2 was consistent with panmixia; however, in clade 1-4 the probable cause was insufficient sampling since only two locations were sampled. Lack of resolution was rejected in this case since the marker was informative in several other clades. As a result we excluded clade 1-4 from further analysis. In the geographic distance analysis, the widespread clade 1-1 conformed to restricted gene flow with isolation by distance. Clades 2-1 and 2-2 both exhibited a signature of contiguous range expansion, albeit on vastly different geographical scales, and the total cladogram was consistent with allopatric fragmentation of clades 2-1 and 2-2. It was not necessary to assign interior and tip status for clades 2-1 and 2-2 to interpret the biological inference of the cladogram.

Genetic data grouped *Iridomyrmex* ants into seven clades (Fig. 2), which aligned with independent morphological determinations. Among taxa the 585-bp fragments had a mean sequence divergence of 8.50% (range = 0.17–11.97%), and within-taxa divergence was 0.65% (range = 0.0–2.90%). Mean AT content for all ants was 70.45%. The seven ant taxa were identified as *Iridomyrmex mattiroloi* group species I, *I. gracilis*, *Iridomyrmex* complex A species A (associated with *J. e. eubulus*), *Iridomyrmex* complex A species B, *Iridomyrmex* complex A species C, *Iridomyrmex* complex A species D, and *I. septentrionalis* species F. All taxa are closely related, ecologically dominant, and have similar gross morphology and behavioral characteristics (A. Andersen, pers. comm.)

Mismatch distribution plots for the three butterfly haplotype groups are shown in Figure 3. Tajima's *D* and Fu's *Fs* tests for selective neutrality (Fig. 3) indicate that groups 1 and 3 populations do not differ significantly from expectations under neutrality (Tajima 1989). Group 2, however, has a significantly negative value in both tests ($D = -1.297$, $P = 0.046$; $Fs = -3.350$, $P = 0.003$), which coupled with almost no genetic variability is consistent with a population expansion after a bottleneck; however, a selective sweep cannot be ruled out (Rand 1996). The shape of the model frequency wave front moving away from the y-axis also conveys biological and temporal information about the population under consideration. For example, steepness of the wave front is inversely correlated with the age of the expansion event (Rogers and Harpending 1992; Ray et al. 2003) so it can be seen that group 2 has recently undergone the population expansion. The extreme steepness of the group 2 mismatch distribution is also consistent with a small initial population

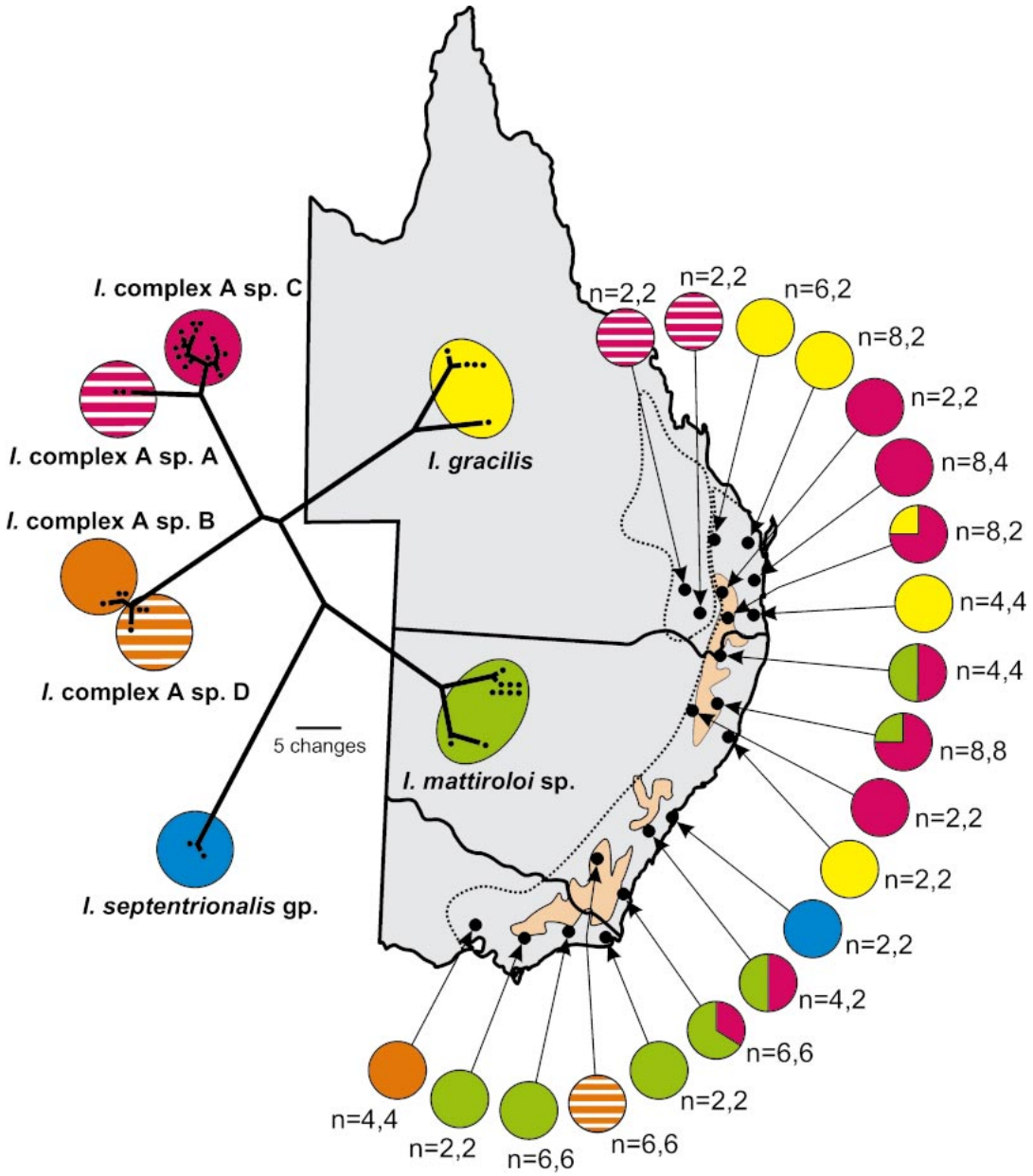

FIG. 2. *Iridomyrmex* ant species groups attending *Jalmenus evagoras* populations. Diagram at left is an unrooted neighbor-joining phylogram based on absolute nucleotide differences among all ants sampled (numbers of terminal taxa are indicated by dots). Species group determinations based on morphology are overlaid as colored circles. Distribution of species groups is shown on the map; *n* indicates the number of specimens from each site used, respectively, for morphological and genetic analysis (586 bp fragment of mitochondrial cytochrome oxidase I gene). All other details as in Figure 1.

size or bottleneck, and the high intercept on the y-axis suggests that the population has undergone a large increase in size (Rogers and Harpending 1992). The estimated time for *J. evagoras* population separation across the Cassillis Gap is 167,000–244,000 years.

AMOVA results (Table 2) indicate that significant levels of genetic partitioning among *J. evagoras* populations can be explained by biogeographical regions, taxonomy, tending ant species, and/or host plants. The strongest predictor was geography, which explained 80.0% of total genetic variation and, importantly, was the only partition in which Φ_{CT} (among regions) exceeded Φ_{SC} (among sites within regions) thus providing further support that geography is the best predictor of genetic discontinuity. Subspecies defined by morphology accounted for only 64.8% of total genetic variation, even though there were no shared haplotypes. Partitioning the data among the 14 host-plant species explained 60.4% of genetic variability, and partitioning the data among the seven attendant ant taxa accounted for 47.2%. Clearly, these variables are not independent since all partitions are themselves correlated with geography (i.e., subspecies, host plants, and attendant ants; see Figs. 1 and 2 for subspecies and ants), and thus geography would account for a significant proportion of molecular variance in all groupings.

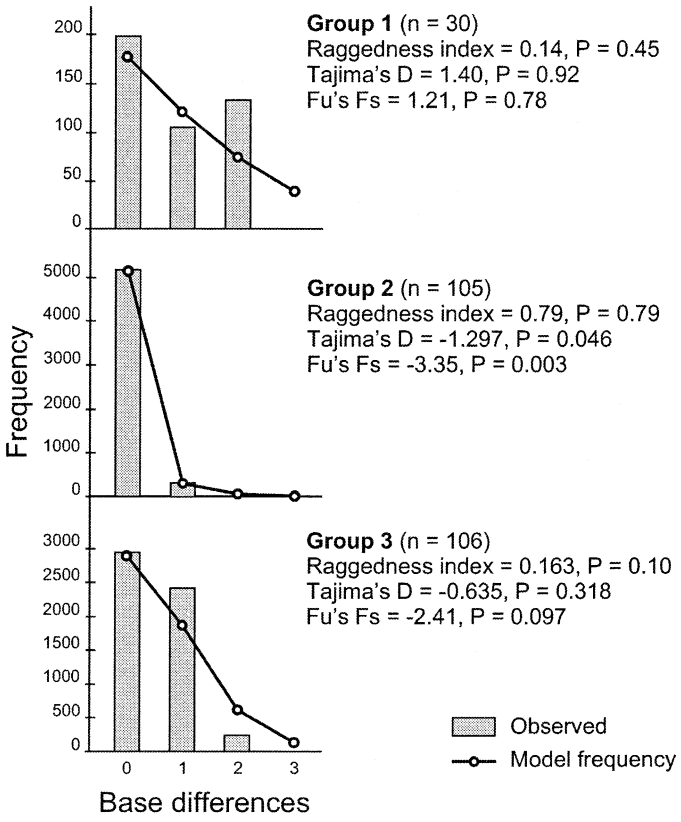

FIG. 3. Mismatch distributions for three subgroups of *Jalmenus evagoras* haplotypes. Group 1, *J. evagoras eubulus* (northwest); groups 2 and 3, *J. e. evagoras* north and south of the Cassillis Gap, respectively. Raggedness index and probability levels are given for each group (Tajima's [1989] *D* and Fu's [1997] *Fs* statistics) under the null distribution (model frequency) consistent with a sudden population expansion.

DISCUSSION

Effects of Ants on Population Structure

An unexpected finding of this study was that *J. evagoras* associates with at least seven ant taxa across its range, and none of these include the previously identified ant associates, *I. anceps* and *I. rufoniger* (for a review see Eastwood and Fraser 1999). These new findings may be explained by the difficulty of identifying morphologically similar *Iridomyrmex* ants at the species level in this large and complex group (Andersen 1995). Although the specific epithets of associated ants may not be of biological importance, the fact that *J. evagoras* associates with a number of closely related ant species (is oligomyrmecophilous) shows that it is less of an ant specialist, at least on a broad geographical scale, than was previously thought (Pierce et al. 1987; Eastwood and Fraser 1999; Pierce and Nash 1999). It appears to be sufficiently labile in its attendant ant requirements that a range of ecologically similar, locally dominant *Iridomyrmex* species can be accepted as partners (see also Fraser et al. 2002). Such regionalized association with different ant species has been observed in other obligately ant-associated lycaenids (e.g., *Maculinea*; Als et al. 2002) and closer scrutiny of the ants tending other widespread obligate myrmecophiles may reveal a similar pattern.

Data gathered in this study do not support a model of butterfly population fragmentation due to the stringent requirements of suitable attendant ant species overlapping with a relatively narrow range of host plants. This holds at both the regional level and on a broader scale that takes into account the entire range of the species. Although there was one signal of isolation by distance in the southern region (Table 1), this was driven by grouping of a derived haplotype in populations north of the Lake George Gate biogeographical barrier, and by two localized populations in southern New South Wales. Enhanced structure in southern Australian lycaenid populations was detected previously (Eastwood and Hughes 2003b) and may be a function of reduced voltism. However, southern populations were also characterized by

TABLE 2. Nested AMOVA results for *Jalmenus evagoras* cytochrome oxidase I haplotypes partitioned by geographical regions, host plants, taxonomy, and attendant ant species.

Partition	df	% variation	Φ	*P*
Biogeography				
Among regions	3	80.04	$\Phi_{CT} = 0.800$	<0.001
Among sites within regions	18	11.87	$\Phi_{SC} = 0.595$	<0.001
Within sites	221	8.09	$\Phi_{ST} = 0.919$	<0.001
Host plants				
Among plant species	13	60.45	$\Phi_{CT} = 0.604$	<0.001
Among subpopulations within host plant species	15	31.45	$\Phi_{SC} = 0.795$	<0.001
Within sites	154	8.10	$\Phi_{ST} = 0.919$	≅0.001
Taxonomy/morphology				
Between subspecies	1	64.79	$\Phi_{CT} = 0.648$	<0.001
Among subpopulations within butterfly subspecies	20	29.91	$\Phi_{SC} = 0.849$	<0.001
Within sites	221	5.30	$\Phi_{ST} = 0.947$	<0.001
Attendant ants				
Among ant species	6	47.23	$\Phi_{CT} = 0.472$	<0.001
Among subpopulations within attendant ant species	18	43.35	$\Phi_{SC} = 0.822$	<0.001
Within sites	197	9.42	$\Phi_{ST} = 0.906$	≅0.001

geographically widespread, shared haplotypes, suggesting contemporary or recent historical matrilineal gene flow over extensive areas. Widespread gene flow is also evident in the northeastern population (clade 1-2) where there is clearly no geographical structure, and this is supported by significantly negative Tajima's *D* and Fu's *Fs* statistics. Finally, contiguous range expansions interpreted by NCA in the higher clades 2-1 and 2-2 indicates historical gene flow as populations expanded into new areas. Signals of limited among-site genetic variation due to panmixia, range expansion, or a selective sweep are consistent with a butterfly that is able to disperse extensively and efficiently to find suitable breeding sites.

These data support the smaller spatial scale findings of Costa et al. (1996). Efficient female dispersers are more likely to expand the species range into new habitats, thereby increasing the potential for encountering and adapting to different ecological conditions or to different attendant ant species. Colonization of novel habitats from genetically homogenous populations is consistent with a propagule pool model of colonization (Slatkin 1977), which is more likely to lead to population differentiation than a migrant pool model (Slatkin and Wade 1978) that would be the case if source populations were fragmented and hence genetically heterogeneous. Thus, diversification might occur more frequently through ecological shifts or long-distance dispersal, for example, during Pleistocene climatic oscillations. The subspecies *J. e. eubulus* may be an example of this expansion process where a subgroup of butterflies has expanded into a novel habitat resulting in ecological specialization, and this possibility is discussed in greater detail below.

We found no evidence of shifts to ecologically different attendant ants (i.e., novel associations) that could lead to specialization with the new ant. All the ants identified in this study were closely related *Iridomyrmex* species, even though ecologically different ant species, known to attend other lycaenids, occur within the range of *J. evagoras*. Furthermore, *J. evagoras* has been documented in nonpersistent associations with a significantly wider taxonomic range of ant species in outbreak years when the butterflies are extremely common (Pierce and Nash 1999; Eastwood and Fraser 1999). Subpopulation shifts to ecologically different ants would be rare events (Rand et al. 2000) and thus would be difficult to detect; however, since there is ample phylogenetic evidence of butterfly speciation coincident with ecologically different ant taxa (Thomas et al. 1989; Elmes et al. 1994; Pierce and Nash 1999; Rand et al. 2000; Eastwood and Hughes 2003a; Megens et al. 2005), lack of evidence in this survey does not mean that this could not happen.

The greatest percentage of variance (80.0%), and the strongest predictor of butterfly genetic diversity, was explained by geography. Since ant species and butterfly haplotypes are both distributed with a geographical bias (Figs. 1 and 2), speciation events resulting from vicariance may result in phylogenetic concordance of butterfly and ant species. This finding is consistent with the hypothesis of diversification resulting from fragmentation (or dispersal) of the lycaenid population coincident with a locally adapted or geographically restricted ant partner. In the case of *J. e. evagoras*, several ant partners currently coexist in each of the two geographical regions, so there is no pattern of geographic specialization with different ants. However, fragmentation could result in allopatric diversification and a phylogenetic pattern of lycaenid sister taxa associating with similar attendant ant species.

Although codiversification of butterflies and attendant ants among *J. e. evagoras* populations is unlikely since several ant species are involved across the species range, the allopatric populations of *J. e. eubulus* uniquely found with the ant *Iridomyrmex* complex A species A do show a signal of codiversification. The ant is closely related genetically to *Iridomyrmex* complex A species C (see Fig. 2), which tends contiguous populations of *J. e. evagoras*. This could indicate that populations of *J. e. eubulus* and their attendant ants have recently become differentiated from their respective conspecifics. However, diversification of the two butterfly subspecies is likely to be influenced by a range of different biotic and abiotic factors. If a habitat shift is a significant driving force in the diversification of an ant-tended lycaenid, and the new habitat contains a novel attendant ant that has diversified contemporaneously, then speciation of the butterfly and attendant ants could coincide. In this scenario, the ants would play only an incidental role in the lycaenid diversification process, but could ultimately appear as specialized symbiont partners in the butterfly phylogeny.

Additional Influences on Population Structure

Habitat

Genetically discrete populations of the two morphologically distinct subspecies could be maintained in the contiguous zone (Fig. 1; Table 2) if nuclear and mitochondrial genes had been evolving while the populations were in allopatry (e.g., Hughes et al. 2001). This suggests that the two subspecies, *J. evagoras eubulus* and *J. evagoras evagoras*, have recently reunited in secondary contact. However, since the ranges and habitats of both these taxa meet at an ecotone, and there is no evidence that a geographical barrier ever separated them, an alternative explanation is that a shift in habitat preferences has resulted in ecologically divergent selection isolating the populations in parapatry (Coyne and Orr 2004). Our mitochondrial data cannot distinguish between these two possibilities.

Host plants

Although a significant amount of genetic variation among butterfly subpopulations was explained by local host-plant preferences, there is clearly a geographical bias to host-plant distribution and butterfly subpopulations. Unlike facultative myrmecophiles, which are frequently host-plant specific, obligate myrmecophiles characteristically associate with multiple host-plant species, often across several families (Pierce and Elgar 1985). Furthermore, obligate, mutualistic lycaenids, including *J. evagoras*, also use attendant ants as oviposition cues (Atsatt 1981a,b; Henning 1983; Pierce and Elgar 1985; Fiedler and Maschwitz 1989; but see van Dyck et al. 2000), so a shift in their host plant would typically be ant dependent. As a result, the influence of host-plant shifts may

not be as important as ant shifts for diversification in obligately ant-associated lycaenids.

The Cassillis Gap

The pioneering acacia host plants of *J. evagoras* flourish within the open eucalypt forests in montane regions immediately to the north and south of the Cassillis Gap, especially after fire has cleared the undergrowth and provided regrowth opportunities (Smith 1982). It is also the preferred habitat for attendant *Iridomyrmex* ants (Andersen 1991; Shattuck 1992). In contrast, during late Pleistocene times, east coast lowland areas such as the Cassillis Gap were characterized by wide expanses of wet sclerophyll or temperate rainforest (Kershaw 1981; Walker and Singh 1981; Galloway and Kemp 1984). Thus, the paucity of suitable ants and the floristics and vegetation structure within the Cassillis Gap would have been a significant barrier to dispersal. Indeed, the disruptive effects of this barrier have been noted previously for several groups including butterflies (Waterhouse 1922), flies (Mackerras and Fuller 1942), birds (Keast 1961), and, to a lesser degree, frogs (Schäuble 2004). Our data suggest that the Lake George Gate biogeographical barrier has not impeded gene flow among butterfly populations as comprehensively as the Cassillis Gap.

The lack of genetic variability in *J. e. evagoras* samples from northern coastal areas (Fig. 1, group 2) is consistent with a population that has undergone a recent and rapid range expansion. It is possible that this range expansion happened subsequent to a bottleneck, as indicated by the shape of the mismatch distribution and the significantly negative Tajima's *D* and Fu's *Fs* statistics. Missing haplotypes on either side of the widespread haplotype Y in the network are consistent with either a bottleneck hypothesis where haplotypes have been lost, or a selective sweep. If the observed pattern does reflect a range expansion following a bottleneck, then it must have taken place within the last 167,000–244,000 years, that is, within the time since population separation across the Cassillis Gap. The time frame is suggestive because it coincides with heightened burning levels and vegetation changes that took place in Australia from the late Pleistocene (Pyne 1991; Knox et al. 1995), and subsequently by aboriginal firestick farming practices in the last 40,000 years (Kershaw 1981; Pyne 1991; Adam 1994; Flannery 1999). Acacias quickly colonize new areas exposed by fire (Smith 1982), and are efficiently located by female *J. evagoras* as suitable breeding sites become available (Smiley et al. 1988). In addition, attendant *Iridomyrmex* ants respond rapidly to environmental change (Vanderwoude et al. 1997), and would benefit from expanding dry sclerophyll-dominated forests coupled with shrinking wet sclerophyll and rainforest areas as a result of burning (Smith and Guyer 1983). Thus, increased burning levels would have influenced the distribution and age structure of suitable acacias (Kershaw 1981; Bowman 1998), creating a more continuous habitat for the expansion of *J. evagoras*. Other more recent anthropogenic activities such as farmland clearing and road building also provide ideal habitat for pioneering acacias and *Iridomyrmex* ants.

Conclusions

This study demonstrated that *J. evagoras* is a wide-ranging species with efficient female dispersal whose caterpillars associate with a variety of taxonomically and ecologically similar ant species that exhibit a regionalized bias in their distribution. There was no evidence of population structure consistent with low levels of dispersal due to the stringent requirements of suitable host plants and appropriate attendant ant species. On the contrary, it is possible that the highly specialized lifestyle of obligate myrmecophiles results in selection for efficient dispersers and that population structure is homogenized as a result. Attendant ants were not shown to influence *J. evagoras* population structure directly; however, regional isolation of *J. evagoras* populations coincident with locally adapted ant species could generate a phylogenetic footprint in which related lycaenids would be seen to associate with related or ecologically similar ants. Ecological shifts in habitat preferences by lycaenids may lead to codiversification with habitat specialist ants even though the ants may play only an incidental role in the diversification process itself. Population genetic structure of *J. evagoras* is consistent with fragmentation due to historical isolation of populations along the east coast of Australia during the Pleistocene, or to diversification following a shift in habitat preferences. Vegetation changes since the late Pleistocene, as well as heightened levels of burning and other anthropogenic activities, are likely to have enhanced recent dispersal patterns.

Acknowledgments

A. Andersen (CSIRO Darwin) identified the ants in this study and provided useful advice. Additional specimens and data were supplied by M. F. Braby, S. Brown, G. Forbes, D. Merrill, C. McCubbin, D. Schmidt, and M. A. Travassos. We thank J. Ma (Griffith University) and A. Kaliszewska (Harvard University) for laboratory support and M. Ponniah, J. Wakeley, and A. Fraser for comments on an earlier draft of this manuscript. Butterflies and ants were collected under Queensland Department of Environment and Heritage permit WISP00490302, Queensland National Parks permit WITK0040602, and Queensland State Forests permit TWB/17/2001-3 issued to the Queensland Entomological Society. This project was supported by an Australian Geographic Science Sponsorship, an Australian Postgraduate Award, a Griffith University Top-up Scholarship, and a W. G. Walker Memorial Fulbright Postgraduate Award to RE, and National Science Foundation grant DEB 0447242 and a grant from the Putnam Expeditionary Fund of the Museum of Comparative Zoology, Harvard University, to NEP.

Literature Cited

Ackery, P. R., R. de Jong, and R. I. Vane-Wright. 1998. The butterflies: Hedyloidea and Papilionoidea. Pp. 263–300 *in* N. P. Kristensen, ed. Lepidoptera, moths and butterflies. 1. Evolution, systematics and biogeography. Handbook of zoology 4(35). Lepidoptera. de Gruyter, Berlin.

Adam, P. 1994. Australian rainforests. Oxford Biogeography Series 6. Oxford Univ. Press, Oxford, U.K.

Als, T. D., D. R. Nash, and J. J. Boomsma. 2002. Geographic variation in host-ant specificity of the parasitic butterfly *Maculinea alcon* in Denmark. Ecol. Entomol. 27:403–414.

Andersen, A. N. 1991. The ants of Southern Australia. A guide to the Bassian fauna. CSIRO Publishing, Melbourne.
———. 1995. Measuring more of biodiversity: genus richness as a surrogate for species richness in Australian ant faunas. Biol. Conserv. 73:39–43.
Atsatt, P. R. 1981a. Ant dependant foodplant selection by the mistletoe butterfly *Ogyris amaryllis* (Lycaenidae). Oecologia 48: 60–63.
———. 1981b. Lycaenid butterflies and ants: selection for enemy-free space. Am. Nat. 118:638–654.
Bowman, D. M. J. S. 1998. Tansley review no. 101: The impact of aboriginal landscape burning on the Australian biota. New Phytol. 140:385–410.
Braby, M. F. 2000. Butterflies of Australia: their identification, biology and distribution. CSIRO Publishing, Melbourne.
Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657–1660.
Costa, J. T., J. H. McDonald, and N. E. Pierce. 1996. The effect of ant associations on the population genetics of the Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae). Biol. J. Linn. Soc. 58:287–306.
Cottrell, C. B. 1984. Aphytophagy in butterflies: its relationship to myrmecophily. Zool. J. Linn. Soc. 79:1–57.
Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland, MA.
DeBaar, M. 1977. New records for butterflies in Queensland and northern New South Wales. Aust. Entomol. Mag. 4:11–12.
Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19: 11–15.
Eastwood, R., and A. M. Fraser. 1999. Associations between lycaenid butterflies and ants in Australia. Aust. J. Ecol. 24: 503–537.
Eastwood, R., and J. M. Hughes. 2003a. Molecular phylogeny and evolutionary biology of *Acrodipsas* (Lepidoptera: Lycaenidae). Mol. Phylogenet. Evol. 27:93–102.
———. 2003b. Phylogeography of the rare myrmecophagous butterfly *Acrodipsas cuprea* (Lepidoptera: Lycaenidae) from pinned museum specimens. Aust. J. Zool. 51:331–340.
Elgar, M. A., and N. E. Pierce. 1988. Mating success and fecundity in an ant-tended lycaenid butterfly. Pp. 59–75 *in* T. H. Clutton Brock, ed. Reproductive success: studies of selection and adaptation in contrasting breeding systems. Chicago Univ. Press, Chicago.
Elmes, G. W., J. A. Thomas, O. Hammarstedt, M. L. Munguira, J. Martin, and J. G. vanderMade. 1994. Differences in host-ant specificity between Spanish, Dutch and Swedish populations of the endangered butterfly, *Maculinea alcon* (Denis et Schiff.) (Lepidoptera). Mem. Zool. 48:55–68.
Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.
Fiedler, K. 1991. Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae (Insecta: Lepidoptera: Papilionoidea). Bonner Zool. Monogr. 31:1–211.
Fiedler, K., and U. Maschwitz. 1989. Adult myrmecophily in butterflies: the role of the ant *Anoplolepis longipes* in the feeding and oviposition behaviour of *Allotinus unicolor* (Lepidoptera, Lycaenidae). Tyô to Ga 40:241–251.
Flannery, T. F. 1999. The future eaters: an ecological history of the Australasian lands and people. Reed New Holland, Sydney.
Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3:294–299.
Ford, J. 1987. Hybrid zones in Australian birds. Emu 87:158–178.
Fraser, A. M., T. Tregenza, N. Wedell, M. A. Elgar, and N. E. Pierce. 2002. Oviposition tests of ant preference in a myrmecophilous butterfly. J. Evol. Biol. 15:861–870.
Fu, Y.-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.
Galloway, R., and E. Kemp. 1984. Late Cainozoic environments in Australia. Pp 53–80 *in* M. Archer and G. Clayton, eds. Vertebrate zoogeography and evolution in Australia: Animals in space and time. Hesperian Press, Carlisle, WA.
Henning, S. F. 1983. Chemical communication between lycaenid larvae (Lepidoptera: Lycaenidae) and ants (Hymenoptera: Formicidae). J. Entomol. Soc. South. Afr. 46:341–366.
Hill, C. J., and N. E. Pierce. 1989. The effect of adult diet on the biology of butterflies 1. The common imperial blue, *Jalmenus evagoras*. Oecologia 81:249–257.
Hinton, H. E. 1951. Myrmecophilous Lycaenidae and other Lepidoptera: a summary. Proc. Soc. Lond. Entomol. Nat. Hist. Soc. 1949–50:111–175.
Hughes, J. M., A. M. Baker, G. De Zylva, and P. B. Mather. 2001. A phylogeographic analysis of southern and eastern populations of the Australian magpie: evidence for selection in maintenance of the distribution of two plumage morphs. Biol. J. Linn. Soc. 74:25–34.
Keast, A. 1961. Bird speciation on the Australian continent. Bull. Mus. Comp. Zool. 123:305–495.
Kershaw, A. P. 1981. Quaternary vegetation and environments. Pp. 82–101 *in* A. Keast, ed. Ecological biogeography of Australia. W. Junk Publishers, The Hague.
Knowles, L. L., and W. P. Maddison. 2002. Statistical phylogeography. Mol. Ecol. 11:2623–2635.
Knox, B., P. Ladiges, and B. Evans. 1995. Biology. McGraw-Hill Book Company, Sydney.
Mackerras, M. B., and M. E. Fuller. 1942. The genus *Pelecorhynchus* (Diptera: Tabanoidea). Proc. Linn. Soc. NSW 67:9–76.
Maddison, W. P., and D. R. Maddison. 1992. MacClade: Analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.
Malicky, H. 1969. Versuch einer Analyse der ökologischen Bezienungen zwischen Lycaeniden (Lepidoptera) und Formiciden (Hymenoptera). Tijdschr. Entomol. 112:213–298.
———. 1970. New aspects of the association between lycaenid larvae (Lycaenidae) and ants (Formicidae, Hymenoptera). J. Lepid. Soc. 24:190–202.
Maschwitz, U., M. Wüst, and K. Schurian. 1975. Bläulingsraupen als Zuckerlieferanten für Ameisen. Oecologia 18:17–21.
Mayr, E. 1942. Systematics and the origin of species. Columbia Univ. Press, New York.
Megens, H.-J., R. de Jong, and K. Fiedler. 2005. Phylogenetic patterns in larval host plant and ant association of Indo-Australian Arhopalini butterflies (Lycaenidae: Theclinae). Biol. J. Linn. Soc. 84:225–241.
Nicholls, J. A., and J. J. Austin. 2005. Phylogeography of an east Australian wet-forest bird, the satin bowerbird (*Ptilonorhynchus violaceus*), derived from mtDNA, and its relationship to morphology. Mol. Ecol. 14:1485–1496.
Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. Genetics 158: 885–896.
Pierce, N. E. 1984. Amplified species diversity: a case study of an Australian lycaenid butterfly and its attendant ants. Pp. 197–200 in R. I. Vane-Wright and P. R. Ackery, eds. The biology of butterflies. Academic Press, London.
Pierce, N. E., and M. A. Elgar. 1985. The influence of ants on host plant selection by *Jalmenus evagoras*, a myrmecophilous lycaenid butterfly. Behav. Ecol. Sociobiol. 16:209–222.
Pierce, N. E., and D. R. Nash. 1999. The imperial blue, *Jalmenus evagoras* (Lycaenidae). Pp. 279–315 *in* R. L. Kitching, E. Scheermeyer, R. E. Jones, and N. E. Pierce, eds. Monographs on Australian Lepidoptera. 6. Biology of Australian butterflies. CSIRO Publishing, Melbourne.
Pierce, N. E., R. L. Kitching, R. C. Buckley, M. F. J. Taylor, and K. F. Benbow. 1987. The costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras*, and its attendant ants. Behav. Ecol. Sociobiol. 21:237–248.
Pierce, N. E., M. F. Braby, A. Heath, D. L. Lohman, J. Mathew, D. B. Rand, and M. A. Travassos. 2002. The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). Annu. Rev. Entomol. 47:733–771.

Posada, D., and K. A. Crandall. 2001. Intraspecific gene genealogies: trees grafting into networks. Trends Ecol. Evol. 16:37–45.
Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Mol. Ecol. 9:487–488.
Pyne, S. J. 1991. Burning bush: A fire history of Australia. Henry Holt and Co., New York.
Qiagen 2001. QIAquick spin handbook. Qiagen Pty. Ltd., Victoria.
Qiagen 2002. Taq PCR handbook. Qiagen Pty. Ltd., Victoria.
Quek, S.-P., S. J. Davies, T. Itino, and N. E. Pierce. 2004. Codiversification in an ant-plant mutualism: stem texture and the evolution of host use among *Crematogaster* (Formicidae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution 58:554–570.
Rand, D. B., A. Heath, T. Suderman, and N. E. Pierce. 2000. Phylogeny and life history evolution of the genus *Chrysoritis* within the Aphnaeini (Lepidoptera: Lycaenidae), inferred from mitochondrial *cytochrome oxidase I* sequences. Mol. Phylogenet. Evol. 17:85–96.
Rand, D. M. 1996. Neutrality tests of molecular markers and the connection between DNA polymorphism, demography, and conservation biology. Conserv. Biol. 10:665–671.
Ray, N., M. Currat, and L. Excoffier. 2003. Intra-deme molecular diversity in spatially expanding populations. Mol. Biol. Evol. 20:76–86.
Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.
Schäuble, C. S. 2004. Variation in body size and sexual dimorphism across geographical and environmental space in the frogs *Limnodynastes tasmaniensis* and *L. peronii*. Biol. J. Linn. Soc. 82: 39–56.
Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin. Ver. 2.000. A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
Shattuck, S. O. 1992. Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). Sociobiology 21:1–181.
———. 1999. Australian ants: their biology and identification. CSIRO Publishing, Melbourne.
Shields, O. 1989. World numbers of butterflies. J. Lepid. Soc. 43: 178–183.
Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.
Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theor. Popul. Biol. 12:253–262.
Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.
Slatkin, M., and M. J. Wade. 1978. Group selection on a quantitative character. Proc. Natl. Acad. Sci. USA 75:3531–3534.
Smiley, J. T., P. R. Atsatt, and N. E. Pierce. 1988. Local distribution of the lycaenid butterfly, *Jalmenus evagoras*, in response to host ants and plants. Oecologia 76:416–422.
Smith, J. M. B., ed. 1982. A history of Australasian vegetation. McGraw-Hill, Sydney.
Smith, J. M. B., and I. J. Guyer. 1983. Rainforest-eucalypt forest interactions and the relevance of the biological nomad concept. Aust. J. Ecol. 8:55–60.
Swofford, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, MA.
Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.
Templeton, A. R. 2004. Statistical phylogeography: methods of evaluating and minimising inference errors. Mol. Ecol. 13: 789–809.
Templeton, A. R., E. Routman, and C. A. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140: 767–782.
Thomas, J. A., G. W. Elmes, J. C. Wardlaw, and M. Woyciechowski. 1989. Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. Oecologia 79:452–457.
van Dyck, H., J. G. B. Oostermeijer, W. Talloen, V. Feenstra, A. Hidde, and I. Wynho. 2000. Does the presence of ant nests matter for oviposition to a specialized myrmecophilous *Maculinea* butterfly? Proc. R. Soc. Lond. B 267:861–866.
Vanderwoude, C., A. N. Andersen, and A. P. N. House. 1997. Ant communities as bio-indicators in relation to fire management of spotted gum (*Eucalyptus maculata* Hook.) forests in southeast Queensland. Mem. Mus. Victoria 56:671–675.
Walker, D., and G. Singh. 1981. Vegetation history. Pp. 26–43 *in* R. H. Groves, ed. Australian vegetation. Cambridge Univ. Press, Cambridge, U.K.
Waterhouse, G. A. 1922. Presidential address. Proc. Linn. Soc. NSW 47:1–17.

Corresponding Editor: O. Pellmyr

APPENDIX 1

Jalmenus evagoras collection data, sampling locations, host plants and GenBank accession details. AL, Tony Leavesley; DL, David Lohman; DS, Dan Schmidt; KD, Kelvyn Dunn; MAT, Mark Travassos; MFB, Michael Braby; MT, Martin Taylor; NP, Naomi Pierce; RE, Rod Eastwood; SB, Steve Brown. Haplotypes are as in Figure 1.

Site	Locality	*Acacia* host plant	Collector	Date collected	*N*	Haplotype	Accession no.
1	Wallan, VIC	—	NP	Feb., Mar. 1992	5	B	DQ249942
	Gresswell Forest, Macleod, VIC	*A. dealbata*	RE	Nov. 2002	7	Y	DQ249947
2	Glenmaggie, VIC	—	KD	Feb. 1996	2	B	DQ249942
	Briagalong, VIC	*A. mearnsii*	RE	Dec. 2002	4	B	DQ249942
	Wellington R., Licola, VIC	*A. dealbata*	MAT	Feb. 1998	5	B	DQ249942
3	Buchan, VIC	*A. dealbata*	MAT	Feb. 1998	5	B	DQ249942
	Murrangower, VIC	*A. mearnsii*	MAT	Feb. 1998	5	B	DQ249942
	Mt. Raymond, VIC	—	MAT	Feb. 1998	5	B	DQ249942
	10 km W of Orbost, VIC	*A. mearnsii*	RE	Nov. 2002	2	B	DQ249942
4	Tidbinbilla, ACT	*A. dealbata*	MAT	Feb. 1998	3	Q	DQ249943
	Tidbinbilla, ACT	*A. dealbata*	MAT	Feb. 1998	3	B	DQ249942
	8 km N of Queanbeyan, ACT	*A. mearnsii*	RE	Nov. 2002	2	B	DQ249942
	8 km N of Queanbeyan, ACT	*A. dealbata*	RE	Nov. 2002	2	B	DQ249942
5	3 km E of Genoa, VIC	—	RE	Nov. 2002	8	B	DQ249942
	3 km E of Genoa, VIC	—	RE	Nov. 2002	2	C	DQ249944
6	1 km N of Tanja, NSW	—	RE	Nov. 2002	2	D	DQ249949
	Tanja, NSW	—	RE	Nov. 2002	3	B	DQ249942
	Stony Ck, S of Bodalla, NSW	—	RE	Nov. 2002	1	B	DQ249942
	Stony Ck, S of Bodalla, NSW	—	RE	Nov. 2002	4	E	DQ249945
7	Bowral, NSW	*A. longifolia*	SB	Feb. 2000	2	B	DQ249942
	Bowral, NSW	*A. longifolia*	SB	Feb. 2000	2	G	DQ249948
	Bowral, NSW	*A. longifolia*	RE	Dec. 2002	1	B	DQ249942
	Mt. Gibraltar, Bowral, NSW	*A. melanoxylon*	RE	Dec. 2002	1	G	DQ249948
	Mt. Gibraltar, Bowral, NSW	*A. melanoxylon*	RE	Dec. 2002	2	B	DQ249942
	East Mittagong, NSW	*A. longifolia*	RE	Dec. 2002	3	G	DQ249948
8	Royal National Pk, NSW	—	MT	1988	3	B	DQ249942
	Menai, NSW	*A. mearnsii*	RE	Dec. 2002	6	G	DQ249948
	Menai, NSW	*A. mearnsii*	RE	Dec. 2002	2	B	DQ249942
9	Wilberforce, NSW	*A. parramattensis*	MAT	Feb. 1998	5	G	DQ249948
	Wilberforce, NSW	*A. parramattensis*	MAT	Feb. 1998	1	B	DQ249942
	Cattai, NSW	*A. parramattensis*	MAT	Feb. 1998	4	G	DQ249948
	Cattai, NSW	*A. parramattensis*	MAT	Feb. 1998	1	B	DQ249942
10	27 km S of Macksville, NSW	*A. binervata*	RE	Dec. 2002	10	B	DQ249942
11	Ebor district, NSW	*A. melanoxylon*	MAT	Feb. 1998	10	Y	DQ249947
	Ebor Junction, Ebor, NSW	*A. melanoxylon*	MAT	Feb. 1998	1	B	DQ249942
	Ebor district, NSW	*A. melanoxylon*	NP	1992	6	Y	DQ249947
	3.8 km S of Ebor, NSW	*A. melanoxylon*	RE	Nov. 1999	2	Y	DQ249947
12	Armidale, NSW	*A. filicifolia*	MAT	Feb. 1998	5	Y	DQ249947
	5 km S of Guyra, NSW	*A. dealbata*	RE	Dec. 2002	4	Y	DQ249947
13	The Summit, Stanthorpe, QLD	*A. leucoclada*	KD	Apr. 1994	1	Y	DQ249947
	Mt. McKenzie, Tenterfield, NSW	*A. decurrens*	RE	Dec. 2002	3	Y	DQ249947
	Passchendaele, QLD	—	RE	Dec. 2002	6	Y	DQ249947
14	Emu Vale, nr Killarney, QLD	—	RE	Feb. 2000	3	Y	DQ249947
	Toowoomba, QLD	—	AL	Feb. 2000	3	Y	DQ249947
	Crows Nest, QLD	—	AL	Mar. 2000	3	Y	DQ249947
	Toowoomba, QLD	*A. irrorata*	RE	Jan. 2003	2	Y	DQ249947
15	Griffith University, Nathan, QLD	*A. macradenia*	MAT	Feb. 1998	1	Y	DQ249947
	Griffith University, Nathan, QLD	*A. leiocalyx*	MAT	Feb. 1998	2	Y	DQ249947
	Mt. Nebo, QLD	*A. irrorata*	MAT	Feb. 1998	5	Y	DQ249947
	Mt. Nebo, QLD	*A. irrorata*	RE	Mar. 2000	1	Y	DQ249947
16	Wild Horse Mtn., Beerburrum, QLD	*A. melanoxylon*	RE	Feb. 2000	3	Y	DQ249947
	Ford Road, Mooloolah, QLD	*A. leiocalyx*	RE	Jan. 2000	2	Y	DQ249947
	Tuckers Ck, Nambour, QLD	—	RE	Feb. 2000	3	Y	DQ249947
	Mapleton Falls, QLD	*A. melanoxylon*	RE	Jan. 2003	3	Y	DQ249947
17	Maryborough Rifle Range, QLD	*A. leiocalyx*	MAT	Mar. 1998	6	Y	DQ249947
	Action Park, Maryborough, QLD	*A. complanata*	RE	Jan. 2000	5	Y	DQ249947
	Action Park, Maryborough, QLD	*A. complanata*	RE	Jan. 2000	1	Z	DQ249946
	Action Park, Maryborough, QLD	—	RE	Jan. 2000	2	J	DQ249952
18	Bunya Mountains, QLD	—	RE	Jan. 2003	11	Y	DQ249947
19	6 km S of Binjour, QLD	*A. melanoxylon*	DS	Mar. 2003	12	Y	DQ249947
20	6 km W of Taroom, QLD	*A. harpophylla*	RE	Apr. 2003	7	R	DQ249953
	6 km W of Taroom, QLD	*A. harpophylla*	RE	Apr. 2003	3	P	DQ249950
21	Kogan, QLD	*A. harpophylla*	MT	1988	1	P	DQ249950
	Kogan, QLD	*A. harpophylla*	MT	1988	2	O	DQ249951
	Wilkie Ck, Dalby, QLD	*A. harpophylla*	RE	Jan. 2003	7	O	DQ249951
22	Oakey, QLD	*A. harpophylla*	DL	2001	3	O	DQ249951
	Jondaryan, QLD	*A. harpophylla*	RE	Jan. 2003	**7**	**O**	DQ249951

APPENDIX 2

Jalmenus evagoras attendant *Iridomyrmex* ant sampling locations, collection and tissue data. *N*, number of butterfly aggregations from which ants were sequenced. All samples were collected by R. Eastwood in November–December 2003 unless otherwise indicated (DL, David Lohman [Jan. 2000]; SB, Steve Brown [Feb. 2000]).

Site	Locality	*N*	*Iridomyrmex* species	GenBank accession nos.
1	Macleod, VIC	2	*I.* complex A sp. B	DQ249954 to DQ249956
2	Briagalong, VIC	1	*I. mattiroloi* group sp. I	DQ249976
3	Murrangower, VIC	1	*I. mattiroloi* group sp. I	DQ249959
	Mt. Raymond, VIC	1	*I. mattiroloi* group sp. I	DQ249958
	10 km W of Orbost, VIC	1	*I. mattiroloi* group sp. I	DQ249957
4	Tidbinbilla, ACT	1	*I.* complex A sp. D	DQ249965
	Queanbeyan, NSW	2	*I.* complex A sp. D	DQ249973, DQ249974
5	3 km E of Genoa, VIC	1	*I. mattiroloi* group sp. I	DQ249960
6	1 km N of Tanja, NSW	1	*I.* complex A sp. C	DQ249961
	Tanja, NSW	1	*I. mattiroloi* group sp. I	DQ249962, DQ249963
	Stony Ck, Bodalla, NSW	1	*I. mattiroloi* group sp. I	DQ249964
7	Bowral, NSW (SB)	1	*I. mattiroloi* group sp. I	not sequenced
	Mittagong, NSW	1	*I.* complex A sp. C	DQ249966
8	Menai, NSW	1	*I. septentrionalis* sp. F	DQ249967, DQ249968
10	27 km S of Macksville, NSW	1	*I. gracilis*	DQ249969
11	Ebor district, NSW (DL)	1	*I. mattiroloi* group sp. I	DQ249990
	Ebor district, NSW	3	*I.* complex A sp. C	DQ249970, DQ249986, DQ249989
12	5 km S of Guyra, NSW	1	*I.* complex A sp. C	DQ249971
13	Passchendaele, QLD	1	*I.* complex A sp. C	DQ249975
	Tenterfield, NSW	1	*I. mattiroloi* group sp. I	DQ249972
14	Emu Vale, Killarney, QLD	1	*I.* complex A sp. C	not sequenced
	Toowoomba, QLD	1	*I.* complex A sp. C	DQ249985
	Crows Nest, QLD	1	*I. gracilis*	not sequenced
15	Nathan, QLD	2	*I. gracilis*	DQ249977, DQ249987
16	Beerburrum, QLD	1	*I.* complex A sp. C	DQ249978
	Mooloolah, QLD	1	*I.* complex A sp. C	not sequenced
	Tuckers Ck, Nambour, QLD	1	*I.* complex A sp. C	not sequenced
	Mapleton Falls, QLD	1	*I.* complex A sp. C	DQ249979
17	Maryborough, QLD	1	*I. gracilis*	DQ249980
18	Bunya Mountains, QLD	1	*I.* complex A sp. C	DQ249982, DQ249983
19	6 km S of Binjour, QLD	1	*I. gracilis*	DQ249988
21	Wilkie Ck, Dalby, QLD	1	*I.* complex A sp. A	DQ249981
22	Jondaryan, QLD	1	*I.* complex A sp. A	DQ249984