CHAPTER 3

GENERIC RELATIONSHIPS

[Some material in this chapter, including photographs on Plate 3.1, is reproduced from Smith (2005) courtesy of the Journal of Arachnology]

3.1. INTRODUCTION AND AIMS

Whilst an examination of the placement of Poltys with respect to other araneid genera was not a primary aim of this study, I thought it would be useful to have some idea of the likely relationships of the genus to assist in choosing one or more outgroups for the analysis of Poltys species. As discussed under generic affiliations (Chapter 1), Poltys was eventually placed in the subfamily Argiopinae as the nominative member of the tribe Poltyini (as ‘Poltyeae’) (Simon, 1895). Also included were the genera Cyphalonotus, Homalopolys, Kaira and Pycnacantha. The genera Ideocaira and Micropolys were described later, and, due to the general appearance of Ideocaira and the eye arrangement of Micropolys, their authors either implicitly (Simon) or explicitly (Kulczyński) suggested that they might be related to Kaira and Poltys, respectively (Simon, 1903; Kulczyński, 1911). More recently, they were listed as part of the Poltyini (as ‘Poltyeae’) (Dippenaar-Schoeman and Leroy, 1996). Archer (1951) recognised that the male pedipalp of Cyphalonotus was far more complex than that of Poltys and proposed a new tribe, the Cyphalonotini, for the former, although later he decided it belonged in the ‘Dolophini’ (Archer, 1965). None of these tribal classifications is currently in regular taxonomic use, and I am using the Poltyini grouping in the broadest sense, including all the above genera as the basis for this section of the study.
The phylogenetic analysis of araneid taxa by Scharff and Coddington (1997) was based on taxa selected from Simon’s tribes (or the earlier subfamily versions thereof), and *Kaira*, which was revised by Levi (1993), was used as the representative of the Poltyini. The results (Fig. 3.1) suggested that *Kaira* should be placed in the ‘*Hypsosinga* clade’ in the mid-basal araneines. If Simon were correct in his affiliations of taxa this is where *Poltys*, and the remaining Poltyini taxa, should also belong. Scharff and Coddington, however, also found that some of Simon’s taxa were polyphyletic. As Archer may have realised during his work on *Cyphalonotus*, the possibility of errors in Simon’s grouping of the Poltyini was compounded by his lack of knowledge of the males of almost all the genera in the tribe. Simon’s assemblage was apparently based on the irregular form of the abdomen, slightly unusual eye arrangements and the strong macrosetae on the legs of the three genera that are now known to prey mainly on moths (*Kaira*, *Poltys* and *Pycnacantha*) (Stowe, 1986; Dippenaar-Schoeman and Leroy, 1996). There is a confusing mixture of similarities and contradictions amongst characters within the genera of this putative group and also with respect to genera elsewhere in the Araneidae. These conflicts make the assessment of the likely placement of *Poltys* within the Araneidae problematic.

The primary motivation for this part of my work was to attempt to establish some possible relatives of *Poltys* from which could be drawn a sensible outgroup taxon for an analysis of the Australasian *Poltys* taxa. Most of the other putative Poltyini would not be suitable for this, even if they were closely related, because of the problems of obtaining suitable recent material for destructive techniques such as the extraction of DNA. Nevertheless, I was still intrigued by some of the characters exhibited by these taxa and
Figure 3.1. The preferred topology of the Araneidae and outgroups presented by Scharff & Coddington (1997), adapted from their figure 82; only node numbers relevant to the current work are included; see Scharff & Coddington (1997) for details.
their superficial similarities to Poltys. Therefore, there were two goals to this section. The first aim was to test whether Poltys might indeed belong in the ‘Hypsosinga clade’ of Scharff and Coddington (1997), and if not, where. The second aim was to find out whether, without any changes or additions to the characters used by Scharff and Coddington, the Poltyini would emerge as a monophyletic grouping within the context of the taxa examined in that study.

3.2. CLADISTICS

Cladistic analyses are used in two areas of this thesis. This chapter presents an examination of the possible generic relationships of Poltys based on an existing study (Scharff and Coddington, 1997). Chapter 5 presents the use of cladistic methods to reconstruct phylogenies of the Australasian Poltys species from which the relationships of the endemic P. laciniosus-group might be inferred. These phylogenies are based on genetic and morphological characters.

3.2.1. A brief background to cladistics

The use of cladistic methodology in biology was pioneered by Hennig (1950, in Rainer Zangerl’s preface to Hennig, 1966). Cladistic analysis forms taxa into interrelated groups that a taxonomist can interpret as genera, families and so on, depending on the level of interrelation. With the addition of certain assumptions, this cladogram can be understood as a phylogeny, which purports to reflect genealogical relationships. This process is achieved through an analysis of the attributes, or states, observed to occur in
each single (homologous) character both within and between taxa in the group under study. The terms “state” and “character” are clearly explained by Watrous and Wheeler (1981, based on the definition of Platnick, 1979). It is assumed that states are discrete, heritable, that they are homologous between taxa, and that changes in the character states represent evolutionary events. Plesiomorphic character states are ancestral (or “primitive”) while apomorphic states are derived; these terms are not absolute but are relative to the group under consideration. Taxa are grouped based on the presence of synapomorphies, derived changes shared by the group. Terminal taxa are characterised by autapomorphies, unique changes.

3.2.2. Character polarity and outgroups

A number of authors have stipulated that the direction of change, or character polarity, needs to be assessed before proceeding with an analysis of the included taxa, i.e. a priori. This could be achieved through comparison with fossil taxa (the stratigraphic criterion, discussed by Kitching et al., 1998), by the ontogenetic criterion (Nelson, 1978), by outgroup comparison (as defined by Watrous and Wheeler, 1981), or methods such as ingroup commonality where it is postulated that the most widespread condition is primitive within the group. All of these methods have problems in this context (summarised by Kitching et al., 1998). Nevertheless, the use of one or more outgroups to polarise characters is now the most widely accepted method for cladistic analysis, but based on a posteriori methodology, such as outlined by Nixon and Carpenter (1993).
Nixon and Carpenter (1993) pointed out that the original concepts of polarity, rooting and outgroups, as formalised by Farris (1972) had been largely misconstrued by more recent advocates of a priori methods. They stressed that there is no need for an a priori assessment of character polarity. In fact to do so is to force (or constrain) monophyly on the ingroup, when in fact the test of this should be part of the process, not an initial assumption. Instead, characters should be defined that are scoreable for both the postulated ingroup and the outgroup(s). This data matrix should then be subjected to an unconstrained, simultaneous analysis to produce a cladogram, which would then be rooted between the ingroup and the outgroup(s). The polarity of characters should then be derived from the analysis.

Nixon and Carpenter (1993) also clarified some other important points about outgroups:

(i) outgroup(s) need not be (or include) the sister group of the ingroup;

(ii) there is no need for the outgroup(s) to be a monophyletic group with respect to the ingroup;

(iii) there is no need for the outgroup(s) to be “primitive” with respect to the ingroup.

3.2.3. Tree-building algorithms

Two methods of cladogram construction are used in this study, parsimony (also called maximum parsimony, or MP) and maximum likelihood (ML). In this chapter only parsimony is used but both methods are employed in Chapter 5. Bayesian analysis, based
on parametric statistics, should also be mentioned as this has recently become popular as an analytical tool, implemented through the program MRBAYES (Huelsenbeck and Ronquist, 2001). All of these techniques have strong followings and, particularly for MP and ML, there have been many years of debate over their relative merits and shortcomings. In recognition of these strengths and weaknesses, the authors of most molecular studies use two alternative criteria to examine their data. In the present case, the choice of likelihood and parsimony over Bayesian analysis was due to pragmatic reasons of accessibility at the time these analyses were carried out.

**Parsimony**

This criterion uses the aim of reconstructing the shortest, or most parsimonious, tree diagram that accounts for all the observed characters and their changes. Several different sets of operational rules have been developed, most of which constrain the direction of permissible changes, or differentially facilitate one type of change over another. All the parsimony analyses in this work use the criterion known as Fitch Parsimony (Fitch, 1971). This treats all multistate characters as unordered, and allows free reversibility. This is the only logical approach for DNA sequence data, and it also makes the fewest implicit assumptions of character development for morphological characters. Other criteria are sometimes used for morphological characters. For instance, Wagner Parsimony (Wagner, 1961 cited in Kluge and Farris, 1969; Farris, 1970) treats multistate characters as ordered, so that moving from state 0 to state 2 would be two steps, rather than
the single step jump in Fitch Parsimony. In practice, many data sets are analysed using a mix of both of these criteria, with some ordered and some unordered multistate characters.

Parsimony methods are useful, in that there are few assumptions (at least in constructing a cladogram—translating this to a phylogenetic tree involves more): (i) the application of “Occam’s Razor”, i.e. that the most simple explanation (the cladogram involving the least number of steps) is the most likely; (ii) that structures (or sequences) to which characters pertain are homologous between taxa; (iii) that any change is as likely as any other (this applies to both direction and rate of changes). Assumption (ii), character state homology, can often be treated as a testable hypothesis, whilst the other assumptions can be tested by comparison with a modified method (such as the introduction of character weighting) or against other methodologies. Parsimony methods for DNA analysis have sometimes been considered to be less effective for reconstructing phylogenies than some other methods, such as neighbour-joining or maximum likelihood. This is partly because only informative sites are used, which is often only around one third of the base positions. The claim that no assumption is necessary about the process of evolutionary change has been questioned in work based on simulation models (Nei, 1991). Nei states that, “for the MP method to work well, approximate constancy of evolutionary rate and a small number of nucleotide substitutions per site are necessary”. In contrast Hillis et al. (1994) concluded that nearly all of the methods they analysed, which included both weighted and unweighted parsimony, could give accurate reconstructions of phylogenies provided the rates of change in observed characters were appropriate for analysis. They also found that weighting could substantially improve performance and that weighted parsimony
outperformed all other methods for finding the correct four-taxon tree with a small data set (ca 10–1000 nucleotides). Recently, the parsimony versus likelihood controversy was re-examined from a statistical perspective (Goloboff, 2003). Goloboff concluded that the methodology of parsimony was supported at levels of complexity varying between the simplest (with few assumptions) through to ‘realism’ (with the addition of parameters to attempt to emulate real systems). Parsimony, with or without weighting, is currently one of the most widely used analysis methods in molecular phylogenetics. This versatility, and the ability to deal with both morphological and DNA data in the same data set, make parsimony an ideal method for the present study.

**Maximum likelihood**

This statistical algorithm was developed to deal with DNA sequence data (Felsenstein, 1981) and the method uses all the data, whether or not the site is variable across the taxa. The tree topology that is chosen is the one found that is most likely to have given rise to the distribution of characters in the data set using a particular model of character evolution. A major weakness is sensitivity of the algorithm to violations of the assumptions used in the evolutionary models. A program is now available, Modeltest (Posada and Crandall, 1998), to test a combination of models and variables against the data set and report the goodness of fit of each model to the data. The top-ranking models are returned by the program along with the suggested values for variables, such as rate heterogeneity, for the recommended model. These variables can be implemented and the analysis carried out under the Likelihood criterion in PAUP* (Swofford, 2001). Modeltest
has helped produce a consistent approach to the selection of the evolutionary model utilised and is currently one of the standard tools used in molecular analysis. Maximum likelihood is used here to provide an alternative methodology to MP for DNA data, in particular to compare a tailored model against the assumption that all character changes are equally likely.

3.2.4. Consensus trees

Likelihood methods usually only result in a single tree because it is unlikely that two or more arrangements will share exactly the same probability of occurrence, however MP frequently results in several, or even many thousand, equally parsimonious arrangements. A number of different methods for deriving consensus trees from these multiple solutions have been proposed. All have certain problems of interpretation and inevitably lose some potentially useful information, but several methods may be useful in different circumstances, providing their limitations are taken into account.

Strict consensus trees show only clades that are resolved in every individual topology. All conflicting nodes are collapsed to polytomies. In data sets with relatively few conflicting arrangements and where no taxa are particularly unstable, a strict consensus is the most appropriate consensus method. In many situations, however, a strict consensus reveals only a ‘bush’ of unresolved taxa.

Other kinds of consensus trees are often more useful when a data set contains ‘rogue taxa’ that appear in a number of different positions. A majority-rule tree depicts those clades that occur in more than a certain percentage of trees. The default (and
minimum possible) setting is >50%. Majority-rule trees are most often used to show the results from bootstrapping or jackknife analysis. But used with suitable care, they may also be useful in their own right when a data set produces many trees. The main caveat with such a method is that it is beguilingly simple to believe in a single tree, yet there is no reason why the ‘true’ topology should be represented by this compilation of numerically dominant parts. One useful part of the generation of a majority-rule tree in PAUP* is the character partition table. This summarises how often each taxon appears in a clade with any other taxon and so provides a certain amount of information on clades which appear below the set level for acceptance in the screened tree.

The other kind of consensus tree used in this study is the Adams consensus tree (Adams, 1972). This method retains detail by removing conflicting elements to their lowest common node whilst leaving non-conflicting parts of clades in place. This is a useful method of picking out consistent associations of taxa, but often clades may be shown that do not actually appear as such in any one original topology.

In all cases it is important to remember that no consensus tree should be interpreted as a phylogeny. Any topology resulting from a consensus method is simply a statement about areas of agreement among trees (Swofford, 1991).

3.2.5. Polytomies and zero length branches

Before using any consensus method in PAUP* or Hennig 86 (Farris, 1988), it is desirable to check through the topologies and delete any with zero-length branches (Scharff and Coddington, 1997). The algorithms used in NONA (Goloboff, 1993) are
better in this regard but the program can still produce uncollapsed polytomies that are
suboptimal once collapsed. Scharff and Coddington (1997) also advocate the filtering of
tree sets to remove those trees containing polytomies for which there is a more resolved
solution present. With the solution present in another, otherwise identical tree, it is
reasonable to support the interpretation as a ‘soft’ polytomy, i.e. irresolution due to a lack
of data, rather than as a ‘hard’ polytomy that implies an assertion of simultaneous
cladogenesis (Coddington and Scharff, 1996). The tree data set can be filtered in PAUP*
but the removal of trees containing zero-length branches is more problematic. Two
methods used here are the manual removal of the topologies with assigned zero-length
branches from a saved PAUP* tree file, or alternatively using WinClada (Nixon, 1999–
2002) by a process of collapsing unsupported nodes then removing suboptimal trees. The
tree set produced by NONA can also be ‘cleaned up’ using WinClada, but cannot easily be
filtered. Whilst tree data sets from either PAUP* or NONA can be imported into WinClada
and back into NONA, once exported from PAUP* retrieving them is difficult. In the study
below, an Adams consensus (which can be implemented in PAUP*) was required to
examine whether clades might be recovered that would otherwise not be found by more
simple consensus methods. Consequently, the tree set primarily used is that produced by
PAUP*’s filtering and the manual removal of topologies with zero-length branches. This is
not, however, the same as the set obtained by passing the filtered trees through the
WinClada routine. It was decided that both methodologies should be used to confirm that
any conclusions drawn were supported in both cases.
3.2.6. Tree support indices

Jackknife and bootstrap

These are two methods often used to test the robustness of a tree topology and can be used in both MP and ML. Both aim to achieve a better estimate of the true sample distribution than may be represented in the real data by resampling using pseudoreplicates. This procedure is usually repeated many times. Results are presented in the form of a majority-rule consensus tree showing the percentage of replicates in which particular clades were found. As with Bremer support (below) the interpretation of the level of support indicated is problematic, although 70% is often considered to be a reasonable confidence level.

The jackknife method removes a random section of data from the original data set on each run and uses the remaining data as a pseudoreplicate; hence it uses a smaller data set than the original. This may be an important consideration when working with large DNA data sets. In its original usage in a systematic context only one taxon at a time was removed (Lanyon, 1985), termed ‘first-order’ jackknifing. The alternative or ‘higher-order’ jackknifing method now used involves deleting multiple characters, rather than taxa. The jackknife used in Chapter 5 is based on the method of Farris et al. (1996), which can be implemented in PAUP*. The following commands were used (implementation method suggested by G. Edgecombe, pers. comm.):

```
jack pct=37 nreps=1000 search=heuristic resample=jac/
addseq=random nchuck=5 chuckscore=1 nreps=200;
```
`jac` resampling is the PAUP* implementation of the method used in Hennig86. The resampling proportion `pct=37` is equivalent to the removal probability of \( e^{-1} \), which is 0.3679 (Farris et al., 1996). The jackknife analyses run on DNA data under likelihood criteria presented in Chapter 5 used only 500 replicates and 100 sub-replicates to cut down the run-time (which was still 13 days on a 3.0 GHz personal computer for the largest data set).

The bootstrap method has probably been used more frequently than the jackknife, and consequently its pros and cons have received considerable attention. The pseudoreplicates are also generated using a subsample, but the missing data are replaced by reusing data already present, i.e. effectively weighting some characters at random by including them twice. Bootstrapping has more serious limitations than the jackknife, particularly with respect to the inclusion of uninformative characters, such as are common among DNA data, and the size of the data set (see Kitching et al., 1998 for a summary). It is also much slower than jackknifing. For these reasons it has not been used in this study.

**Bremer support**

First suggested by Bremer (1988), this is another commonly used method for examining the support for clades on a given tree topology. Firstly the most parsimonious tree(s) are calculated. Then successive iterations of the search are performed each time keeping trees that are less than optimal by one further step. The resulting cladogram illustrates at what step any particular clade is lost from the consensus. The higher the number of steps that can be taken before the clade drops out, the greater the robustness of
the associated clade. There is, however, no objective measure of what constitutes a satisfactory level of support. Bremer support may be implemented using NONA and has been used here only for the simultaneous analysis of all the data sets presented in Chapter 5.

3.3. METHODS

3.3.1. Taxa

Scharff and Coddington (1997) used type species as generic exemplars where available, but they also compared other species from each genus to confirm that codings were typical. Because Scharff and Coddington developed the character descriptions and selected suitable genera as exemplars, they could ensure that there were no polymorphisms, resulting in a single terminal per genus. Unfortunately, adding a new taxon may prove to be problematic if the genus does not fit neatly with respect to the character states used. This is a case in point in Poltys, where several characters vary across the Australian species. It is usually recommended that enough exemplars are included in the terminals to adequately express all the character states (e.g. Schuh, 2000), but it may not be appropriate to include three terminals for one genus if all the other genera are only represented by a single terminal. In the current study, an initial analysis that included representatives from all three distinct Poltys species-groups confirmed that they held together as a clade. For the main analysis presented here, only the type species, P. illepidus, was included to test the placement in the strictest sense of the generic definition.
The genus *Pycnacantha* was excluded, as no male specimens were available. *Kaira* was recently revised by Levi (1993) and was included by Scharff and Coddington (1997) in their study. The other genera of Poltyini are generally poorly known and it was first necessary to identify males for *Homalopolys*, *Ideocaira* and *Micropoltys*, which are described only from females. When *Homalopolys* males were found it became apparent that this genus in fact belongs in the Tetragnathidae in the current sense of the family (Hormiga et al., 1995) (Fig. 3.2.a–e), and will probably prove to be congenereic with

**Figure 3.2.** *‘Homalopolys’* sp. ex Kalimantan (RMNH ex coll. CLD): a, male, general lateral view; b, male pedipalp, ventral; c, female general lateral view; d, epigynum, ventral; e, epigynum, posterior view. Scale lines: a, b, 1 mm; c–e, 0.25 mm. See Abbreviations section for key.
Paraebius Thorell, which is currently synonymised with Dolichognatha O.P.-Cambridge (Levi, 1981). This genus was therefore excluded from further analysis here. The female type of Ideocaira transversa Simon has been examined, and unpublished drawings of the female type of Micropoltys placenta Kuczyński were supplied by H. Levi. Unfortunately, none of the species in which males could be matched to females represented the type species of the genus. For Cyphalonotus, the expanded pedipalp is from a different species to that used for scoring general characters (necessitated by the need to use material from the only vial that contained more than a single male). The structures visible on the unexpanded pedipalp of the species against which other male and female characters were scored appear to be similar; there are also no scoreable differences in the general attributes in the males of both species. Neither Cyphalonotus species has been identified. The type species, C. larvatus (Simon) is recorded from Congo and East Africa (Platnick, 2006). This leaves Poltys illepidus as the only type species used in this analysis. Although this is far from ideal, the nature of this data set, with a rather high proportion of taxa to characters, meant robust results were unlikely even before adding additional taxa (Scharff and Coddington, 1997). Therefore, I did not expect to achieve precise results in this tentative exploration of these genera. These issues would need to be addressed for a more rigorous analysis in the future.
3.3.2. Characters

The specimens examined for each character attribute are listed in Appendix 2. Specimens were examined and attributes scored according to the methods of Scharff and Coddington (1997) (Appendices 1 and 3). Most character states are illustrated in Figures 2.1–2.4, Plate 2.3 and Figures 3.3–3.5. Some characters, listed below, require comment on their interpretation in relation to Scharff and Coddington’s analysis.

Figure 3.3. Cyphalonotus sp.: a–d male: a, general lateral view; b, left pedipalp, prolateral; c,d, left pedipalp expanded, prolateral and retrolateral (different species to a & b). e–g, Cyphalonotus sp., female: e, general lateral view; f, g, epigynum, ventral and lateral. Scale lines: a, e, 1 mm; b–d, f, g, 0.5 mm. See Abbreviations section for key.
Characters 11 and 12. Median apophysis of male pedipalp with bifid prong or threadlike spur. The apically directed hook-like portion of the Poltys MA is distinctive (Fig. 2.4.f, Plate 2.3.b), but it does not conform totally to either of the diagnoses for these character states.

Character 19. Stipes absent or present. In Micropoltys the sperm duct appears to pass from the radix, through the base of the distal haematodocha and straight into the embolus. There is apparently no sclerite as such between the two, so this is scored absent [0] (Fig. 3.5.c).

Character 23. Tip of male pedipalp embolus simple or with cap. Only Poltys and Micropoltys pedipalps have been examined under SEM (Plates 2.3.b, 3.6.a). There is no indication on either of these that any part is designed to break off, or has already done so. These are scored as simple [0]. The attributes of the other genera are unknown so they are scored [?].

Character 30. Scape with pocket near tip, absent or present. Poltys illepidus have a broad turned-over rim along the whole of the posterior margin of the epigyne (Fig. 2.3.a). I have interpreted this as a (rather wide) pocket present [1]. Micropoltys females have at least a sharp depression, which is tentatively also scored here as a pocket present [1] (Fig. 3.5.e).

Characters 33 and 34. Coxa I hook and femur II groove, absent or present. Amongst these genera, all of the males with similarly sized females have these features (e.g. coxal hook arrowed in Plate 3.1.b, Micropoltys).
Figure 3.4. *Ideocaira triqueta*. a–c. male: a, general lateral view; b–c, left pedipalp, prolateral and expanded, dorsal view. d–f, female: d, general lateral view; e, abdomen, dorsal; f, epigynum, ventral. Scale lines: a, d, e, 1 mm; b, c, 0.5 mm; f, 0.25 mm. See Abbreviations section for key.

**Character 46.** Clypeal tooth of females absent or present. Both males and females of the *Micropolys* species figured have a rather rounded clypeal tooth. The male is shown in Plate 3.1.b, but the tooth is more developed in females. This character is not present in Levi’s drawing of the type female of *Micropolys placenta* but I have scored it as present [1].

**Character 50.** Ratio of lateral eye-median eye separation, <1 or >1. *Poltys* and *Micropolys* are unusual amongst araneids in that they have widely separated lateral eyes,
so there is no lateral eye group as such (Figs. 2.2.a, 2.4.a, 3.5.a, d). In applying this character to these genera I took Scharff and Coddington’s instructions literally, and used the distance at the widest point, i.e. that to the posterior eye, so that the separation is scored as >1 [1].

Figure 3.5. Micropoltys sp. a–c, male: a, general lateral view; b, c, left pedipalp, prolateral and expanded, apico-dorsal view. d, e, female: d, general lateral view; e, epigynum, ventral. Scale lines: a, d, 1 mm; b, c, e, 0.25 mm. See Abbreviations section for key

Characters 59 and 60. Abdominal shape. Both male and female Ideocaira triqueta Simon have strongly triangular abdomens, which are widest anteriorly (Fig. 3.4.e, female). The females of I. triqueta vary in their relative dimensions, some being wider than long
and some the reverse. The female of *I. transversa*, the type species, is distinctly wider, however, so I have used this to decide the matter and scored Character 60 as wider [1].

**Character 67.** Tactile setal bases on carapace and abdomen, normal or gasteracanthine-shaped. *Micropolyts* has rather distinctive setal bases over much of the prosoma, including the basal chelicerae (Plate 3.1.b). There is none on the dorsum of the abdomen, but they occur around the pedicel on the venter. Some of these bases and the setae themselves (Plate 3.1.c) are similar to those figured by Scharff and Coddington (1997) and I have scored them as gasteracanthine-like [1]. Those on the sternum (Plate 3.1.d) and around the eye region and chelicerae are further modified, with an anteriad-projecting lamella and deep pits on each side.

**Characters 74 and 75.** Orb web and sticky spiral. Joseph Koh has provided me with a photograph of *Cyphalonotus* in an orb web (identified by me from the photograph and confirmed by JK using the specimen). I cannot see anything to suggest that it is not a normal araneid web and so have scored Character 75, sticky spiral, as present [0]. (This character makes no difference to the position of *Cyphalonotus* in the results).

**Character 78.** Sticky-spiral (SS) localization: outer leg 1, inner leg 1 or leg 4. In the *Poltys laciniosus*-group species that I have observed spinning webs, leg 4 is mostly used to monitor the position of the spider with respect to the sticky spiral, especially closer to the hub where the distance between radii is short. The leg used by *P. illepidus* was not noted but the web is similar to those of the observed species and this character is therefore scored as L4 [2]. These *P. laciniosus*-group species also move around the web in a similar way to the larger nephilines (Scharff and Coddington, 1997; Eberhard, 1982), constantly facing
between the hub and the direction of travel. Like these nephiline spiders, Palystis makes a finely meshed web, which probably influences the most efficient way of moving around the web (Eberhard, 1982).

3.3.3. Analysis

The full set of data (74 taxa, 82 characters, original data available from Treebase—see Appendix 3) was run in PAUP* using a heuristic search with the commands:

```
  hsearch addseq=random nchunk=5 chuckscore=1 nreps=1000 randomize=trees;
  hsearch start=current nchunk=0 chuckscore=0;
```

The first line keeps only five trees from each island sampled, preventing the tree buffers from filling with thousands of trees and increasing the chances of finding all islands of trees. One thousand replicates are carried out, each time with the taxa added in a random order. The default branch swapping algorithm TBR (tree bisection reconnection) is used. The order of the resulting trees is randomised before entering the second line of command. The second line swaps on the trees that were kept from the first search through to completion.

The trees were filtered and trees containing zero-length branches were removed manually, or using WinClada, as discussed above. Strict, majority-rule and Adams consensus trees were produced in PAUP* and all topologies were examined using WinClada.
Figure 3.6. Strict consensus of the Araneidae for the data of Scharff & Coddington (1997) and taxa from the Polytini (in bold). Clade numbers show relevant areas of agreement with Scharff & Coddington fig. 82.
Figure 3.7. Majority-rule consensus of the Araneinae for the data of Scharff & Coddington (1997) and taxa from the Poltyini (in bold). Numbers show the percentage of topologies containing the particular clade (>50% only).

All data were also run in NONA using the standard commands, as recommended by Miller (2000):

```
mult*1000;
max*; or jump*1;
```

Replacing the `max*` command with `jump*1` helps to find different islands of trees by searching suboptimal arrangements up to two steps longer than the optimal. The most comprehensive tree set found by NONA was compared to that found by PAUP*.
Even before adding extra taxa, the nature of this dataset, with rather a high proportion of taxa to characters means robust results are rather unlikely (Scharff and Coddington, 1997). No method of assessing tree support by methods such as bootstrap or jackknife was presented in the original analysis by Scharff and Coddington. Since the data set is now further destabilised by the addition of extra taxa, it was deemed inappropriate to use these methods in this study.

3.4. RESULTS

PAUP* initially found 948 minimal length trees (300 steps). This was reduced to 376 trees by filtering and finally 156 trees after manual removal of topologies with zero length internal branches (referred to subsequently as the ‘manual tree set’). After passing the filtered set through WinClada, 132 topologies remained (the ‘WinClada tree set’). NONA found 344 initial trees using the jump*1 command (length 300, as PAUP*), which is reduced to 232 trees after collapsing polytomies in WinClada. These topologies are the same as those in the PAUP* data set (shown by putting the unfiltered PAUP* tree set through WinClada: the same 232 trees are found). Using the max* swapping algorithm was less effective and only recovered 308 trees, or 192 trees post WinClada.

All the consensus trees maintain the outgroup structure and basal araneid placement of Chorizopes O.P.-Cambridge, found by Scharff and Coddington (1997) (Figs. 3.1, 3.6–3.8). The araneines become a bush beyond this point in the strict consensus tree (Fig. 3.6), although with a few resolved terminal clades. All the Poltyini examined here are found within the Araneinae (sensu Scharff and Coddington, except for Scoloderus Simon). The
Figure 3.8. Adams consensus of the Araneinae for the data of Scharff & Coddington (1997) and taxa from the Poltyini (in bold). Clade numbers show relevant clades analogous to those found by Scharff & Coddington.

A majority-rule tree produced from the WinClada tree set is slightly less resolved than that shown from the manual tree set (Fig. 3.7): two additional levels are collapsed in the araneines, so that Hypsosinga Ausserer and Dolophones Walckenaer are in the main araneine ‘bush’.

The position of Polty within the araneines is unresolved by all the consensus methods (Figs. 3.6–3.8). The character partition table from PAUP® indicates that Polty
pairs with \textit{Zygiella} F.O.P.-Cambridge (31\% of trees) or \textit{Kaira} (15\%) in the manual tree set, and there are several combinations of a clade involving \textit{Polty} and some or all of \textit{Zygiella}, \textit{Kaira}, \textit{Metepheira}, \textit{Singa} C. L. Koch and \textit{Larina} Simon. Examining trees, these sub-arrangements add up to 61\% of topologies. This group is all of Scharff and Coddington’s ‘\textit{Hypsosinga clade}’ (clade 44, Fig. 3.1), except \textit{Hypsosinga} itself and with the addition of \textit{Larina}, which also frequently came into this clade in Scharff and Coddington’s analysis. In other topologies there is usually a series of single taxon ‘steps’ in the basal araneines, in which \textit{Polty} occurs, often with other parts of the ‘\textit{Hypsosinga clade}’ emerging as adjacent steps. In many trees with this type of topology, \textit{Witica} O.P.-Cambridge and \textit{Arachnura} are also present in the very base of the araneine branch. In the WinClada tree set, 55\% of topologies placed \textit{Polty} with various permutations of this modified ‘\textit{Hypsosinga clade}’, and the figures for pairing with \textit{Zygiella} or \textit{Kaira} are 27\% and 18\%, respectively. \textit{Polty} never appears in clades with any other taxa in either tree set.

The only Poltyini taxon to be resolved within the araneine ‘bush’ in the strict consensus is \textit{Cyphalonotus}, which is the sister taxon to Scharff and Coddington’s clade 60 of \textit{(Araneus + Aculepeira} Chamberlin and Ivie) (Fig. 3.6). The majority-rule and Adams consensus trees both suggest \textit{Ideocaira} may belong among or near Scharff and Coddington’s clade 57 (but now also containing \textit{Cyphalonotus} and possibly without \textit{Larina}) (Figs. 3.7–3.8). In every topology \textit{Ideocaira} occurs in a trichotomy with \textit{Neoscona} Simon. The majority-rule tree shows \textit{Kaira} as sister to \textit{Metepheira}, as previously found by Scharff and Coddington (clade 47). \textit{Micropolty} is best resolved by the Adams tree, which recovers a clade where it is sister to \textit{Alpaida} O.P.-Cambridge + (\textit{Bertrana}
Keyserling + *Enacrocoma* Mello-Leitão) (Scharff and Coddington clade 64, Fig. 3.8).

Examination of the trees indicates that *Micropolys* is always found either at the base of a clade composed of the above clade plus its sister group, or at the base of its sister clade. These results are the same for either tree set.

**3.5. DISCUSSION AND OUTGROUP SELECTION FOR PHYLOGENETIC STUDIES**

The question of whether *Poltys* should be included in the ‘*Hypsosinga* clade’ remains uncertain. In these results it is most frequently associated with one or more of the genera *Zygiella, Kaira, Metepeira, Singa* and *Larinia*, most of which are indeed from this clade. The inclusion of *P. illepidus* in the data set destabilises the arrangement found by Scharff and Coddington (1997), however, reducing the former clade to a loose association of genera with variable placement within the Araneinae. Despite this, one of these genera would provide the best choice of outgroup given the current evidence. Another might be from the broader araneine grouping. Nevertheless, a cautionary comment about other *Poltys* species is required. As mentioned earlier, the use here of only *Poltys illepidus* as the only exemplar in a genus that is polymorphic in several characters is far from ideal. Although the type species seems to exemplify the ‘basic’ *Poltys* body plan, and lacks some of the apparently more derived character states seen elsewhere in the genus, it is possible that the genera that appear as potential relatives in the scenario above might be different if one or more of the other *Poltys* species were included or substituted. Unfortunately, the inclusion of more *Poltys* species was not possible in this case.
The second aim of this study was to test whether the taxa formerly included in the Poltyini would appear as a group when included with the taxa analysed by Scharff and Coddington (1997). Even ignoring *Homalopoltyx*, which appears to be a tetragnathid, it is extremely unlikely that the remaining taxa form a monophyletic grouping, although they may all occur scattered among a broader group of araneines. *Cyphalonotus* is the most consistently placed of these taxa, close to *Araneus*, and *Ideocaira* may also belong in the same area of the araneines (Scharff and Coddington clade 57). *Micropoltyx* may belong in the sister clade to these two (which would be clade 62 in Scharff and Coddington’s topology, Fig. 3.1), and, as already discussed, *Poltys* may belong in or near the ‘*Hypsosinga* clade’. Given the limitations of this study noted above, however, these preliminary findings should be subjected to further analysis when the opportunity becomes available.
CHAPTER FOUR
DNA SEQUENCING AND THE APPLICATION OF DNA DATA TO SPECIES
SEPARATION PROBLEMS

[Some material in this chapter is reproduced from Smith (2006) courtesy of The Records of the Australian Museum]

4.1. INTRODUCTION AND AIMS

The differentiation of genes is the underlying mechanism of speciation. Genes are also responsible for producing and controlling the expression of the unique traits and characters, which are used to delimit species in traditional morphological taxonomy. Two populations that can be perceived as separate species by traditional taxonomic methods would therefore be expected to exhibit significant differences in their genetic material if this separation really is a reflection of genetic isolation. What is more, differences that may be more subtle than some of those which are apparent from gross morphology might be useful as a taxonomic tool in their own right. Potentially, not only should it be possible to distinguish cryptic species, i.e. those that have diverged genetically but not in obvious morphological traits, but it should be possible to glean information on the relationships between and within taxa by comparing the patterns of divergence between the same set of genes in different organisms.

Molecular data have been accessed in a number of ways. The development of starch gel electrophoresis (Smithies, 1955 cited in Murphy et al., 1996) enabled protein-based studies to be carried out, based on the differences between proteins produced by a gene being reflected by their mobility on a gel when an electric current is applied. The major advantages of this method, even today, are that the technique is relatively cheap, although quite time consuming, and can be carried out using relatively
simple and inexpensive equipment. The disadvantages, compared against more recent methods, are that electrophoresis produces comparative, or distance, data based on phenotypic variation rather than absolute, or character data of the genotype, and that fresh or frozen material must be used as preservation by other methods affects protein structure. With the elucidation of the double-helix structure of DNA (Watson and Crick, 1953), the development of techniques for more direct exploration of genes and protein structure was not far behind. Common techniques that also produce distance data are DNA–DNA hybridisation and analysis of fragments and restriction sites. Direct DNA sequencing is the only technique that produces character data, but the other techniques are powerful tools in appropriate situations (Werman et al., 1996; Dowling et al., 1996).

Early direct sequencing of DNA was a slow and laborious process. The polymerase chain reaction (PCR), which facilitated multiple copying of DNA fragments (DNA amplification), was developed in the early 1970s (Kleppe et al., 1971). The full potential of this technique was realised several years later with the discovery and purification of a heat-stable polymerase from an extremely thermophilic bacterium (Mullis and Faloona, 1987). Although other methods are available, PCR has been the major factor contributing to the sudden increase in the use of molecular information in many systematics projects. Continued advances in automatisation of parts of the process and the availability of “off the shelf” reagents and information on primer sequences have now made DNA sequencing an almost routine procedure for many genes.

Many arguments have arisen over the uses of molecular data and how results compare to those generated from morphological attributes. Some conflicts are due to
still unresolved differences in opinion over species concepts (see the debate presented by Wheeler and Meier, 2000). Other disagreements revolve around the suitability of certain parts of the genome—whilst some support the use of non-recombining loci such as mitochondrial genes (Davis and Nixon, 1992), others claim that these should not be used for definition of species due to differing geographical patterns in genetic history. It has been apparent from many studies that different genes give apparently conflicting information (e.g. Navajas and Boursot, 2003 who used the same genes as the current study). Nevertheless, as commented by Sperling and Harrison (1994), this does not mean that the information is not valid. Each gene can legitimately reflect a different aspect of the organism’s history. So, it is most important to get a balanced view from several different sources of information, and most studies use at least two different genes. Morphological data can also be used in the same studies; in the view of Moritz and Hillis (1996), “In general, studies that incorporate both molecular and morphological data will provide much better descriptions and interpretations of biological diversity than those that focus on just one approach.”

In this study of Poltys, both molecular and morphological data have been used. The aims were two-fold; firstly comparison of DNA sequences between specimens could support the species separations indicated by morphology. This is the main concern of this chapter. Secondly, the DNA data could be used to generate a phylogeny; this is the subject of Chapter 5. Whilst DNA sequencing is quite widely available at a number of institutions, the equipment to facilitate the process is expensive, it is still relatively time consuming, and the cost of consumables is significant. For this reason it was still not the default method at the time this study was commenced and other techniques, although producing less informative data, may still
have been as appropriate for some questions (Baverstock and Moritz, 1996). DNA sequencing was chosen for this study in preference to protein electrophoresis, which could have otherwise fulfilled the requirements of the study, largely because of the uncertainties surrounding the availability of fresh material. DNA–DNA hybridisation (discussed by Werman et al., 1996) provides an estimate of genomic sequence (rather than just single gene) divergence between organisms and has been used for many phylogenetic studies. Despite this strength, it is a complex and relatively expensive technique requiring the use of radioisotopes. Another caveat is the controversy surrounding the choice of distance metric (Werman et al., 1996). Overall, these difficulties made it an inappropriate choice for the current study. Restriction fragment analysis is most commonly used in population studies and is most efficient where there are large numbers of individuals to sample (Dowling et al., 1996). In this study, where relatively few individual organisms were available, and the specimens were slowly added into the study as it progressed, direct DNA sequencing was the most appropriate methodology.

4.2. PERCEIVED PROBLEMS IN POLTYS AND THE RELEVANCE OF SEQUENCE DIFFERENCES IN THIS CONTEXT

The process of separating the Australian Poltys species by morphological methods was initially problematic due to inter and intraspecific variation. Even after most of the levels of variation became apparent some residual uncertainties persisted. In relation to the phylogenetic applications of molecular data, five situations have been identified in which it has been demonstrated that morphological attributes alone may not provide adequate information for species identification (Baverstock and Moritz,
1996). The problems presented by Poltys at different times throughout the project can be mapped onto four of these:

(i) two sympatric or parapatric species may be so similar in morphology that specific status is not detected. (P. noblei cf. P. laciniosus and further confused by descriptions of P. mammmeatus, P. bimaculatus and P. salebrosus);

(ii) two allopatric populations may be morphologically different but it is unclear whether they are biologically distinct. (P. grayi cf. P. noblei; P. laciniosus ex the NT/Kimberley region);

(iii) two parapatric populations may be morphologically distinct but show clinal variation. (Epigynal size and shape in P. laciniosus; modified patellar spines in P. illepidus);

(iv) two (or more) morphologically distinct forms may represent polymorphisms within a single interbreeding population. (Any of the species with distinct variations of abdominal shape; investigated specifically in P. noblei, although these can be seen to represent a continuum when more specimens are examined).

The variability of mitochondrial DNA (mtDNA) sequences has made certain areas of this genome a target for phylogeographic, population and interspecific analyses. There has been some discussion on the suitability of non-recombining loci for species separation in the phylogenetic sense (e.g. Davis and Nixon, 1992; Moritz et al., 1992), nonetheless mtDNA has been demonstrated to be a useful tool to aid or confirm species recognition in a number of studies such as those on araneoid spiders (Hedin, 1997a), skinks (Moritz et al., 1993), onychophorans (Trewick, 2000) and carabid beetles (Pawson et al., 2003).
Recently, broad success has been claimed in using COI sequences as ‘species barcodes’ (Hebert et al., 2002, 2003, 2004). In particular, Hebert et al. (2003) demonstrated that more than 98% of congeneric species pairs of animals showed more than 2% divergence (uncorrected pairwise comparisons), and that for chelicerates (1249 pairs of species examined) the mean interspecific divergence was 14.4%. In contrast, intraspecific divergences for various taxa (drawn from those used as exemplars by Avise, 2000) were reported to be rarely greater than 2% and often less than 1% [interpreted by Hebert, 2003—the original studies have not been checked here]. The suggestion by Hebert and others that the divergences of COI sequences alone are sufficient to separate taxa for taxonomic purposes has received considerable criticism (e.g. Mallet and Willmott, 2003; Bond, 2004; Will and Rubinoff, 2004); DeSalle et al., 2005). These authors support the use of molecular data, but suggest a variety of more derived approaches that use evidence from several different genes as well as morphological data. Here I am unable to use complex algorithms for molecular species separations due to the extremely limited size of the dataset. Whilst recognising the limitations of the percentage divergence approach, I use Hebert’s figures as a baseline for comparison to the inter- and intra-specific divergences in the COI sequences of Poltys specimens. This information in combination with information from the ITS2 sequences is used to support or question the species separations suggested by morphological data.

4.3. CHOICE OF SEQUENCES USED IN THIS STUDY

The sequence first chosen was from the mitochondrial gene cytochrome c oxidase subunit 1 (COI). As indicated above, this has been widely used in invertebrate
studies both at a specific and sub-specific level and is usually one of the easier genes to sequence, even from less ideally preserved specimens, because mitochondria are abundant in the cytoplasm. I first tried to use primers ACO1AF and ACO602R (Colgan et al., 2001). These had proven usable in some arthropods but a clean signal could not be achieved in Poltys. By chance, a project on wolf spiders (Lycosidae) was just reaching completion at the Australian Museum (Colgan et al., 2002). Degenerate primers from within this section of COI had been designed for this project, and these primers were made available to me. These primers worked well in Poltys, producing a 212bp section which was adequate for the initial usage of species differentiation. In hindsight, it would have been better to experiment with a combination of these and other universal primers in turn in order to obtain a longer sequence (as has now been done by T. Blackledge (pers. comm.) for specimens of *P. laciniosus* and *P. illepidus*). Unfortunately this option was not appreciated at the time and the opportunity to obtain a longer sequence was missed. It was also intended that the 12S ribosomal mitochondrial gene used in a study of *Kaira* (by Piel and Nutt, 1997) would be sequenced for the phylogenetic analysis. There was insufficient time to undertake this part of the study, however.

The second sequence was suggested by Dr M. Arnedo when I requested information on the primers and sequences used for a project on *Orsonwelles* Hormiga (Linyphiidae). This was the ribosomal gene ‘internal transcribed spacer 2’ (ITS2). This is a non-coding sequence between the ribosomal 28S and 5.8S units; parts of these genes are adapted for primers (Hedin, 1997b). ITS2 is often quite variable, so it is useful at the specific level, but may also provide information at higher taxonomic levels. This information is often lost in COI due to saturation of base changes,
especially at position 3, thus ITS2 could provide a good balance for COI in a study such as this.

There can be considerable problems associated with the use of ITS2, which make it unsuitable for use in some circumstances. In particular, there are many copies of the gene in the ribosomes in the cytoplasm and because ITS2 is not directly read to code a protein, the evolutionary pressures that might otherwise conserve the sequence are reduced. Polymorphisms are common in some organisms; when these involve only single bases this may not be a problem, but unconnected areas of genetic material often seem to get spliced in. This can result in undecipherable superimposed sequences when more than one version is present in a single organism. Another problem is that there may be great differences in the length of the sequence even between quite closely related taxa. As well as making alignment extremely difficult, it can then become almost impossible to be sure that the sequences are homologous between species.

Unfortunately, ITS2 appeared to be working quite well in Poltys until the last few sequences either failed, or were found to be impossible to align. By this stage of the project it was too late to change to a more suitable gene. Thus for both COI and ITS2, the analyses presented here, and in Chapter 5 in particular, are far from ideal and are intended to illustrate the learning of methodology rather than a polished final product. Despite this, much of the content of this present chapter was found to be suitable for publication in support of the taxonomic work of Chapter 2 (Smith, 2006).
4.4. METHODS OF DNA EXTRACTION AND SEQUENCING

4.4.1. DNA extraction

All specimens and taxa sequenced for each gene are shown in Appendix 4. Two to six legs were removed from each specimen, the number depending on size and method of preservation. These were used directly for DNA extraction (fresh frozen specimens), washed in ddH₂O (initial spirit specimens, all in 70% ethanol), or rehydrated through a series of solutions (later spirit specimens, some 70% and some 96% ethanol). DNA was extracted by one of three methods. For the initial batch of 9 specimens examined from the Sydney area (frozen fresh material) a Gentra PUREGENE®DNA Isolation Kit was used, with slight modifications to the manufacturer’s method: RNase digestion was omitted and a wide-bore pipette was used to minimise shearing. Extractions from all specimens stored in ethanol were performed either by the method of Saghai-Maroof et al. (1984) (“CTAB”) or using a QIAGEN DNeasy™ Tissue Extraction Kit (04/99), following protocol B for insects with the substitution of 200 µl CTAB for PBS (the reagent provided in the kit) in step 2. DNA pellets were rehydrated in 50 µl ddH₂O except those extracted using the Qiagen kit, which were eluted in 100 µl. Samples of 2 µl volume were run on 1.2% agarose gels containing ethidium bromide (in TBE buffer) and examined for UV induced fluorescence. Depending on the apparent quantity of DNA, samples were either used undiluted or diluted 10 or 20 times.
4.4.2. Primers and PCR

COI

Spider-specific nested primers designed by Colgan et al. (2002) were used: Spider COI F 5’ CCTGGGAGTTATTTAGGGGATGATC and Spider COI R 5’ GGATATACAGTTCAACCAGCCTCC. Initial amplifications were carried out using 1 µl of appropriately diluted sample product, 3.5 mM MgCl₂, Buffer IV (10X, 20 mM (NH₄)₂SO₄, 750 mM Tris-HCl pH9.0, 0.1% Tween) (Advanced Biotechnologies), 0.05 mM dNTP, 12.5 pmol primers and 0.5 Units of Red Hot™ thermostable DNA polymerase in a total volume of 25 µl with oil overlay. A control using 1 µl ddH₂O instead of DNA was included in each batch. The exact reagents used were varied at later stages of the study depending upon the recommended practices of the Australian Museum EBU at that time (see reagents listed for ITS2 below). The PCR profile was 94°C for 3 min, 45°C for 1 min, 72°C for 1 min—one cycle; 94°C for 30 sec, 55°C for 30 sec, 72°C for 45 sec—35 cycles; and 94°C for 30 sec, 55°C for 30 sec, and 72°C for 3 min—one cycle. Two µl of each reaction product was resolved and UV visualised on 2% agarose gels containing ethidium bromide. PCR products were purified using the QIAquick™ PCR Purification Kit, following manufacturer’s instructions varying the amount of ddH₂O used for elution (step 8) depending on the concentration of DNA.

ITS2

Primers were based on information given by Hedin (1997b): ITS2-28S TCCTCCGCTTATTGATATGC and ITS2-5.8S GGGACGATGAAGAAGCGCAGC. Amplifications were carried out using 1 µl of appropriately diluted sample product, 1.5 mM MgCl₂, Buffer IV (as above), 0.05 mM dNTP, 12.5 pmol primers and 0.25 Units
of QIAGEN Taq DNA polymerase in a total volume of 25 μl. The PCR profile was 94°C for 2 min—one cycle; 94°C for 30 sec, 57.5°C for 30 sec, 72°C for 45 sec—30 cycles; and 72°C for 5 min—one cycle.

4.4.3. Cycle sequencing

Samples were sequenced in both directions using the Big Dye DyeDeoxy™ terminating sequencing method, initially in 10 μl reactions, using 1 μl purified PCR product, 1 μl BigDye™ Terminator Mix, 0.8 μl primer (5.0 pM/μl) and 7.2 μl Buffer IV (made up as before) with an oil overlay. Later samples (including all ITS2) were in 20 μl reactions, using 2 μl BigDye™ Terminator Mix Version 3, 1.0 μl primer (3.2 pM/μl), 0.5–2.0 μl purified PCR product, 4 μl sequencing buffer, 10 μl 10% DMSO (ITS2 only) and ddH₂O up to 20 μl. Samples were cleaned by ethanol/sodium acetate precipitation (or ethanol purification for BigDye™ v.3) and run on an Applied Biosystems 310 Gene Analyser®.

4.4.4. Sequence editing and alignment

COI

Sequences were edited and assembled using Sequencher™ 4.1. Sequences were imported into a block and their alignment was checked using Se-Al v.2.0 (Rambaut, 1996) then output in NEXUS format for analysis using PAUP* 4.0b10 (Swofford, 2001).
ITS2

This is a non-coding gene and hence has rather variable areas that require the insertion of gaps for alignment. This is a practice that has theoretical problems because the artificially introduced gaps are not necessarily equivalent to insertions and deletions, which arise by mutational events (Olsen, 1988). It is sometimes suggested that any sections that cannot be unambiguously aligned should be deleted. Unfortunately, in this case this action would have involved discarding most of the variable parts of the sequence, so an alignment algorithm was required. A simplification of the alignment method outlined by Mindell (1991) was used because variable areas were relatively short and well defined. An initial sequence alignment from Clustal X (Thompson et al., 1997) (manually adjusted) was used to generate uncorrected pairwise similarities. A second alignment was then built, starting with the two most similar sequences and adding and aligning each remaining sequence in turn according to its similarity to the first. Sequence alignment in Clustal X used gap penalties of 15 to open a gap and 6.6 for lengthening. A few further adjustments to the alignment were made manually. The manual adjustments were each tested and those that produced the shortest tree in a simple parsimony analysis were accepted.

4.5. METHODS FOR SPECIES SEPARATIONS

For COI, each putative Poltys species was represented by at least two samples from geographically separated sites (if possible). Species that exhibited interspecific variation in characters other than abdominal shape were represented by a wider range of samples. The 212bp fragment of sequenced COI from each individual sampled was compared to all others to obtain uncorrected pairwise overall similarity percentages
(100x number of substitutions/total number of bases). Sampling for ITS2 was much more limited due to time constraints and because many samples used for COI did not amplify successfully for ITS2. Pairwise similarities were obtained from PAUP* from the aligned sequence.

4.6. RESULTS

4.6.1. The sequences

The sequences obtained and their alignments are shown in Appendices 5 (COI) and 6 (ITS2). All unique sequences have been submitted to GenBank (Accession numbers in Appendix 4). Unfortunately, no ITS2 sequence could be obtained at all for _P. milledgei_, and only a rather poor short single-stranded sequence was obtained for _P. noblei_, despite good quality extracts. Most of the failed sequences for _P. noblei_ were jumbled, appearing to comprise two different superimposed sequences. Several different specimens from the original nine Sydney specimens were tried to check that sample quality was not an issue. The one sequence that was almost readable was from an early optimising PCR run and the conditions should have been less, rather than more, specific. Unfortunately, in this case it would appear that the alternative sequence was also optimised by the same conditions as the target sequence. Lack of time prevented the resolution of this problem. In the case of _P. milledgei_, poor extracts from 70% alcohol preserved material was most likely the cause of the problem as _P. jujorum_ also showed no product at all until a fresh extraction from recent 96% ethanol preserved material was obtained.
4.6.2. Species separations

**COI** (Table 4.1 below diagonal, Fig. 4.1)

Intraspecific variation is 0–5.19% (mean=1.81), if the two populations of *P. illepidus* are considered as conspecific. If the nine *P. illepidus* North to South pairwise comparisons are removed (but the within population figures retained) the range is 0–2.36% (mean=0.93). Between species, the range is 7.55–20.75% (mean=14.49) if *P. illepidus* is considered as a single species, or 4.25–20.75% (mean=14.26) if the two populations are considered separately. Either of these interspecific values is comparable with those reported for chelicerates (Hebert *et al.*, 2003). The intraspecific values are also comparable to those reported by other studies, and suggest that based on this COI data set, two cryptic species might be present within the Australian distribution of *P. illepidus*.

**ITS2** (Table 4.1 above diagonal)

No intraspecific variation was detected, including between northern and southern *P. illepidus* (see Appendix 4 for specimens sequenced). Interspecific variation ranged from 0.75% (*P. illepidus*—*P. stygius*) to 12.18% (*P. grayi*—*P. jujorum*), mean 9.57%. The extremely low variation between the former pair reflects their morphological similarity but is at odds with the high differentiation found by COI.
Table 4.1. Mean uncorrected pairwise differences x 100% for COI and ITS2 (averaged between specimens within populations or species).

<table>
<thead>
<tr>
<th>ITS2 above</th>
<th>(P.) frenchi</th>
<th>(P.) grayi</th>
<th>(P.) illepidus (northern)</th>
<th>(P.) illepidus (southern)</th>
<th>(P.) jujorum</th>
<th>(P.) laciniosus</th>
<th>(P.) milledgei</th>
<th>(P.) noblei</th>
<th>(P.) stygius</th>
</tr>
</thead>
<tbody>
<tr>
<td>COI below</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P.) frenchi</td>
<td>–</td>
<td>10.91</td>
<td>10.69</td>
<td>10.69</td>
<td>12.05</td>
<td>6.92</td>
<td>no data</td>
<td>no data</td>
<td>10.94</td>
</tr>
<tr>
<td>(P.) grayi</td>
<td>11.79</td>
<td>–</td>
<td>11.08</td>
<td>11.08</td>
<td>12.18</td>
<td>5.84</td>
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<td>no data</td>
<td>11.84</td>
</tr>
<tr>
<td>(P.) illepidus (N)</td>
<td>13.36</td>
<td>12.66</td>
<td>–</td>
<td>0</td>
<td>8.84</td>
<td>10.46</td>
<td>no data</td>
<td>no data</td>
<td>0.75</td>
</tr>
<tr>
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<td>15.25</td>
<td>13.60</td>
<td>4.56</td>
<td>–</td>
<td>8.84</td>
<td>10.46</td>
<td>no data</td>
<td>no data</td>
<td>0.75</td>
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<tr>
<td>(P.) jujorum</td>
<td>18.71</td>
<td>16.90</td>
<td>13.52</td>
<td>12.42</td>
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<td>10.05</td>
<td>10.63</td>
<td>10.91</td>
<td>13.43</td>
<td>–</td>
<td>no data</td>
<td>no data</td>
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<tr>
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<td>16.43</td>
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<td>14.47</td>
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<td>–</td>
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<tr>
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<td>7.78</td>
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<td>15.57</td>
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</tr>
<tr>
<td>(P.) stygius</td>
<td>16.51</td>
<td>15.80</td>
<td>14.47</td>
<td>14.31</td>
<td>15.57</td>
<td>13.49</td>
<td>15.88</td>
<td>15.09</td>
<td>–</td>
</tr>
</tbody>
</table>
FIGURE 4.1. Frequency histogram of within and between species distances (% difference) calculated from uncorrected pairwise distances.

4.7. DISCUSSION AND CONCLUSIONS

The separation of species by morphology presented in the taxonomic section is consistent with DNA evidence overall. The COI data alone are equivocal for *P. illepidus* northern and southern populations, however, and give different levels of separation for some taxon pairs to that suggested by other data (e.g. *P. illepidus* c.f. *P. stygius*, Table 4.1). As mentioned above, such nonconcordance of data from different genes has been reported in a number of other studies. Yet another example emphasizes the need for the cautionary approach, which requires evidence from several sources, as espoused by several authors (e.g. Moritz *et al.*, 1992; Sperling and Harrison, 1994).
Most of the uncertainties outlined in Section 4.2 above can be resolved using the pairwise comparison technique backed up by other evidence. The paragraphs below refer back to those points:

(i) Cryptic taxa. The possible presence of cryptic taxa in *P. laciniosus* was certainly resolved by COI, and *P. noblei* has now been separated. In retrospect these were not truly cryptic species, but the variation present in abdominal shape is far more obvious, and in opposition to, the more subtle signals found in the epigyne morphology. Once the results from the initial DNA sequencing validated the levels of variation present within one species (reported in Smith, 2003), and males of *P. laciniosus* were finally collected, the problem dissolved. The results also indicate a possible cryptic taxon in the southern populations of *P. illepidus*; this is discussed further under (iii), below.

(ii) Morphological variation between populations. Whether the allopatric populations of *P. noblei* v. *P. grayi* and *P. laciniosus* from the Kimberley v. elsewhere, are separate species is not completely resolved, due to a lack of ITS2 data for critical specimens. Overall, the data support separation of *P. grayi* and *P. noblei*, although with 7–8% difference in COI sequences, they are the most closely related species amongst the taxa examined. The morphological differences are subtle, but quite definite considering the lack of genitalic differentiation shown between some other species pairs. The partial ITS2 sequence obtained for *P. noblei* also appeared to have a few base changes compared to *P. grayi*, but this could not be corroborated due to the high noise level in the data. The case of conspecificity of *P. laciniosus* from the NT and Kimberley regions with those from the rest of Australia is less strongly defined. The COI data indicate that there is no specific differentiation, yet the morphology of the
epigynes of the two recorded females is quite distinctive. No males are yet known from this area. The DNA extraction from the 75% ethanol preserved NT specimen was not assayed for the ITS2 gene as attempts from other specimens of a similar age had not been successful. Further work needs to be carried out in this area.

(iii) Clinal variation. The problem of interpreting clinal variation in the genitalia of *P. laciniosus* and the modified leg macrosetae of *P. illepidus* is also only partially resolved. Specimens of *P. laciniosus* from across Australia showed a variation of approximately 1–2% in the COI gene (many more specimens than shown in Appendix 4 were actually sequenced; the five shown here were chosen as exemplars as they covered the range of variation in COI, in epigynal features and also represented most regions of Australia). There was no obvious pattern to the COI variation, either geographically, or with respect to epigyne morphology. With the possible exception of the NT specimen, discussed above, it is highly probable that *P. laciniosus* is pan-Australian in distribution and without any cryptic species present in the specimens examined. Conversely, some uncertainties remain with respect to *P. illepidus*. Overall, given the lack both of morphological variation, and of differentiation in the sequences of ITS2 examined, these are most likely to be a single species despite the consistent 4–5% variation in COI. Nevertheless, when suitably preserved specimens are available from the geographic area between the two extremes sampled so far for DNA, it would be desirable to carry out further work.

(iv) Polymorphic species. This has indirectly been dealt with under (i), above, as it was the basis for the possible presence of one or more cryptic species. It is now apparent that this situation is not really applicable to *Poitys*, as the abdominal variation can be seen to be continuous, rather than discretely polymorphic, once enough
specimens have been examined. Nevertheless, historically it has certainly caused confusion, and resulted in the species recognised here as *P. laciniosus* being described four times, whilst *P. noblei* was overlooked. Evidence for the variable morphology within several *Poltys* species was supported by the COI data set, and was backed up by other morphological data and rearing experiments (Chapter 7).
CHAPTER 5

CLADISTIC ANALYSIS—RELATIONSHIPS OF THE POLTYS LACINIOSUS-
GROUP AND P. FRENCHI

5.1. INTRODUCTION AND AIMS

Poltys is a sizable genus, with 44 extant species currently listed by Platnick (2006). As has been demonstrated, most of the Australian Poltys species also appear to be distributed into SE Asia and one, or both, of the P. illepidus-group species may also be widespread in mainland Asia. This means a phylogeny of a small part of the genus will be compromised at best, and potentially could give misleading results as not all of the species-groups can be included. With this caveat in mind, it may be possible to usefully examine a clearly defined subset of the Australasian taxa: the P. laciniosus-group, which seems to be restricted to mainland Australia. Poltys frenchi, from northern Australia and New Guinea, presents a combination of morphological attributes which in many ways are intermediate between these endemic taxa and the P. illepidus-group. Support for the hypothesis of a relationship between the Poltys laciniosus-group and P. frenchi, and these taxa and the P. illepidus-group will be tentatively examined using the two short DNA sequences and morphological characters.

5.2. OUTGROUPS

The general area of the Araneidae that outgroups should ideally be taken from was identified in Chapter 3. The choice within this was controlled by practical considerations:
(i) that both males and females were available for coding morphological characters;

(ii) fresh or 96% ethanol specimens should be available for DNA sequencing;

(iii) preference would be given to taxa that were identified in the literature.

After some experimentation with several taxa that were later dropped because sequences failed to amplify for one or both genes (Arachnura, Dolophones and Eriophora Simon), Lipocrea tabida (L. Koch) and ‘Araneus’ eburnus (Keyserling) (both identified from Davies, 1988, the former as Larinia t.) were selected as outgroups. Nephila plumipes (Latreille) was also successfully sequenced for use as a distant outgroup. Unfortunately, the ITS2 sequence was far shorter than that in the araneid genera and sequence alignment proved problematic, so this too was eventually dropped. The specimens used for sequencing and those examined for morphological characters are listed in Appendix 4.

5.3. THE TREATMENT OF DATA FROM DIFFERENT SOURCES

There has been some disagreement over the best way to treat sets of data derived from different sources. There are several reasons why DNA data from different genes might produce conflicting phylogenies. The most obvious is the different patterns of inheritance of genes that are passed on from a single sex only, such as the mitochondrial genomic material. Other differences might arise from processes such as lineage sorting, incomplete speciation and hybridisation. The data from morphology can also be compromised by homoplasious convergence or other inappropriate
definition of characters and their states. It has been argued that demonstrably heterogeneous data sets should not be combined in an analysis that assumes character homogeneity (Bull et al., 1993). Nevertheless, with more and more studies producing multiple sets of information, it is often desirable to combine data to give meaningful results if possible. A test for incongruence between data sets was developed by Farris et al. (1995).

Two methods of combination have received most attention. Taxonomic congruence analyses the data sets separately and then combines these results into consensus trees to find common hierarchical groupings. The consensus trees, which may be produced in the first round as well as in the second, are derived from cladograms however, not original data, and global parsimony is sacrificed in the process (Miyamoto, 1985). Simultaneous analysis is now more often recommended (Kitching et al., 1998) and several studies have demonstrated that apparently incongruous individual data sets can actually combine to more resolved, and better supported, trees than those found by individual data sets alone (Vane-Wright et al., 1992; Sullivan, 1996; Morgan-Richards and Gibbs, 2001, amongst others). Simultaneous analysis including morphological data can also provide results that are more stable with respect to sensitivity to parameter variation than molecular data alone (Prendini et al., 2003). Furthermore, there is no harm in both examining the data sets individually and in combination (Miyamoto, 1985, and as demonstrated by Wheeler et al., 1993) and this is the approach I take in this study.

Any method of combining data suffers from problems when not all taxa, or individuals, are represented in all the data sets. Here, the initial separate analyses of each set of data allow all the available data to be explored and also allow different
treatments that may be applicable to one set but not another. No consensus tree can be attempted in this case, because the taxa cannot be retrospectively omitted or added to make data sets match each other. Secondly, the data for taxa represented in all three sub-sets are combined and analysed using a simple parsimony analysis, irrespective of apparent differences in parameters such as rates of genetic change. The significance of incongruence between data sets is tested (Farris et al., 1995).

5.4. DATA AND ANALYSES—SEPARATE DATA SETS

5.4.1. DNA methodology

Before proceeding with analysis, both DNA data sets were tested for base homogeneity between taxa using the “basefreqs” command in PAUP* and for saturation of substitution events by plotting the number of substitutions against uncorrected pairwise distance, also generated from PAUP*. Unrooted phylograms were produced using heuristic search methods under both maximum likelihood (ML) and parsimony criterion settings in PAUP*. Taxa were joined by random addition; tree-bisection-reconnection branch-swapping was used, the heuristic search algorithm was as described in Chapter 3; 500 or 1000 replicates were carried out for ML analyses, 1000 for parsimony. For ML, the model of sequence evolution that best fitted the data according to the Akaike information criterion (Akaike, 1974, cited in Posada and Crandall, 1998) was chosen using likelihood ratio tests implemented by Modeltest 3.6 (Posada and Crandall, 1998). Jackknife resampling was used in PAUP* to test the robustness of nodes, as described in Chapter 3.
COI saturation and homogeneity

The separation of the Australian *Polyps* species as indicated by the COI sequence has already been discussed. This was the primary function of this sequence and it was not ideally suited for phylogenetic use, being rather short. Examination of substitutions at the different base positions showed that almost 90% of all substitutions occur at position 3 (Fig. 5.1.a). Saturation plots examined substitutions at position 3 (Figs. 5.1.b-d). Whilst transversion events show no sign of saturation, the plots of transition events at this position indicated that if nucleotides were to be used for analysis, outgroup taxa should be omitted (Figs. 5.1.c, d). The alternative would be to translate the nucleotides to proteins, but these sequences were too short to provide enough information for this option to be viable. Therefore all the following analyses include only the 20 ingroup specimens with unique sequences. [Note: although some variation within the species that is here described as *P. noblei* was reported in Smith (2003), no full sequences have been obtained for either of these specimens so they have not been included here. One specimen was from the limit of the northern range of distribution in far north Queensland, the other from the southern end in SE Victoria. The variation was observed in two base positions, i.e. 0.94%, and involved the same positions and bases in each specimen.]

Base frequencies are biased away from C and towards T (A=0.2375, C=0.1229, G=0.1993, T=0.4403), but homogeneity between taxa is high ($\chi^2$ test, p > 0.99, 57 d.f.). For ML Modeltest suggested the “HKY85+I+G” model of character evolution best fitted the data. This is the model of Hasegawa *et al.* (1985) plus specified values for the invariable proportion of bases and gamma—see Appendix 8.

Parsimony searches were either unweighted or transversions weighted 2 x transitions.
Figure 5.1. DNA sequence base substitutions with respect to position and saturation. a–d, COI: a, proportions of base pair substitutions occurring at positions 1–3; b–d, position 3 only, saturation plots: b, transversions; c, transitions, all taxa; d, transitions in ingroup (*Poltys*) only. e, ITS2, saturation plot, all substitutions. Legends shared for axes in b–e.

**ITS2 saturation and homogeneity**

Analysis of the sequences shows that saturation may be starting but is not yet significant (Fig. 5.1.e), base frequencies are almost equal (A=0.2368, C=0.2605,
G=0.2747, T=0.2280), and homogeneity between taxa is high ($\chi^2$ test, p > 0.99, 21 d.f.). Modeltest 3.6 suggested the “SYM+I+G” model to be most suitable for this data set. SYM is the symmetrical model of Zharkikh (1994) plus specified values for the invariable proportion of bases and gamma—see Appendix 8. Parsimony analyses used unweighted data, with gaps coded as missing data.

5.4.2. Morphological data

5.4.2.1. Methods

All characters are unordered and unweighted. Searches in PAUP* were carried out as above using the parsimony criterion. The same data were also run in Hennig 86 and NONA. WinClada was used to examine character state changes and produce the cladogram. Characters were ACCTRAN, or ‘accelerated transformation’ optimised. ACCTRAN places the change of character state as close as possible to the root. This practice favours, “the acquisition of a character with subsequent homoplasy accounted for by reversal” and is said to maintain the “original conjecture of the character as a putative synapomorphy” (see Kitching et al., 1998 and references therein). The opposite option is DELTRAN, or ‘delayed transformation’, where character changes are applied as far towards the branch ends as possible. This favours parallelisms, or independent derivations of character attributes. This can be viewed as rejecting the original hypothesis of primary homology (Kitching et al., 1998).

5.4.2.2. The Characters

Characters are explained below and the attribute codings are given in Appendix 7. Some characters used for araneoid taxa by Scharff and Coddington (1997) have been
included, but most were not relevant for this small and mostly closely related set of taxa. Those that are used are referenced for background information.

Finding reliable characters among taxa which are inherently variable has proven quite problematic. The 28 which have been used are mostly qualitative (in the sense of Thiele, 1993) and in the context of the taxa here there are no problems with overlapping data. Nevertheless, if other Poliys taxa were to be included some of these characters would probably prove unworkable. They are representative of what I have informally called species groups, which are well separated in Australia, but it is unlikely that the same boundaries will exist in a more central area of the taxon’s range. For instance, *P. pannuceus* from Myanmar aligns with the *P. illepidus*-group in general morphology, but the appearance of the epigyne (Fig. 2.22.h) is reminiscent of many *P. columnaris*-group species.

Another potential problem concerns the coding difference between an inapplicable entry ‘-’, and an unknown state due to incomplete data, ‘?’ . The meanings of the two are quite different but many programs, including PAUP*, do not distinguish between them. This can lead to characters being assigned unresolved potential attributes that cannot possibly apply to the taxa concerned (Platnick *et al.*, 1991).

Strong and Lipscomb (1999) critically examine all the coding methods currently used and show that there are potential problems with all of them. Here I decided to follow the composite coding methodology (e.g. as applied by Davies and Lambkin, 2000) wherever possible. This method avoids inapplicable entries by coding such characters as ‘absent’ in a multistate character; thus, any ‘?’ s are unknown, but possible, characters. In all the characters where this is used (Characters 6 and 16, 24) absence is considered primitive and there are no secondary losses; the conditions that are
suggested to be prerequisites for successful tree reconstruction by Strong & Lipscomb (1999).

For palpal characters, the ventral side is considered to be as seen when the palpal femur points towards the viewer (unexpanded palp). ‘Scharff & Coddington’ refers to Scharff and Coddington (1997). Davies (1988) and Figure 5.2.a and b show most characters for ‘Araneus’ eburnus and Lipocrea tabida.

Male sexual characters

1. Embolus base. Dorsal [0], ventral [1] or retrolateral [2]. In the unexpanded palp in ventral view the embolus origin may be in different positions (e.g. Figs. 2.4.f [0], 2.13.g [2]).

2. Conductor position. Normal [0], displaced prolaterad [1]. In many taxa the conductor arises from the retrolateral apex of the tegulum. In Australian Polysis, the conductor often arises apicoventrally but in the P. columnarinis-group in particular, the base is displaced ventrally and the conductor itself is seen on the prolateral side of the palp (Fig. 2.9.e).

3. Conductor shape. With divided tip [0], entire [1]. ‘Araneus’ eburnus and L. tabida (Fig. 5.2.a, b) and a number of other araneine taxa have conductors with a divided apex (although rather narrow in ‘A.’ eburnus) [0]. There is no evidence of this division in Polysis species [1].

4. Terminal apophysis. Present [0], absent [1]. The only taxa in the present analysis without a terminal apophysis are the three species in the P. laciniosus-group.

5. Terminal apophysis shape. Bulbous or lobed [0], short paddle (c. twice as long as wide) [1], long paddle (>> twice as long as wide) [2]. The Polysis taxa with a
TA have simple paddle-shaped sclerites (e.g. Figs. 2.9.k [1], 2.18.e [2]). ‘Araneus’ *eburnus* has a similar structure but with a lateral lobe (Fig. 5.2.a) [0]. *Lipocrea tabida* has a large sac-like TA with a hooked end (Fig. 5.2.b) [0].

6. **Median apophysis.** Directed retrolaterally, robust and heavily sclerotised [0], directed apically, triangular base with extended hook [1]. All the *Poltys* males examined so far have a distinctive MA, with a roughly triangular basal plate that is drawn out apically into a lightly sclerotised elongate hook (Fig. 2.4.f). The other taxa have rather more complex, robust and heavily sclerotised sclerites.

7. **Paramedian apophysis.** Absent [0], lobe [1], fold [2] or textured plate [3]. *Araneus* *eburnus* and the *P. illepidus*-group species have a PM that appears as a lobe or fold (Fig. 5.2.a; Plate 2.3.d). The *P. laciniosus*-group taxa and *P. frenchi* both have a sculptured plate (Plates 2.5.e, 2.6.b (both left of centre)). As discussed by Scharff and Coddington (character 18) the sclerites referred to in that work as PM may not be homologous between taxa and thus care needs to be exercised in the use of this as a character. Here the PM of ‘A.’ *eburnus* appears to arise from the same point in the membrane of the tegulum, directly adjacent to the MA, as the PM of the *Poltys* taxa, so I have considered them to be homologous.

8. **Embolic shape.** Normal [0], long, free-standing and wire-like [1]. Males of the *P. laciniosus*-group have a long wire-like embolus (E in Fig. 2.19.b).

9. **Patellar macrosetae.** Present [0], absent [1] (adapted from Scharff and Coddington character 4).

10. **Femoral tubercle and endite tooth.** Present [0] or absent [1]. These characters have been combined. All taxa with large males in this data set possess a
strongly developed endite tooth and femoral tubercle (Scharff and Coddington characters 3 and 45).

Female sexual characters

11. Epigynum shape. Broad [0], spade-like [1], sharp ‘V’-shape [2], tongue-shape [3] or with reflex [4]. The epigynes of Australian *Poltys* fall into three broad categories: ‘spade-like’ includes all the species that have a distinctly narrower base (e.g. Figs. 2.3.g, 2.12.a); *P. columnaris*-group species are included in the ‘broad’ category (e.g. Fig. 2.9.a). The third category has been subdivided to separate the
pointed from the blunt shapes (e.g. Figs. 2.17.e [2], 2.16.a [3]). Only ‘A.’ *eburnus* from these taxa has a classic ‘araneine’ reflexed scape [4].

12. **Form of the copulatory ducts.** Long [0], short [1] or a pore [2]. Well formed ducts (such as illustrated by Davies, 1988 for ‘A.’ *eburnus* and *L. tabida*) are considered ‘long’ [0]. Some *Poltys* species apparently lack ducts completely, it is assumed there is a pore into the dorsal lobe of the spatheca (e.g. Fig. 2.1.b [2]).

13. **Position of exterior foveal opening.** Posterior [0], dorsal [1] or ventral [2]. This character separates the position of the foveal openings in the *P. columnaris*-group, which are almost on the posterior margin of the epigyne (Fig. 2.1.d), from that in the other Australian *Poltys* species, which are on the posterior plate. This is usually held close against the underside of abdomen when at rest, hence the description of ‘dorsal’ here (e.g. Fig. 2.1.f).

14. **Foveal shape.** Paired pockets [0], paired channels [1], paired scoops [2], single scoop [3]. Foveal shape is a useful character to distinguish between some *Poltys* species. (e.g. Figs. 2.9.i [0], 2.3.b [1], 2.3.h [2], 2.16.d [3]).

**Somatic characters**

15. **Female/male size ratio.** <2x [0] or >2x [1]. (Scharff and Coddington character 2.6.f).

16. **Female ALE position.** Close to PLE [0], equidistant between ALE and AME [1], distinctly closer to AME [2]. All Australian *Poltys* species have widely separated lateral eyes and the ALE is often nearer to the median eyes than to the other lateral eye (e.g. Fig. 2.2.a).
17. **Female eye tubercle.** Absent [0], present, gently convex anteriorly [1],
    anterior extended to a distinct point [2]. Australian *Poltys* species have a distinct eye
    tubercle. This is extended to a blunt point in the *P. columnaris*-group (Fig. 2.7.b).

18. **Modified setae in ocular area.** Absent [0], present [1]. Both Australian *P.
    columnaris*-group species have distinctive setae in three tufts on the eye tubercle (Plate
    2.5.a).

19. **Female carapace profile.** Flat [0], double dome [1]. *Lipocrea tabida* and ‘A.’
    *eburnus* have carapaces that are low or flattened towards the rear. The *Poltys* taxa have
    a peak both anterior and posterior to the fovea (Plate 2.3.c).

20. **Female carapace colour.** Pale [0] or dark [1]. This is one of the few
    reasonably constant areas of colour on *Poltys* females. Species with intermediate
    pigmentation have been included under pale. This contrasts the generally pale or lightly
    pigmented state found in the majority of species with the characteristically very dark
    pigmentation shown by *P. laciniosus* and most Australian specimens from the *P.
    illepidus* group.

21. **Female prolateral cheliceral teeth.** Four [0] or three [1] (Figs. 2.2.m [0],
    2.14.f [1]).

22. **Female sclerotised false eyespots.** Absent [0] or present [1]. These black,
    shiny spots on the dorsal abdomen of *P. columnaris*-group species may resemble false
    eyes (Fig. 2.7.k). They are also found in some other araneid taxa such as some
    *Cyphalonotus* species.

23. **Abdominal angle.** 45 degrees or more to the body. Absent [0] or present [1].
    In most spiders the pedicel joins the abdomen near the anterior so the abdomen is
    carried only slightly inclined from horizontal. In *Poltys* and some other spiders the
joining point is much closer to the spinnerets so the abdomen is angled over the posterior carapace (e.g. Fig. 2.6.a).

24. Abdominal shape. Intraspecific variation in shape. Small [0], great [1]. Most species show a limited amount of variation in abdominal shape. A few, such as some Poltys species (and Caerostris), include much more extreme variants. Here, a clear distinction has been noted between the species in which humeral tubercles may or may not be present and those that have never been observed with this attribute (the P. columnaris-group species and both outgroup taxa).

Leg characters

25. Modified leg macrosetae. Absent [0], present [1] elongate [2]. Several Australian Poltys species may have flattened and/or elongate flattened macrosetae on the patellae and anterior tibiae (e.g. Figs. 2.4.e [1], 2.8.i [2]).

26. Female anterior leg prolateral macrosetae. Normal [0], strong [1]. Poltys species have numerous strong, erectile macrosetae on the prolateral faces of the tibia and metatarsus of legs I and II (e.g. Fig. 2.14.g).

27. Female anterior tibia and metatarsus shape. Normal [0], D-shaped [1]. Poltys species have distinctively angular tibiae and metatarsi in cross section. They are rather flattened dorsally and rounded to triangular ventrally.

Behavioural characters

28. Egg sac shape and placement. Sac-like on a twig [0], camouflaged along a twig [1], or fluffy mass on a leaf [2]. Different Poltys species have several quite distinct
styles of egg sacs (Plate 8.4.b–e). Unfortunately, the structure and placement of ‘A.’
*eburnus* and *L. tabida* egg sacs are not known to me.

5.5. DATA AND ANALYSES—COMBINED DATA SETS

5.5.1. Preparation

Firstly all the data sets were reduced to the species for which ITS2 data were
available. Next the multiple COI sequences that represented polymorphism within
species were compared with the aim of keeping a single representative sequence for
each taxon. It was decided to omit *P. illepidus* southern populations altogether, due to
the questionable status of the population. Polymorphic sites could either be coded as
such, or these bases could be deleted from the data set. This latter option is
recommended by Kitching *et al.* (1998) and is the approach used here. Ten
polymorphic loci were therefore removed. When added to the ITS2 data the remaining
sites give a total of 614 characters from DNA. The DNA data were transcribed from
alphabetic base characters to numerals by simply replacing every ‘A’ with 0, ‘G’ with
1, ‘C’ with 2 and ‘T’ with 3 (this was the order the letters came up in the first taxon).
The 28 morphological characters were then appended giving a total of 642 characters.

5.5.2. Analysis

All characters are unweighted and unordered. The parsimony search strategy
and jackknife test for node support used above was implemented in PAUP®. The
heuristic search was repeated in NONA, and Bremer support was calculated. The
significance of incongruence (*Farris et al.*, 1995) was calculated using the HomPart
command in PAUP® (*Swofford*, 1998) with either 100 (default) or 1000 replications.
5.6. RESULTS

5.6.1. COI data

Maximum Likelihood

The single tree is shown in radial form (Fig. 5.3.a from TREEVIEW Page, 1996) to avoid arbitrary rooting. Poltys laciniosus is the sister taxon to a clade containing P. grayi, P. noblei and P. frenchi. The P. columnaris-group species also form a clade, as do P. illepidus northern and southern populations. Poltys stygius arises between the P. laciniosus and P. illepidus-group clades. Jackknife 50% majority values supporting species-level and higher nodes are superimposed on Fig. 5.3.a: support is strong for the P. columnaris-group clade (92%) and the P. frenchi–P. noblei–P. grayi clade (79%), the broader P. laciniosus association with the latter is less well supported (66%) and the P. laciniosus specimens do not hold together below this level.

Parsimony

The unweighted search produces six trees that differ only in the arrangement of terminals in the P. laciniosus and P. columnaris-groups (Fig. 5.3.b shows tree #5). All six trees have equal character statistics. The arrangement within the P. laciniosus–P. frenchi–P. noblei–P. grayi clade is as found by ML. The only major difference is the position of P. stygius, which is the sister taxon to the P. columnaris group. With transversions weighted x 2 over transitions two trees are obtained (length 195), which only vary in the P. laciniosus terminals and otherwise agree with the unweighted analysis. Figure 5.3.b also shows the jackknife support values for the weighted analysis. Support is strong for the overall P. laciniosus–P. grayi, P. noblei and P.
Figure 5.3. Relationships within *Polystis* as indicated by COI sequences, unrooted trees, jackknife support for species-level and higher nodes superimposed: a, from ML; b, one of six trees from unweighted parsimony, variations all in terminals of *P. laciniosus* and *P. milledgei*. See Appendix 4 for specimen codes.

*frenchi* clade (80%), but rather weak for the *P. columnaris*-group and the position of *P. stygius*. In the weighted jackknife (not shown) support for all clades is increased but the specific-level support for *P. illepidus* and *P. laciniosus* is slightly lower, although still over 80%.
5.6.2. ITS2 data

Maximum Likelihood. (Fig. 5.4.a, includes jackknife support values).

*Pollys* is found to be monophyletic with respect to the selected outgroup taxa. *P. frenchi* is the sister taxon to *P. laciniosus* and *P. grayi*; *P. jujorum* is the sister to *P. illepidus* and *P. stygius*. Jackknife support is strong for the *P. frenchi–P. laciniosus* group clade (95%) and for *P. illepidus–P. stygius* (94%) but weak for the association of *P. jujorum* with the latter (54%), and for the monophyly of *Pollys* (52%).

![Phylogenetic tree](image-url)

**Figure 5.4.** Relationships within *Pollys* as indicated by ITS2 sequences, with jackknife support superimposed and tree statistics inset: a, ML; b, parsimony. See Appendix 4 for specimen codes.
Figure 5.5. Relationships within Poltys: a, as indicated by morphology and behaviour characters, one of two trees, other unresolved within the *P. laciniosus*-group; jackknife support superimposed and tree statistics inset. See text for character descriptions. Symbols indicate type of change, ■ = autapomorphy, ● = non homoplasious synapomorphy, ○ = homoplasious synapomorphy; b, as indicated by combined data set, with jackknife and Bremer support superimposed.

Parsimony

A single tree with the same conformation as produced by ML (Fig. 5.4.b). Support is strong for both major clades and also the monophyly of Poltys (97%).
5.6.3. Morphological data

Unweighted and unordered parsimony analysis gives two trees with identical character statistics, one fully resolved (Fig. 5.5.a) and one without resolution within the P. laciniosus-group. Poltys frenchi is sister to the three P. laciniosus-group taxa; the P. illepidus-group is sister to this clade and the P. columnaris-group is sister to all. Poltys is monophyletic. The same result is produced by all the programs used. Jackknife support is strong for all species-groups and the monophyly of Poltys, although slightly weaker (71%) for the inclusion of P. frenchi in a clade with the P. laciniosus-group.

5.6.4. Combined data

PAUP* and NONA both find a single tree (Fig. 5.5.b, includes jackknife and Bremer support values), length 369 steps, which has the same arrangement as that derived from ITS2 data. Jackknife support for all nodes is above 70%, although barely so for the P. frenchi–P. laciniosus-group. Bremer support is minimal for this clade and also low for P. jujorum–P. illepidus-group, and moderate for the P. laciniosus-group clade. Support is highest for the monophyly of Poltys and also the P. illepidus-group clade.

The test of incongruence suggests that these data should probably not, in fact, be combined. The probability of error due to incongruence is only significant at 10% for 100 replications, but rises to a highly significant 1% for 1000 replications.

5.7. DISCUSSION

The separate data sets give largely incongruent results, and it is not surprising that the test of incongruence should be significant. Nevertheless, both of the DNA data
sets strongly indicate that, despite its intermediate morphological characters, *P. frenchi* is probably more closely related to the *P. laciniosus*-group than was assumed in the loose division into species groups presented in the taxonomic section of this paper. The monophyly of this clade is reasonably well supported, at least by DNA data, but there is no firm consensus as to the relationships within it, or on a likely sister group within the Australian *Poltys*.

Of the three components comprising the combined data, the ITS2 data set originally contained the greatest number of informative characters, although only a few more than the COI data set. The morphological data set has only about half as many informative characters as the COI. How many from each component set are informative when combined has not been assessed, but the overall total is a smaller number than the sum from the separate parts. The resulting phylogram is similar to that from ITS2, the numerically dominant source of data but the branch-lengths in different areas of the tree are much more equal (Fig. 5.5.b).

The initial hypothesis of relationships was based on the apparent intermediacy of certain character pairs. For instance, the presence of a *P. illepidus*-like terminal apophysis in the male palp is combined with an elongate, prolaterally-based embolus, the long epigyne is spade-shaped, and the rather light build is combined with a broad low carapace: all these are combinations of states from the two groups. In addition, although *P. frenchi* has the usual four prolateral cheliceral teeth, there is a clear tendency towards reduction seen in *P. timmeh*, from New Caledonia, which is polymorphic for this character but otherwise appears to be most closely related to *P. frenchi*. 

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The morphological data set strongly supports the monophyly of the *P. laciniosus*-group. Within the group the arrangement of taxa is not supported by jackknife resampling, and must be considered unresolved at present. Three of the four morphological synapomorphies supporting the grouping are well-defined and may be strong, namely the lack of a terminal apophysis, the long, wire-like embolus and the form of the egg sacs. Character 21, reduction of the cheliceral tooth pattern from four to three, is less robust, as occasional exceptions have been noted. At the next node support for *P. frenchi* as a sister taxon is only provided by the synapomorphies of embolus base position and paramedian apophysis becoming a textured plate. Whilst both may be good characters, the form of the PM is actually rather broadly defined, as the texturing is different in *P. frenchi* compared to the other three (c.f. Plates 2.5.e and 2.6.b). Weakness in the morphological analysis at this level is inevitable, given that the basis for apparent intermediacy in form uses the same interpretation of morphological characters as that on which the data set is based. The two DNA data sets, in contrast, are independent from visible characters. It may therefore be significant that they mostly give much stronger support for a monophyletic clade containing *P. frenchi* and the *P. laciniosus*-group, despite offering conflicting and/or poorly supported resolution within this clade. Note however, that the rooting points of the COI cladograms are not defined. Other potential sources of error, or misinterpretation, in DNA data stem from the degree of saturation with regard to transitions at base 3 in COI, and sensitivity to the precise alignment between sequences in ITS2.

Apart from the *P. frenchi* clade, there is no overall agreement on other relationships between species groups. Morphological characters support the monophyly of the *P. frenchi* clade and the *P. illepidus*-group, whilst ITS2 and combined results
indicate that all other Australian Pollys are the sister group to the *P. frenchi* clade. This latter result, and the general level of conflict between the separate data sets, might suggest that the closest relatives of the *P. frenchi* clade may not be represented in the Australian fauna. The original hypothesis postulated that the *Pollys laciniosus*-group was related to *P. frenchi* and these taxa collectively were most closely related to the *P. illepidus*-group. Whilst the hypothesis is not disproved, the consensus data only support half of the argument.
CHAPTER 6
FIELD STUDIES

6.1. INTRODUCTION AND AIMS

This chapter describes field studies that document aspects of the biology and behaviour of Poltys noblei in bushland situations in the Sydney region. The overall goal is to begin to understand some of the ways in which these spiders have adapted behaviourally to exploit this habitat.

The first study investigates web-site tenacity. It stemmed from initial observations that indicated that some specimens of Poltys noblei appeared to use the same web-site for an unusually long period of time. The survival of a nocturnally active spider such as this is dependent on its finding a suitable hiding place during the day. The usual diurnal microhabitat of Poltys is bare, dead twigs on standing or fallen vegetation, a structural element that the spiders’ body shape and patterning seems to mimic. Nevertheless, this microhabitat is extremely exposed and thus a good match to the substrate is critical. I suggest that that this might impose an extra constraint on these spiders to remain in a known, safe site for longer than might otherwise be normal for orb-weaving spiders.

The second study aimed to examine the cryptic hiding positions taken up during the day by P. noblei of various sizes. I hypothesize that larger spiders should present their best camouflaged aspect towards the local source of light, as this is the direction from which potential predators can see best when approaching. Unfortunately, this study could not be completed but the data collected are presented as a preliminary exploration or pilot study, which could be used as a basis for future work.
6.2. WEB-SITE TENACITY

6.2.1. Background

Predator-prey interactions have long been of interest in ecological studies as evidenced by the sections and references in many standard ecology text books (e.g. Ricklefs, 1980) as well as more focused reviews (e.g. New, 1991). Optimal foraging theory, which developed rapidly during the 1970s and early 1980s, suggests that predators should optimise their hunting techniques to the most energy efficient method (reviewed by Pyke, 1984). Arising from these considerations, the marginal value theorem predicts that foragers should move on from a patch when the success rate falls to the average success rate for the environment as a whole (Charnov, 1976). Two assumptions were made in Charnov’s analysis: firstly that the forager actually depletes an area of prey; secondly, that the forager has some method of assessing the current patch against past experience. Spiders from a number of families have been used as models for studies of predator-prey interactions, both as the predator (e.g. Olive 1982, Janetos, 1982a) and as the prey (e.g. Rypstra, 1984; Wise and Chen 1999). In particular, orb weaving spiders have proven useful subjects. Orb-weavers:

(i) are easily located in the field;

(ii) can easily be kept and experimentally manipulated in the laboratory;

(iii) foraging investment can be calculated from the amount of silk used in the orb web, which is used to catch prey;

(iv) mostly renew their webs on a daily basis so differences in shape or investment between old and new webs can reflect factors operating on the spider almost in real time;
(v) are reasonably sedentary but not entirely so, moving within a useful working time-frame.

These characteristics make orb-weaving spiders ideal subjects for examining the predictions of the marginal value theorem, albeit with modifications to allow for the assumptions made on both prey depletion and memory of past experiences (Janetos 1982, a, b). The use of memory by spiders has since received some attention with quite different results reported in different taxa, which would make neither the original assumptions, nor Janetos’ modified version necessarily applicable in many cases (Vollrath, 1985; Vollrath and Houston, 1986; Nakata and Ushimaru, 1999; Nakata et al., 2003).

Of course, in reality there are many factors that may affect the suitability of any particular web-site for any particular spider (Riechert and Gillespie, 1986), so it is likely that the decision to move on to a new site comes from a complex combination of stimuli of which foraging success makes up only a part. Nevertheless, many studies have attempted to tease out the effects of one or more factors. Prey availability (e.g. McNutt and Rypstra, 1997; Bradley, 1993), the age of the spider and moulting (Enders, 1975), habitat suitability (Hodge, 1987a), web destruction and habitat disturbance (Enders, 1976), moving costs (Jakob et al., 2001) and conspecific interactions (Smallwood, 1993) have all been shown to have effects in at least some species.

Part of the interest in orb-weaving spiders is that, although the web represents a significant investment of resources on a regular basis, many species ingest all, or most, of the web before either renewing it in the same location or moving on to a new site. Therefore, the proteins are recycled and so the moving costs in terms of lost silk resources are minimal. Except for the loss of time that might otherwise be available for
foraging, it can be argued that provided other known stimuli can be discounted in some way (for instance by keeping spiders in a uniform cage habitat) the choice to relocate a web is purely a matter of foraging parameters. Even without taking other factors into account, Janetos (1982a) demonstrated that a model derived from the ideas of marginal value theorem could be meaningful in a field situation. He compared an “orbweaving guild” of mixed species that recycle their webs, with a “sheetweaving guild” of several linyphiid species that abandon their webs when they relocate. The orb-weaving guild were found to spend either less or more time than expected at any particular web-site, when compared against a random hypothesis. This was interpreted as evidence that behavioural mechanisms were the primary factors influencing web-site tenacity. The sheet-weavers, however, had a distribution of residence times close to random, indicating that random environmental factors or disturbance were more likely the dominant factors causing the relocations of these species.

Different aspects of the risks of not finding good sites in terms of prey abundance have been addressed both implicitly in the models already discussed and explicitly (occasionally rather controversially) in a number of other studies (Olive, 1982; Gillespie and Caraco, 1987; Schuck-Paim and Alonso, 2001; Nakata and Ushimaru, 2004). In contrast, the cost of moving sites in terms of increased predation risk has rarely been addressed. In a notable exception, Nephila clavipes (Linnaeus) was shown to be unexpectedly averse to moving away from prey-poor habitats and appeared to tend towards a random distribution of residence times (Vollrath, 1985; Vollrath and Houston, 1986). It is postulated that in this species, the costs of suboptimal growth resulting in a smaller size at maturity and therefore lowered fecundity are less than the costs due to predation risk when moving to a new web-site.
Other species of *Nephila* Leach are recorded as recycling their webs prior to moving sites (Miyashita, 2005), so in this material respect the cost of moving should be negligible and it might be expected that *Nephila* should tend towards the movement patterns shown for other orb-weavers (Janetos 1982a; Hodge, 1987a, b; Nakata and Ushimaru, 1999). Although web recycling behaviour is not explicitly stated for *N. clavipes*, the important factor in this case is suggested to be the time taken to establish an effective barrier web at a new site (Vollrath, 1985). The barrier web is probably an important safety feature for the spider, which would make a substantial meal for a bird. This interpretation of risk avoidance in *N. clavipes*, which is facultatively variable in growth rate and size (Vollrath 1985), is supported by recent work on two other *Nephila* species, *N. pilipes* (Fabricius) (as *N. maculata*) and *N. clavata* L. Koch (Miyashita, 2005). Miyashita demonstrated that the larger and faster growing *N. pilipes* is much more likely to risk moving in search of a high quality web-site than the smaller and slower growing *N. clavata*. Thus the life history strategy of a species is also demonstrated to affect risk taking and web-site tenacity.

All of the species examined in the studies discussed above are diurnal species. Janetos (1982a) does not state whether the orb-weavers he studied are at the hub of the web during the day or monitor the web from a retreat, but all the other species spend the day at the hub of their webs. The only species amongst these for which camouflage may play a part is *Cyclosa octotuberculata* Karsch, which hides amongst the debris that it builds in across the centre of the web (Nakata and Ushimaru, 2004). The spider takes this debris with it when it relocates, however, so there is no risk associated with lack of camouflage at a new web-site. Nocturnally active orb-weaving spiders, for which camouflage during the day might be important, are almost unrepresented in web-site
tenacity studies. An exception involves two studies on *Tetragnatha elongata* Walckenaer (Tetragnathidae) (Gillespie and Caraco, 1987; Smallwood, 1993). Gillespie and Caraco (1987) found an extreme difference between the relocation patterns of two populations of spiders in habitats with different prey levels. This they attributed to a “risk sensitive foraging strategy”. Smallwood (1993), however, examining one of the same populations of spiders, suggested instead that the effect observed was due to strong intraspecific competition when spiders were present at a high density in favourable habitat. Notwithstanding, the residence times reported by Gillespie and Caraco (1987) at low spider densities are longer than any of those reported in the studies above except for *Nephila clavipes* (Vollrath, 1985) (Table 6.1). Gillespie and Caraco (1987) did not examine the fit of the distribution of residence times to a random hypothesis, and the times reported by Smallwood (1993) at the same location a year later were rather shorter. Other evidence that nocturnal spiders may relocate less frequently than diurnal species can be gleaned from anecdotal sources. Main (1976) and Forster and Forster (1999) discuss nocturnal araneids that apparently use the same resting position and/or web-site for several months. Both discuss spiders that appeared to change colour over a prolonged period so that they ultimately matched the background colour of their day-time resting positions. Main (1976, p. 195) is not specific, discussing orb-weaving spiders in several situations in her garden; the implication that they stayed in the same position is my interpretation. Forster and Forster (1999, pp. 152–153), however, report that a particular *Eriophora pustulosa* (Walckenaer) stayed for six months in a position under the eaves of their house. Like the reliance of *Nephila* on its barrier web, it is worth considering whether the costs of
moving for a nocturnally active spider may be higher if moving carries a significant risk of reducing the effectiveness of its camouflage by day.

Table 6.1 has been compiled as an aide to visualising the range of residence times reported by the various studies discussed above. The format used by different authors varies: some report the mean length of residence, others report the rate of turnover per day, whilst others use the percentage that stay (web-site tenacity in the original sense used by Enders (1973), see below). This makes a straight comparison rather difficult, but in this table all Enders’ figures and turnover ratios have been converted to turnover as a percentage, reducing the format to two types of listing. Many studies do not report the longest residence times recorded, especially those that measure turnover. Nevertheless, this provides some useful baseline data against which a nocturnal and cryptic spider, such as Poltys, can be evaluated.

“Web-site tenacity” as defined by Enders (1973) is, “the percentage probability that, once it has built a web, the spider will remain at the same web-site from one day to the next”. Enders’ precise usage has not usually been followed in later studies. Here, I use the term merely to mean the period of residence, expressed in whatever way is relevant to the context at the time.

6.2.2. Methods

6.2.2.1. Pilot study

Initially a pilot study was conducted to assess the feasibility of monitoring these animals. Transects along tracks at two sites in the Ku-ring-gai National Park north of Sydney (Kalkari and Myall, Fig. 6.1.a; Plate 6.1.a) were walked throughout the night for 8 consecutive nights, recording the positions and activities of Poltys specimens
Table 6.1. Recorded web-site tenacity for various orb-weaving spider species. Entries are approximately ordered shortest (top) to longest (bottom).

<table>
<thead>
<tr>
<th>Species or guild</th>
<th>Average residency / % of spiders moving per day</th>
<th>Longest recorded residency period</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micrathena gracilis</em></td>
<td>1 day</td>
<td></td>
<td>Hodge, 1987a</td>
<td>released into suboptimal habitat</td>
</tr>
<tr>
<td>(Walckenaer)</td>
<td></td>
<td></td>
<td></td>
<td>when in habitat with high average prey availability; nocturnally active only results averaged over 2 years</td>
</tr>
<tr>
<td><em>Tetragenatha elongata</em></td>
<td>1.3 days</td>
<td></td>
<td>Gillepsie &amp; Caraco, 1987</td>
<td></td>
</tr>
<tr>
<td>Diurnal orb-weaving guild</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cyclosa argenteovalba</em></td>
<td>14.1% to 31.7% move</td>
<td>≥8 days</td>
<td>Nakata &amp; Ushimarau, 1999</td>
<td>“natural” relocation rates from familiar and unfamiliar sites respectively</td>
</tr>
<tr>
<td><em>Micratha elongata</em></td>
<td>2.3 days</td>
<td></td>
<td>Janetos, 1982a</td>
<td></td>
</tr>
<tr>
<td><em>Argiope trifasciata</em></td>
<td>4.5 days</td>
<td>≥11 days</td>
<td>Olive, 1982</td>
<td>averaged for all different feeding levels same habitat as Gillepsie &amp; Caraco, 1987, poor quality</td>
</tr>
<tr>
<td>(Forskål)</td>
<td></td>
<td></td>
<td></td>
<td>when in natural habitat released into natural habitat control replicates</td>
</tr>
<tr>
<td><em>Tetragenatha elongata</em></td>
<td>4 to 6 nights</td>
<td></td>
<td>Smallwood, 1993</td>
<td></td>
</tr>
<tr>
<td><em>Micrathena gracilis</em></td>
<td>6.7 days</td>
<td>“weeks”</td>
<td>Hodge, 1987b</td>
<td></td>
</tr>
<tr>
<td><em>Micratha elongata</em></td>
<td>8 days</td>
<td></td>
<td>Hodge, 1987a</td>
<td></td>
</tr>
<tr>
<td><em>Argiope trifasciata</em></td>
<td>8.7 days</td>
<td></td>
<td>McNett &amp; Rypstra, 1997</td>
<td></td>
</tr>
<tr>
<td><em>Argiope trifasciata</em></td>
<td>12.5 days</td>
<td></td>
<td>McNett &amp; Rypstra, 1997</td>
<td>fed (prey supplemented) replicates</td>
</tr>
<tr>
<td><em>Argiope trifasciata</em></td>
<td>1 to 30% move</td>
<td></td>
<td>Enders, 1975</td>
<td>web destruction or control regimes</td>
</tr>
<tr>
<td><em>Argiope aurantia</em></td>
<td>12 to 50% move</td>
<td></td>
<td>Enders, 1976</td>
<td>undisturbed but different feeding regimes</td>
</tr>
<tr>
<td><em>Argiope aurantia</em></td>
<td>10 to 13% move</td>
<td></td>
<td>Enders, 1976</td>
<td>younger animals move more frequently (field results); web site tenacity reduced post molting</td>
</tr>
<tr>
<td><em>Argiope aurantia</em></td>
<td>7 to 38% move</td>
<td></td>
<td>Enders, 1975</td>
<td>juveniles</td>
</tr>
<tr>
<td><em>Cyclosa octotuberculata</em></td>
<td>5.9% move</td>
<td></td>
<td>Nakata &amp; Ushimarau, 2004</td>
<td></td>
</tr>
<tr>
<td><em>Cyclosa octotuberculata</em></td>
<td>3.8% move</td>
<td></td>
<td>Nakata &amp; Ushimarau, 2004</td>
<td>adult females</td>
</tr>
<tr>
<td><em>Nephila clavipes</em></td>
<td>16 days</td>
<td></td>
<td>Vollrath, 1985</td>
<td>when in prey poor habitat</td>
</tr>
<tr>
<td><em>Tetragenatha elongata</em></td>
<td>17.9 nights</td>
<td></td>
<td>Gillepsie &amp; Caraco, 1987</td>
<td>when in habitat with low average prey availability; spiders also at hub during day</td>
</tr>
<tr>
<td><em>Nephila clavipes</em></td>
<td>≥42 days</td>
<td></td>
<td>Vollrath, 1985</td>
<td>when in prey rich habitat</td>
</tr>
</tbody>
</table>
on each pass, as well as web damage. These results indicated that:

(i) many spiders use the same, or a closely adjacent, web-site night after night.

Following individuals at regular intervals from sunset to sunrise minimised the possibility that spiders were swapping sites without my knowledge;

(ii) the likelihood of a similarly sized and shaped spider moving into a vacated web-site soon after departure of the first was low unless there was a high local density. Therefore, with the exception of bushes where several similarly shaped and sized spiders are interacting, it is possible to monitor spiders without marking individuals;

(iii) eighty-seven percent of records were open-ended, i.e. with one or both of the arrival or departure dates outside the monitoring period (Fig. 6.2). Therefore a much longer time scale would be necessary to adequately assess spider movements.

6.2.2.2. The regular long-term transect

A study site was established along the Waitara Creek fire trail, a remnant of urban bushland connected to the Berowra Valley Regional Park, between Hornsby and Normanhurst in the northern fringes of Sydney (Fig. 6.1.a). This was walked at approximately 7–10 day intervals from April 2002 to April 2004 (112 transects at an average of 8.49 day intervals) and then observations were continued on just a few selected spiders until the last had disappeared in late November 2004. In addition, during the time of the full transect, an additional period of overnight monitoring similar to the pilot study was conducted. This reaffirmed that the individual spiders being monitored were not moving between sites, and that approximately weekly transects were unlikely to have a significant proportion of errors due to translocations.
Figure 6.1. Maps of transects: a. the locations of all transects; b. the Waitara Creek transect route.
Figure 6.2. Pilot study results for Myall and Kalkari transects. The length of each bar represents the presence of a particular spider through one or more nights.

The full length of the transect (Fig. 6.1.b) was approximately 400 m, but this was not all included for the whole period of study. One short section, a loop around a clearing, was only included for part of the period and an extra section was added to the far end in late September 2002 when several easily observable specimens were noted.
there. The topography of the transect is rather mixed. The first section (approx 100 m) is approximately level up to and including the clearing, then the track descends, at first steeply, then more gently, towards Waitara Creek (at 360 m). After crossing the creek there is the short relatively open section (to ca 400 m), which was included in the transect after September 2002. The vegetation throughout is a patchy mix of natives and weeds. After an initial open weedy stretch, the vegetation along the top section (Plate 6.1.b) and down the slope (Plate 6.1.c) is Allocasuarina littoralis with native understory including Dwarf Apple (Angophora hispida) and Banksia species. Emergent trees are Sydney Peppermint (Eucalyptus piperita) and Smooth-barked Apple (Angophora costata). All of these frequently have dead low branches, which Poltys use. Spiderlings and small specimens also use denser shrubs. Around the clearing are native shrubs such as Banksia ericifolia, Dwarf Apple, and a quite diverse lower herb layer to the east, but mostly weeds along the western edge. The lowest section near the creek is mainly introduced garden species (there was a dwelling in this area 30–40 years ago) and weeds (Plate 6.1.d, after rain in July 2005). A settlement pond and weir partially block the flow of the creek making this area rather more humid than the slope and top stretch. Poltys noblei usually prefer the drier areas but during this drought period also thrived in this lower area. Night temperatures were typically one to several degrees centigrade cooler down in this valley.

Each transect was started at least one hour after dark, later if possible, ideally on a night with suitable weather conditions for locating spiders in webs. Occasionally, in periods of bad weather, the transect was split over two nights if interrupted by rain during the first night. Each Poltys seen along the route was noted if its position was such that a reliable visual identification to genus could be made (only P. noblei has
been recorded from this area). Hence larger spiders were in general identifiable at a
greater height and distance from the path than smaller ones. The only other genera that
might be confused with Poltys in this area, Heurodes (when hanging on a line) and
Carepalxis L. Koch (in a web or on a line) have rarely been recorded here, and
certainly not frequently enough to significantly affect the accuracy of the analysis. The
approximate size, shape (if distinctive) and position were noted, as well as the activity
of the specimen. The temperature was noted at the beginning and turn-around point and
again on return to the start. Because of public access to the area and not wishing to
draw the attention of potential bird predators to the locations of spiders, web locations
were not marked and I avoided seeking out the specimens during the day (although a
few were easily visible, which allowed further confirmation that the same specimen
was using the site throughout the putative period of residence). Instead, web locations
were described or sketched in relation to vegetation features. Overall this worked well,
especially as I soon became familiar with the vegetation along the track. No attempt
was made to mark specimens as the majority were too small and most were out of
reach. The size of each specimen was estimated by eye in terms of a size class, usually
from a distance. Hence the size ranges used in the analysis are approximate. Slight
changes in web-site (up to about 20–30 cm for a small spider or 50–100 cm for a larger
specimen) were noted but were not considered moves unless there were other reasons
to suspect that the specimen in the new site was not the original, or that the specimen
was now using a different resting position.
6.2.2.3. Data analyses

The weekly data were collected in note form and regularly transcribed to a
pictorial notation. Tabular data were subsequently extracted from the pictorial record.
Both versions are presented in Appendices 11 and 12 because the pictorial record
provides a unique overview of the individual spiders and their interactions. Three
analyses are carried out using these data.

Web-site tenacity

The sample mean and standard deviation were calculated from the persistence
times for all spiders with unambiguous records (these data are printed in black in
Appendix 11). This excluded specimens for which the moving in date was unknown
(i.e. they were already present on the first night of the transect), or for which there was
possible confusion with one or more neighbouring spiders. The records were next
separated into two size classes based on the field estimates, small and (medium +
large). As discussed above, these are approximate but the rationale is as follows. The
division is at the point where males mature and thereafter leave the record, as they
cease making webs and become mobile (abdominal height approximately 1.5–2.0 mm).
Also, at this size female abdomens start to differentiate in shape and camouflage may
begin to play a more important role. Therefore, the larger class comprises only females.
The divisions are not ideal, as subadult males may have different movement patterns to
juvenile females of similar size. Unfortunately, it was not possible to identify juvenile
males in the field, so the resulting small class contains two sub-classes with potentially
heterogenous movement patterns. Adult females may well also have different
requirements to juveniles but there were too few observations of adult females to allow
separation of these either. Some spiders grow from one class into the next whilst resident at a single web-site. The class used here is the size at arrival.

The distribution of residence times of small spiders was compared against those of the (medium + large) class using the $\chi^2$ test on contingency tables and pooling most columns with expected values $< 5$ (it is not necessary to remove all expected values less than 5 (Parker, 1979)). All subsequent tests used the two size classes separately. Finally, the sample mean and standard deviation were calculated for each size class.

Next, the recorded web-site tenacity was compared with a random hypothesis. I attempted to follow the methods of Janetos (1982a) and Hodge (1987a, b) for generating an expected distribution of residence times. This is based on the expectation that compounded random events such as web damage or disturbance by a predator should result in spider movement events that can be explained by a Poisson process (Janetos, 1982a). A negative exponential series was generated for each size class (using the ‘expondist’ function in Microsoft Excel), which models the expected distribution of spider movement events over time according to this random hypothesis. This distribution of class frequencies was then compared with that collated from the recorded data. The estimation of the constant $\lambda$, which describes the probability density function ($y = \lambda e^{-\lambda x}$), was problematic as neither Janetos nor Hodge report on how this was estimated in their studies.

Two methods for estimating $\lambda$ were tested:

(i) based on the whole observed distribution without pooling small classes;

(ii) based on the distribution with classes in the tail of the distribution pooled.

In both cases, $\lambda$ was varied until the $\chi^2$ value was minimised, signifying the closest fit of the estimated series to the observed data. Method (i) was found to be
unsatisfactory because the value of $\lambda$ cannot be consistently applied to both the estimation of small expected values and the overall distribution. Method (ii) risks losing potential information due to the pooling of tail classes. Method (ii) was chosen for the analyses presented here for two reasons. Firstly, based on their figured frequency distributions, this is most likely to be the method used by the previous authors. Secondly, it provides a more conservative estimate of significant deviation from the random hypothesis than that provided by Method (i). This conservatism seems to be justified given the problems with this method.

For each size class the final class of the negative exponential distribution was effectively pooled to infinity so that $N_{\text{exp.series}} = N_{\text{observations}}$. The degrees of freedom are the number of classes minus 2 because two parameters of the expected series are derived from the observed values. [The negative exponential probability series is described by $y = a e^{-bx}$, where $a$ and $b$ are two constants, but in this special case $a = b = \lambda$. The other observed value used is $N_{\text{observations}}$.]

**Population dynamics**

These data are presented primarily to give an overview of the status of the *Poltys* population during the course of the transect study. It should be kept in mind that the transect route is not a closed system and there is no way to accurately assess immigration, emigration, natality or mortality. The figures were derived from a direct count of the spiders observed at each transect period, as shown in the pictorial Appendix 11, and smoothed by a three-period rolling average. Spiders that were not seen were not counted, even though they may have been considered to be present right through a period in the web-site tenacity analysis. The size bars in the source data set
indicate the approximate sizes of the specimens, and the size classes used for this figure are explained on the final page of the appendix. Because the smallest size class covers the whole range where both males and females may be present, this class has been halved for this figure, leaving an estimate for females alone to allow direct comparison with the numbers in the larger size classes. The ratio of males to females has not been examined in *P. noblei*. It appears to be approximately 1:1 at hatching in the species that have been reared from egg sacs (e.g. *P. grayi*, see Chapter 7). Thus, reducing this size class by half is probably an accurate estimate for females in the initial spiderling emergence ‘spikes’, but as the year progresses, the remaining small spiders may well become biased towards males, which may be able to delay maturity until periods when females are likely to be available. The numbers of small spiders shown as females at this time may therefore be an overestimate. The recorded rainfall at Berowra, approximately 14 km N of Hornsby (Bureau of Meteorology, 2002–2004), and the average temperature recorded during each transect provide background environmental information.

**Seasonality of spider movements**

Relatively few spiders are active in winter, but as there is also a drop in the number of spiders recorded at this time, it is difficult to assess whether the proportion of spiders that move at this time is actually lower. Similarly, the emergence of spiderlings in late summer provides a large number of new arrivals, which obscure real movement patterns between classes. Some method of assessing the underlying activity of spiders on a seasonal basis is needed to gain any insight into the timing of movements in each size class.
Specimen data were extracted for each residency—the season a spider moved in to a position and its size, the seasons during which it was resident at a position and the season and size at which it moved on. If a spider had grown between size-classes during its period of residence, the original data were checked to separate the seasons in which the two different size classes were present. For each kind of move, in or out, and for each size class of spider, the total number of moves per season was calculated as a proportion of the numbers of spiders in that class recorded during the season. The size classes for this purpose were small (as described previously), large (subadult and adult females) and medium (everything in between). See the end of Appendix 11 for how these sizes are depicted.

6.2.3. Results

For the complete data set the sample mean is 4.80 recording periods (40.75 days), SD = 5.57, N = 218. The longest recorded residence is 31 recording periods (263 days) (Fig. 6.3.a). When small and (medium + large) residence times are compared using a contingency table (Table 6.2) the distribution of residence times is found to be significantly different between the two size classes ($\chi^2 = 18.09, 0.025 > p > 0.01$). The mean stays are 3.77 sampling periods, (32 days), for small spiders (SD = 4.51, N = 138), and 6.59 sampling periods (56 days), for (medium + large) spiders (SD = 6.69, N = 80).

The distribution of residence times of small spiders is weakly significantly different to the random hypothesis ($0.05 > p > 0.025$) (Fig. 6.3.b). This is not the case for the (medium + large) spiders, the distribution of residency times for these is not significantly different to random ($0.5 > p > 0.1$) (Fig. 6.3.c).
Table 6.2. Contingency table to compare residency time classes between small and (medium + large) spider size classes.

<table>
<thead>
<tr>
<th>Residence class</th>
<th>Size class</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5−7</th>
<th>8−9</th>
<th>10−13</th>
<th>14−</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>Observed</td>
<td>52</td>
<td>28</td>
<td>17</td>
<td>10</td>
<td>12</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Expected</td>
<td>44.94</td>
<td>24.06</td>
<td>14.56</td>
<td>10.13</td>
<td>14.56</td>
<td>12.03</td>
<td>8.23</td>
<td>9.50</td>
<td></td>
</tr>
<tr>
<td>M+L</td>
<td>Observed</td>
<td>19</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>12</td>
<td>8</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Expected</td>
<td>26.06</td>
<td>13.94</td>
<td>8.44</td>
<td>5.87</td>
<td>8.44</td>
<td>6.97</td>
<td>4.77</td>
<td>5.50</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>71</td>
<td>38</td>
<td>23</td>
<td>16</td>
<td>23</td>
<td>19</td>
<td>13</td>
<td>15</td>
<td>218</td>
</tr>
</tbody>
</table>

χ² = 18.09, 0.025 > p > 0.01, 7 df.

These data sets are of course incomplete due to two major omissions. The first of these is the number of spiders that have been missed because they both arrived and left between samples. The second is not counting the spiders that were affected by the actions of conspecifics. The magnitude of the first omission could be estimated from the Pilot Study figures, where 13% both arrived and left within 8-nights. This figure is itself suspect, however, partly because it is likely that some spiders were already present but not noticed until they built a web, and partly because of variations in the seasonality of movements. An easier method is to assume that the spiders currently in the first column are 1/8.49th of those that should be there. The figure 8.49 is the average number of days between sampling events. This would suggest that about 440 small spiders have been missed between sampling periods, and 160 (medium + large) specimens. Using these figures to calculate new mean residence periods, the stays become 1.66 recording periods (14.1 days) for small spiders (N = 578) and 2.86 recording periods (24.3 days) for medium + large spiders (N = 240).
Figure 6.3. The frequency of occurrence of spider tenancy periods: a. small compared to (medium + large) spiders; b. histogram of residency periods of small spiders (tail values pooled) compared to a random hypothesis; c. ditto for (medium + large) spiders. See text for details.
The second omission in numbers is all the spiders that were excluded from the web-site tenacity calculations because they were aggregated and could not be followed individually without marking. These spiders tend to be unusually mobile due to increased interactions with neighbouring conspecifics (Smallwood 1993; pers. obs.). It is not possible to estimate these interactions, which vary between unique encounters and several times per night per spider (personal observations), but it is likely that these would account for many additional moves. These interactions have not always been included in other studies, however, so for comparison between studies their inclusion here would not necessarily be essential.

**Tables 6.3.a and b.** The percentages of spiders which move on a seasonal basis, classed by spider size. Table a, spiders beginning residency (moving in); Table b, spiders ending residency (moving out).

### a.

<table>
<thead>
<tr>
<th>Spider size class</th>
<th>By season (Moving IN) %</th>
<th>Overall % (all seasons)</th>
<th>No. of spiders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>Large</td>
<td>100</td>
<td>83</td>
<td>69</td>
</tr>
<tr>
<td>Medium</td>
<td>30</td>
<td>63</td>
<td>87</td>
</tr>
<tr>
<td>Small</td>
<td>45</td>
<td>62</td>
<td>100</td>
</tr>
<tr>
<td>All spiders</td>
<td>42</td>
<td>67</td>
<td>95</td>
</tr>
</tbody>
</table>

### b.

<table>
<thead>
<tr>
<th>Spider size class</th>
<th>By season (Moving OUT) %</th>
<th>Overall % (all seasons)</th>
<th>No. of spiders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>Large</td>
<td>50</td>
<td>86</td>
<td>93</td>
</tr>
<tr>
<td>Medium</td>
<td>68</td>
<td>88</td>
<td>77</td>
</tr>
<tr>
<td>Small</td>
<td>56</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>All spiders</td>
<td>62</td>
<td>92</td>
<td>85</td>
</tr>
</tbody>
</table>

Note for table: the number of spiders in size classes differs due to specimens which grow from one size class to another during their period of residence. Large = adult plus subadult females; Medium = all other juvenile females; Small = juvenile males and females too small to sex (up to about 3 moult(s)).
Figure 6.4. The Waitara Creek transect through time. a, the population of female spiders in different size classes recorded at each transect period (smoothed curve from 3-period rolling averages); b, rainfall, measured at Berowra, approximately 14km North of Hornsby (Bureau of Meteorology, 2002–2004); c, air temperature averaged between top (start), bottom (turnaround point) and end (return to start) of each transect.

The population trends over the two years of the transect records (Fig. 6.4.a) show that there may be juvenile female spiders of all sizes to be found throughout the year (but see the “Methods” section for a caveat on the possibility of the small
specimens all being juvenile males in spring). The spiderling emergence peaks stand out (the very large peak in the second summer was probably due to the emergence of two egg sacs laid by females close to the track), but most other peaks and troughs roughly correspond to variations in temperature (Fig. 6.4.c), rainfall (Fig. 6.4.b) or both. It is noticeable that there is a trough each spring and early summer where the numbers of medium-large spiders are decreasing, but the numbers of adult females do not increase to the same degree. This may be an indication of predation pressure at this time of year. Other than that, the population sizes appear remarkably stable through 2003, when there was moderate rainfall on a regular basis. In contrast, numbers seem to have been declining through the autumn of 2002, during a severe period of the drought.

The percentages of spiders beginning or terminating residencies are shown in Tables 6.3.a, b. The large and medium-large class contains 23 and 29 spiders respectively, but \( \leq 3 \) of these are found in the autumn or winter seasons and so these percentage figures need to be treated with caution. In addition, all or most adult females die during summer or autumn, so Table 6.3.b, reflects this high exit rate. To avoid these biases, Figures 6.5.a, b, only include movement percentages for small and medium spiders.

Summer and autumn are shown to be the peak seasons for beginning a period of residence, spring and summer are the peak seasons for leaving. There is only a small difference between the behaviour of the two size classes. Winter is confirmed as a period of relatively low mobility, at least for spiders arriving into a new web-site. In terms of departures, there is more activity during winter than there is in autumn for medium spiders. This may in part be due to losing track of the spiders when they do
Figure 6.5. Spider movements as a percentage of the spiders recorded on a seasonal basis: a. the season at the start of recorded residence; b the season at the end of recorded residence. See text for details.

not make webs in cooler weather. If the spider then moves on as soon as the weather warms up it may not be recorded again at the original site despite being there much longer than was apparent. The shift of emphasis between the two graphics of Figures 6.5.a and 6.5.b seems to suggest that the general strategy is to move in spring and summer (rapid growth is often occurring at this time), but by autumn, many spiders are settled into a site where they will stay until the next spring. In fact, 100% of small spiders end their residence during spring, but only 60% begin a residence during this period. The 40% discrepancy in numbers will be partly due to the maturation of males and partly due to spiders growing into the next size class. The difference in the medium size-class is also quite marked (88% to 63%), but whether this is due to predation, movement away from the transect, or growth into the next size class, is unclear.
6.2.4. Discussion

The mean web-site tenacity of 40.75 days, averaged over all the data, is far longer than any time reported in the studies of orb-weaving spiders listed in Table 6.1. Even the recalculated average residence times using the estimated “missing” short term residencies (14.1 days for small spiders 24.3 days for medium + large spiders) are still longer than those recorded for any diurnal species other than *Nephila clavipes* (Table 6.1). Although there is the possibility of overestimation in these figures due to the specimens not being marked, the long period of larger spiders gives some support to these data. The large spiders are those in which error is least likely as they are most recognisable by individual shape. Other biases may arise from the comparison to other studies, which are rather heterogeneous in their methodologies. Although most were field studies, the parameters for what constituted a site move were varied. Some studies were manipulating prey levels, which may have resulted in inadvertent disturbance and some discounted moves that were assessed to have been caused by predation or interference from other spiders. To be strictly comparable this heterogeneity is undesirable, but for the purpose of comparison to my set of rather less rigidly controlled field observations, it is probably more useful to have this broadly applied collection of studies.

There are also differences between the behaviour of largely nocturnal and diurnal spiders that make direct comparisons even more difficult. The diurnal spiders do not normally leave the web-site, but instead the new web is constructed within the frame of the previous one (e.g. Hodge, 1987a). Thus, as long as there was no disturbance, the web-site will be in exactly the same place and repeated occupancy can be assumed to be a direct measure of the suitability of the web-site for the spider.
Polys, in contrast, may spend up to 17 hours each day not in a web and only the bridging line across the top of the web and access lines between and along twigs are commonly left in place. The bridging line may survive the day but it is quite flimsy compared to the tougher silks used by many diurnal species, which need to withstand average day-time conditions; consequently it is often broken in windy weather. The position of anchor lines and thus hub position is dependent upon wind direction and starting point. This means that except in an extremely simple structural situation, or in calm weather, the position of the web is unlikely to be exactly replicated from night to night. In fact, the day resting position of Polys noblei was recorded almost 1 m away from the web-site and was probably further in some cases as some specimens could not be relocated. [It is sometimes over 3 m in P. illepidus, but these use a much stronger bridging thread]. Smaller spiders (of both species) are usually close to their web-sites, so it may be that as they grow, the spider needs to move its resting position onto a larger twig, which may well be towards the interior of the bush, whilst at the same time accessing a larger web-space, likely to be in the opposite direction. This remoteness makes the regular long-term usage of some sites even more remarkable and certainly challenges the statement that, “araneid spiders cannot be said to have any home range outside the confines of the web” (Enders, 1976).

Although the web-site tenacity distributions of both large and small spiders tended towards a random series (the small spiders did deviate significantly, but only weakly), the underlying data show a similar pattern to those recorded for the mixed araneid guild (Janetos, 1982a) and for Cyclosa argenteoalba Bösenberg & Strand (Nakata and Ushimaru, 1999), with rather more short and longer term stays and less medium-term residency periods than expected. But the extension of the recording
period from the days found in other studies to weeks, and the tendency towards a random distribution, both suggest that the costs for moving between sites, especially for larger animals, are extremely high. That there should be a significant difference between the behaviour shown by small and larger spiders is not a surprise. Small spiders in this context have an abdominal length of only 1–2 mm, and are relatively inconspicuous wherever they hide. The sharp drop in numbers of spiderlings in the months immediately post emergence may indicate high mortality due to predation during the day, but is equally likely to be due to the attention of araneophagous spiders (including conspecifics) at night as well as unsuccessful spiderlings starving to death and dispersal (whether Polys spiderlings disperse by ballooning is unknown). It is likely that the costs of high mobility at this stage are less than those of staying in close proximity to other hatchlings, which will attract predators and may be especially risky at moulting time. Conversely, the larger the spider becomes, the more important is effective camouflage in an exposed habitat and the higher the potential cost of moving. Notwithstanding, most spiders do relocate once they moult to adult, although this is often only one or two metres into a larger space in the same tree. I noticed several spiders that tried two or three adjacent sites (a relatively low risk option) before finally moving further afield (a much higher risk). A high incidence of moving after moulting and significant differences in web-site tenacity between spiders of different instars were both recorded for Argiope aurantia Lucas (Enders, 1975). Juvenile Cyclosa octotuberculata were also found to relocate more frequently than adults (Nakata and Ushimaru, 2004).

Seasonal differences in web-site tenacity have not been a focus of previous studies in the sense it is presented here, mainly because most of the species involved
have a distinct annual or biannual cycle, so any differences have been related to instar (e.g. Enders, 1975). In the case of Pollys noblei, where spiders of a range of sizes are present together through most seasons of the year, the correlation between seasons of rapid growth and seasons of frequent movement become apparent. Spring and summer are the periods of greatest activity and generally coincide with a drop in the numbers of larger spiders (as well as of small spiders, which are probably mostly males and many of which mature at this point). It is likely that predation of larger spiders by birds is at a peak in spring due to nesting demands, but it is impossible to tell whether there is any greater likelihood that newly moved spiders are more likely to be found by predators than longer-term residents. This would be predicted by other aspects of this study, but would be a major challenge to follow through in the field.

6.3. SPIDER ORIENTATION WITH RESPECT TO CAMOUFLAGE AND PREDATORS

6.3.1. Background

The crypsis and behaviour of Pollys spiders is typical of the kind of camouflage referred to as “special protective resemblance” (Robinson, 1969a). This differs from “eucrypsis” or pure camouflage, in the mimicry aspect of the disguise, in that even when removed from its background the animal still resembles its model. Thus, as long as it does not move, a Pollys spider that has dropped onto the ground still resembles something other than a prey item—a piece of stick, or a bud or gall—whether or not it matches the ground beneath it in colour. Other behavioural adaptations are typical correlates of crypsis (Tinbergen, 1965, listed in the form below by Robinson, 1969a), these are:
(i) diurnal immobility;

(ii) living on a background that matches the animal’s colouration;

(iii) adopting a position that provides maximum concealment;

(iv) living well spaced out (i.e. at interindividual distances that greatly exceed the distance at which predators usually detect them readily.

The behaviour of Poltys in general appears to agree with these points, however, (iii), in particular, stands out as a statement that should lead to some testable hypotheses and this is the thrust of my study here.

The main predators that are of relevance to an arboreal spider in southern Australia during the day are spider-hunting wasps and birds. Wasps are versatile and common predators and have been suggested to be a significant selective force on the evolution of defensive behaviours in those genera of spiders that are taken as prey (Obin, 1982; Blackledge and Pickett, 2000). All Pompilidae and several genera in the Sphecidae provision their larval cells with spiders, and several of the Ichneumonidae lay eggs on spiders in situ, the spider carrying the attached larva as it grows (Foelix, 1996). Wasps range widely in size, and whilst some will only take prey of a size they can easily fly with, others will drag spiders too large to carry. Spiders are therefore under threat from wasps at almost all stages of growth. In the Sphecidae some wasps may use more than 25 spiders to provision a single cell (e.g. Obin, 1982), others may use fewer larger spiders. Sceliphron laetum (F. Smith) was recorded to use from 3–9 spiders per cell in one study (Elgar and Jebb, 1999) and up to 12 in another (Smith, 1979). Pompilidae use only a single large prey item.

Poltys are definitely preyed upon by wasps, but the only published report I have located records “Poltys sp.” amongst 12 spider species recorded in S. laetum cells in
New Guinea (Elgar and Jebb, 1999). I have examined these specimens (in WAM) and they are medium–large juvenile and subadult females of a *P. illepidus*-group species. Other *Poltys* species, mainly *P. laciniosus* and *P. noblei*, have been recorded from wasp nests in Queensland (from QM loan data) and *S. laetum* is named in some of these records. These specimens were mainly adult, but both species are slightly smaller than adult females from the *P. illepidus*-group. There may be more examples among vials of juvenile spiders, but these have been examined in less detail than adults. I have also occasionally found juvenile *Poltys* parasitised by a larva in the field, most likely that of an ichneumon wasp, but this does not seem to be common. These larvae are typically white, so possibly the adverse impact on the effectiveness of the spider’s camouflage might make *Poltys* a less than ideal target for this sort of wasp predator.

Studies on several different wasp species have shown considerable variation in hunting technique and prey taken, even between individuals of the same species of wasp (comparisons with previous records discussed by Eberhard, 1970 and Coville, 1976; also see Laing, 1988; Edmunds, 1993; Blackledge and Pickett, 2000). Most of these reports concern spiders in webs, or concealed in retreats near webs. Probably the most relevant for cryptic prey such as *Poltys* is the work of Edmunds (1993). This study was concerned with Salticidae rather than Araneidae, and it examined the activities of the spherd wasp *Pison xanthopus* (Brullé) in Ghana. Edmunds found that in proportion to their occurrence in the wasps’ hunting environment, “good” ant-mimics (in the visual sense) were predated less by wasps than “poor” ant-mimics (which were mainly behavioural mimics) or non ant-mimics. Despite most wasps not finding the well-camouflaged spiders, certain individuals specialised almost exclusively in locating these “good” mimics. This seems to suggest that these wasps are using
visual cues and can learn to discriminate quite finely. Another wasp of this genus found
in New Zealand, Pisim morosum Smith, actively searches for prey by hovering and
flying around tree branches, rocks and flowers (Laing, 1988). I have also seen a small
(ca 8 mm long) black wasp flying slowly around the end of dead twigs on which Poltys
spiders were hiding in the Sydney area. These observations suggest that visual
searching techniques are important in at least some wasp hunting strategies and the
evolution of good camouflage for spiders on exposed twigs may be partly driven by
this type of predator.

Other reports of wasp hunting techniques, particularly on some of the larger
Sphecidae, suggest that precise vision may be less important for these species. Coville
(1987) lists four primary hunting techniques for sphecs, two of which are obviously
applicable to Poltys, namely, (i) alighting on bumps and spots, particularly those that
contrast against the background, and (ii), tapping surfaces with their antennae to detect
and flush spiders that are maintaining cryptic postures. The third method, of examining
webs, would not usually be applicable as Poltys usually remove their webs during the
day, but some lines often remain, including the draglines along twigs around the resting
position, so it is possible that wasps could use this as a cue to initiate a more detailed
search. One author reports that two species of wasp, from the families Pompilidae and
Sphecidae, appeared to sting automatically on contact with spider cuticle (Eberhard,
1970). This would remove some of the need to visually recognise cryptic prey,
especially if the spider has fallen to the ground and maintains its cryptic position. It has
also been reported that Poltys (and other cryptic araneids) respond to the tapping
technique (ii) above, as used by S. laetum. The wasp moves down a twig, tapping as it
goes and as soon as the spider moves the wasp pounces (Robert Raven, pers. comm.).
Birds are also primarily visual predators and have been shown to be adept at learning to find cryptic prey (Bond and Kamil, 1998). It has also been suggested that the influence of birds as predators of spiders is often overestimated, although at certain times of year they may become more important (Foelix, 1996). Foelix also comments that spiders recorded from bird stomach contents are nearly always conspicuous species rather than cryptic forms. The relative importance of wasps and birds is confirmed by Blackledge et al. (2003). Based on other published studies, they estimate that one species of wasp alone accounts for 30 times more predation of spiders than 15 species of birds combined. Nevertheless, there is potential for impact at certain critical times. Around Sydney, the Noisy Miner (Manorina melanocephala (Latham)), is common and inquisitive. I have observed this species searching along larger dead twigs, in curled bark and under leaves—all likely spider hiding places and I suspect it has been responsible for eating one or two large specimens that I was observing, after I had been incautious enough to draw attention to the spiders in their daytime resting position. [Indeed, this problem rather reduced the opportunities for day-time observations along the transect routes.] Smaller bird species, which might forage on smaller twigs, mainly seem to forage among living leaves (pers. obs. in Sydney area). This may in part be due to the extreme fragility of thin dead twigs. Although birds may come across Poltys relatively rarely, the spiders they find on the larger dead twigs are most likely to be adults or subadults. As relatively few spiders survive to maturity, a small amount of predation at this stage has the potential to make an impact on a local population of spiders, particularly in spring when predator pressure is high due to nesting demands but before spiders have produced egg sacs. At this time of year the main foragers are
experienced birds, later the inexperienced fledglings are less likely to impact on a cryptic species.

6.3.2. Aims

I hypothesized that spiders that are reliant on camouflage for protection from visual predators should adjust their positions so that their best-camouflaged aspects are presented towards the most potentially dangerous direction of approach. An obvious first consideration is the direction of light. A predator would be able to see best when approaching with the light coming from behind it or from above and so a spider should orient itself to minimise the threat from this direction. I aimed to test this by noting details of the resting positions of spiders and relating the direction of orientation of the abdomen to factors such as vegetation structure and light direction.

6.3.3. Methods

Spiders were located in webs at night and the web position was noted in relation to a small piece of cotton tied to a nearby twig. The following morning the surrounding twigs were searched until the spiders were located on their resting positions. The direction the anterior tip of the abdomen pointed was recorded, with an indication of the inclination away from the horizontal plane, as well as the direction the dorsal abdomen faced and the height of the hiding position. A sketch or photograph recorded the layout and aspect of the immediately surrounding vegetation, as well as the position of the previous night’s web. Most of these searches were carried out in the northern Sydney area and it is assumed the spiders were *P. nobleti*. Some additional information
(not used in these analyses) was also collected for other species around Cairns in NE Queensland.

Time constraints and the difficulty of finding good sites with accessible spiders in the drought period during which the study was undertaken precluded the collection of sufficient data to fully explore this line of enquiry. The following provisional analyses were carried out:

(i) overall orientation with respect to the spider’s spinnerets. The attachment disc spun by the piriform spigots on the ALS is the spider’s primary attachment point to its substrate, although claws on other legs could be used to also hold onto silk lines or the surface of the twig. Purely from mechanics one would expect the spider to effectively pivot from this point, so that its spinnerets should always be higher than the anterior carapace. Nevertheless, it is possible that other positions might be better for predator avoidance. The attitude of the spider was therefore noted for each specimen.

(ii) Overall orientation with respect to light. In the southern hemisphere the sun is roughly to the north of vertical, so bearings from 270° to 359°, and 0° to 89° can be considered well-lit, whilst 90° to 269° are less well-lit. A random hypothesis would suggest that if there is no bias, there should be the same number with their “best” camouflage to the north as to the south. The “best” camouflage, which I define as the aspect with the least recognisable features of a spider, is probably either the dorsal abdomen or approaching the anterodorsal abdomen (compare spiders in natural hiding positions shown in Plates 1.1.a, 8.2 and 8.3). Direction was therefore measured as the way the dorsal abdomen faced. Specimens that were essentially horizontal, with the dorsal abdomen recorded as “up”, were assigned a direction based on the direction the anterior abdomen pointed.
(iii) Orientation with respect to the aspect of the vegetation. No numerical analysis was attempted due to insufficient data. Instead, the orientation of the abdomen in terms of both the anterior and dorsal directions was plotted diagrammatically. Spiders were split into small, medium, and large size categories based on the estimated abdominal height.

6.3.4. Results

The full set of data collected on each spider is transcribed from the diagrams and notes into Appendix 13. Much of these data cannot be used in any quantitative manner at present, but provide habitat information, web information and some general observations, which are discussed in Chapter 8.

(i) Overall orientation with respect to the spider’s spinnerets. For some small vertical specimens and those twisted laterally (about 6 in total) my notes were not clear enough to differentiate whether the spinnerets were higher than the frontal carapace. All other specimens, even those which were essentially pointing vertically upwards or downwards, were using the angle of the twig to keep the spinnerets highest.

(ii) Overall orientation with respect to light. Overall orientation does not vary significantly from 1:1, i.e., the random hypothesis appears to be fulfilled (Table 6.4). Using total values, $\chi^2 = 2.440678$ (with correction for continuity applied (Parker 1979)), $0.5 > p > 0.1$, 1 df. Without needing a test, the small and large spiders can be seen to be almost perfectly distributed between the northern and southern pairs of quadrants; but the medium-size spiders show a significant bias to facing towards a northerly aspect: $\chi^2 = 5.041667$ (correction for continuity, as above), $0.05 > p > 0.01$. 

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**Table 6.4.** The orientation of the dorsal abdomens of *Poltys noblei.*

<table>
<thead>
<tr>
<th>Abdomen facing</th>
<th>North</th>
<th>South</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>14</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Medium</td>
<td>18</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Large</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36</strong></td>
<td><strong>23</strong></td>
<td><strong>59</strong></td>
</tr>
</tbody>
</table>

North is considered well lit, South is less well-lit. Direction in the case of specimens where the dorsal abdomen was upwards is that in which the anterior abdomen pointed. Size classes as in Tables 6.3.a, b. See text for details.

(iii) Orientation with respect to aspect of the vegetation. Orientation compared to aspect also shows mixed results (Figs. 6.6, 6.7.a, b). The small spiders may avoid the northerly aspect of a bush or tree (Fig. 6.6), but there are no apparent patterns in their orientation. Conversely, the medium-sized spiders are evenly spread in their distribution with regards to aspect (Fig. 6.7.a). All of the spiders in the north or north-east parts of bushes held their abdomens more-or-less horizontally, and most pointed in northerly or easterly directions. In the southerly and westerly parts of vegetation the majority of spiders have abdomens inclined away from the horizontal and also have their abdomens pointing or facing in a north-easterly direction. Large spiders again tend to have their abdomens in a horizontal plane in the north-easterly positions, but otherwise appear to be haphazardly positioned (Fig. 6.7.b).
**Figure 6.6.** Diagrammatic representation of spider abdominal orientation with respect to position in a tree or shrub (represented by large circle). Small spiders only. See key, text and Table 6.3. for details.

**6.3.5. Discussion**

As a general rule *Poltys* are positioned with their spinnerets higher than the frontal carapace. This may be part of their defensive posture, but could also be purely mechanical. It does have the general effect of facing the dorsal abdomen roughly upwards, however, and the dorsal abdomen appears to be one of the most effectively camouflaged aspects of the spider.

Apart from this, these preliminary comparisons suggest there may be patterns in several aspects of these records. Even with sufficient information it would be difficult,
Figure 6.7. Diagrammatic representations of spider abdominal orientation with respect to position in a tree or shrub (represented by large circle). a. medium spiders; b. large spiders. See key, text and Table 6.3. for details.
if not impossible, to differentiate between behavioural positioning by the spiders, and the distribution pattern resulting from selective predation on randomly positioned spiders (selective in the sense that some spiders are more easily found than others). This would need to be tested using enclosures that exclude predators. Nevertheless, the preliminary results allow the formulation of more refined hypotheses based on spider size and likely dangers, which could be tested by more rigorous experimental designs in the future.

The wasp predators, which are probably the dominant influence, are searching using two strategies: vision and touch. Those searching visually are approaching from the outside of the vegetation and looking inwards, whilst those searching by touch are using some unknown cue (perhaps the presence of silk) to select a twig to land on, then walking outwards. The reports available indicate that common Pison Jurine species (which are mostly quite small wasps) may primarily use visual strategies, whilst the larger Scelipron laetum may use touch. Bird predators are most likely to come into contact with large spiders on more solid twigs and so are more likely to be looking outwards from the inside of the bush or tree, or across from a neighbouring twig. These differences in hunting strategy should be reflected in the resting positions of spiders of different sizes.

Small spiders (those with abdominal height estimated at 2 mm or less), seem to avoid the northern side of bushes. This may be a response to predators, but may also indicate that small spiders are most prone to desiccation and heat stress. The lack of any particular orientation of their abdomens may indicate that they are too small to be in great danger from predators, and could support the idea that avoidance of the unshaded side of the bush is more important.
The patterns in the orientation of medium-sized spiders appear to suggest that some other factor becomes important as the spiders become larger. Wasps that are primarily visual hunters may be most likely to target this size-range (>2 mm, < 10 mm). As well as further information on this kind of hunting behaviour, in order to thoroughly examine this it would be necessary to somehow quantify the selection of resting site with respect to surrounding twigs and open areas.

Large spiders (> 10 mm) were not represented by enough specimens to draw any but the most tentative conclusions. Fewer spiders were in the southern parts of bushes or trees, however, and like the medium spiders, those in the north-east and east held their abdomens almost horizontally. Sceliphron laetum has been observed hunting by touch, but is likely to also respond visually to lumps, especially if they do not match the background. With potential predators (both wasps and birds) coming from any direction, once a spider gets to this size the individual circumstances of its surroundings may be more important than any generalisations of ambient light direction. Again, the structure of the surroundings needs to be quantified in some way.

Other complicating factors are the other possible defence mechanisms of the spider. As is obvious from Plates 8.2.c and some specimens on Plate 8.3, the anterior eyes have a clear field of view due to protruding beyond the legs on the eye tubercle. What is less obvious is that, like a person looking through their fingers, the lateral eyes also have a path of vision past the leg spines: the anterior laterals probably in a ventral direction, the posterior laterals posterolaterally. In some photographs, glints of light are visible at the points where these eyes would be and unobtrusive movements of the legs would give much clearer fields of view. Araneid eyes do not sharply focus images but are sensitive to light and dark and movement (Foelix, 1996), so a moving predator can
probably be detected in most of the directions for which camouflage is least optimal. This might alter the dynamics of what positions are safest for the spider.

Poltys also show typical secondary defence mechanisms (Robinson, 1969a), which operate after the animal has been detected by a potential predator. Most, if not all, Australian Pollys species have bright yellow or orange areas on the anterior femurs (Plate 8.4.a). Suddenly exposed, these may startle predators and allow the spider to escape by dropping to the ground. I have not observed this used in defence in a natural situation, but in captivity they wave their front legs, exposing a flash of colour, when disturbed by a probing finger. A similar area of colouration on the forelegs of some mantis species is used as part of a threat display as a deterrent against small birds (G. Milledge, pers. comm.) and other vertebrate predators such as lizards (Crane, 1952). An additional possible defence mechanism is seen in the P. columnaris-group species from south-east Asia and the Australian tropics, which have rows of eye-like, black, shiny maculae on the posterodorsal abdomen (Fig. 2.7.k). Several tropical species also have modified leg spines that are flattened and may be elongated with a weak point basally (e.g. Plate 2.5.c). The role of these modified spines is unknown. The females of one species, P. elevatus from Singapore and Malaysia, have what appears to be a stridulatory mechanism (Fig. 2.22.d) that might produce a faint rustling sound. Nothing similar has been observed in any other Pollys species and its use is unknown, but it could conceivably act as a deterrent to predators.

As already discussed in relation to small spiders, thermal constraints on spider positioning should also be taken into consideration. On several occasions I have observed a spider shifting its position round a twig as a hot summer day progresses and nearly all of the larger Pollys specimens that were studied in northern Queensland were
in rest positions where they would be in shade for much of the day. On one day of field work in Sydney (in Wallumatta Nature Reserve) during which the temperature rose to around 40°C, several Poecilus spiders were observed hanging beneath twigs and making no attempt at camouflage. Not all of these spiders would have been in full sun on their normal resting position, but all were in an open situation where there was considerable reflection of heat from the ground. Thermal effects such as this might influence the suitability of different areas of habitat, just as shade or sun may make parts of the same tree or shrub more or less suitable for spiders. The shape and smoothness of the spider might also be a factor here—a tall thin abdomen beset with small tubercles would provide the largest possible surface-area to volume. An analysis of association between factors should help tease this out. As with other aspects of this topic, however, far more base data are necessary to be able to attempt this.
CHAPTER 7

GROWTH AND DEVELOPMENT DURING CAPTIVE REARING

7.1. INTRODUCTION AND AIMS

Captive rearing of spiderlings from egg sacs was essential in this project. Initially it allowed examination of the variation in abdominal shapes that might arise from a single egg sac. This assisted the validation of species separations in the *P. laciniosus*-group, which was initially confounded by the range of abdominal shapes compared to epigynal features. Secondly, rearing males proved useful to confirm male and female pairings in the northern species that were found to have sympatric distributions. Adult females of *P. laciniosus* from a single egg sac were also examined to provide data on the variation in epigyne morphology. This helped establish whether it was likely that northern and southern females were conspecific. Methods for rearing spiderlings and the results of these primary objectives are presented in the first two sections below. Subsequently, the data collected whilst rearing these spiders have been compiled to provide an insight into some aspects of the life history of the species involved, including the number of moults taken to reach maturity, the relative growth rates of males and females and the development of abdominal shape. These results are presented and discussed in the final section of the chapter, especially in relevance to the differential in sizes of males and females and the resulting potential disassociation of generations.
7.2. REARING SPIDERS IN CAPTIVITY

The majority of studies involving rearing orb-web spiders aim to examine web-building behaviour, therefore spiders are reared in large cages where they can spin webs and live food is usually placed into the webs. Indeed, a recent publication provides invaluable advice for laboratory rearing (Zschokke and Herberstein, 2005). Of studies involving the rearing of spiders for other purposes, many minimise the space and facilities required by enclosing spiders in small vials, at least for younger stages, but far fewer have successfully used artificial food mixes (Peck and Whitcomb, 1968 cited in Nentwig, 1987; Amalin et al., 1999; Amalin et al., 2001). A number of instances of spiders scavenging already dead prey, or accepting unlikely food items offered in a captive situation, can be found in the literature (Nentwig, 1987 provides examples). As far as I am aware, however, no studies have reported rearing orb-web building species to maturity either in small containers or entirely on non-living food; but I have not attempted to thoroughly research this point. In the present study, when I first began rearing spiders I merely experimented with the food and containers that were available to me. The food mix I ended up using (described below) is probably overly complex, but most animals thrived on it so I saw no reason to simplify it.

7.2.1. Methods for captive rearing of Poltys

7.2.1.1. Procedure

Egg sacs were allowed to hatch in whatever container they had been laid in. Within a few days of hatching a number of spiderlings were drawn out of this communal pot at random (scooping a pair of forceps through the mass of lines usually picked up quite a few) and each was placed into a separate vial (species that I hoped to
follow in detail) or two spiderlings to one vial (species for which I was only trying to raise a few males). Vials were made of glass, approximately 1.5 cm diameter x 5 cm high. For humidity-loving or -tolerant species, the plastic caps supplied with the vials were used as stoppers but with several small holes punched in each cap; *P. laciniosus* did not thrive when using these and responded better in the drier conditions provided by using caps of a thin cotton cloth secured with an elastic band. Vials were kept upright in commercially available polystyrene trays, which have 10 x 10 rows of holes suitable for vials of this size. Males were reared through to maturity in these, females until about the 5th moult when they were transferred into the next larger size of vials (2.5 x 5 cm). Large females were again transferred into larger containers, usually plastic specimen pots (4.4 x 5.8 cm) either with holes in the lid or with cloth caps for *P. laciniosus*. Water was provided by wetting a small twist of cotton wool; this was stuck on the side of the vial (or fell into the bottom of the pot). *Polys laciniosus* with cloth caps were provided with additional humidity at night in dry weather by the placement of a damp cloth over each tray of specimens. This cloth was removed in the morning. At first, spiderlings were alternately fed freshly squashed flies (*Drosophila* Fallén spp. if available, or houseflies (*Musca domestica* Linnaeus) hatched from commercially available pupae) and a few crushed and dried pollen granules (see below), which were sprinkled onto their lines. It soon became apparent that spiders could only grow through two or three moultss on this diet (similar results were reported for a lycosid species fed on a monotypic diet by Uetz *et al.*, 1992) and so the food mix described in Section 7.2.1.2. was developed. Initially pollen was provided every second feed for the youngest spiderlings in case they could not survive on the food mix alone. The pollen granules rapidly went mouldy in the humid vials so that frequent vial-changing was
necessary. Eventually it was discovered that spiderlings could survive on the food mix from the start so the spiderlings from the last few egg sacs had this alone; this was preferable because changing the vials was less frequently required than with pollen feeds, resulting in less disturbance. Lumps of food mix appropriate to the size of the spider were stuck to the inside of the vial lid for those with plastic lids (which facilitated cleaning or swapping to a clean lid) or to the side of the vial for *P. luciniosus*. All spiders were provided with an appropriately sized twig with side bumps and most chose to use this to sit on rather than the lid of the vial. The twig was stuck onto the base of the vial in a small ball of “Blu Tack”, to keep it in position during cleaning and so help minimise disturbance. The date of each moult was recorded on a label, and cast skins and the label were kept in a separate vial next to the spider. The timing of the introduction of food into the vials was found to be important, especially in warm weather. The mix tended to grow fungus in the more humid vials (control of humidity was a constant problem) which was detrimental to the health of the spiders, and it “went off” to a foul-smelling mess if the quantities in the mix “were out”. The best solution was found to be to introduce the food mix into the vials shortly before dark, so it was fresh when the spiders became active at night, and in hot weather to remove it before the next night. In cooler weather the remains could be left until the next feeding day. Once it was dark, the spiders were active and they became stressed if disturbed, so it was important to control the lighting to switch off at a time that was realistic for the feeding requirements. The room normally used to house the spiders was in deep shade except mid morning and was almost dark for an hour after sunrise and before sunset. Thus with the blinds closed the photoperiod could be controlled within limits. For the southern species the day length was close to that in Sydney, for northern
species (in a separate room if necessary) it was as close as possible to that in the tropics. Temperature could not be totally controlled, but a room heater and thermostatically controlled heating pads were used to keep spiders as close as possible to an appropriate temperature. Each spider was fed every two to three days depending on how many others there were to feed (which sometimes required feeding different batches on different nights) and the time of year.

7.2.1.2. The food mix

The mix was based on houseflies (hatched from commercially available pupae), moths (captured around local street lights), some ingredients that were shown to be of potential use by Amalin et al. (2001), and also pollen, which has been demonstrated to be of important nutritional value to spiderlings in an orb-weaving species that recycles webs (Smith and Mommsen, 1984). The pollen was not pure but is commercially available from health-food shops in Australia as “pollen granules”. I understand that this is harvested from beehives as a by-product of honey extraction. It certainly smells strongly of honey and some granules are rather sticky. I found the only way to make this crumbly was to dry some granules in the oven then crush them using a pestle and mortar. These crushed granules were used for the spiderlings, tapped onto the twig or silk lines off a small paint-brush. Whole granules could be used for the main mix. The amounts of mix ingredients were approximate, but roughly comprised:

- around 50 hatched and frozen houseflies (the nutritional value probably declines quite rapidly when stored in the freezer (D. Smith, pers. comm.), but the bodies provided useful structure for the mix; most nutrition is probably from the other ingredients);
- a few fresh frozen moths, wings removed and as many body hairs as possible washed off;

- two large or three smaller mealworms (the larva of an exotic tenebrionid beetle), killed in the freezer and chopped into small sections (more used if moths were not available);

- two or three pollen granules;

- two or three drops of egg yolk (too much and it “goes off” quickly);

- a few drops of soy-milk to moisten (a sticky consistency is needed to make the mix stick to the vial lid or wall; this was necessary because spiders made no attempt to wrap this food and food that fell to the floor of the vial was not eaten).

The ingredients were mashed and mixed thoroughly using a pestle and mortar and divided into portions in small vial lids. These were wrapped in twists of plastic wrap and stored in the freezer until required.

7.3. ABDOMINAL SHAPES AND SPECIES STATUS

To recap briefly, the aims of this section are:

(i) to examine the variation in abdominal shapes that might arise from a single egg sac;

(ii) to confirm male and female pairings in the northern species;

(iii) to help confirm intraspecific variation in *P. laciniosus*. 
7.3.1. Methods

Spiderlings were raised as described above. The cohorts of female offspring from egg sacs of each of Poltys illepidus, P. laciniosus and P. grayi were used as exemplars to examine variations in abdominal shape and, for P. laciniosus, epigyne shape.

7.3.2. Results

7.3.2.1. Abdominal shapes of spiders from the same egg sac

Figures 7.1 and 7.2 are of Poltys illepidus; Figs. 7.3–7.5 are P. laciniosus; Fig. 7.6 is P. grayi. In both P. laciniosus group species it is shown that spiderlings from a single egg sac can develop into more than one shape, but there is more variation among

\[ \text{Figure 7.1. Poltys illepidus abdominal shapes (dorsal), egg sac 202. a, mother; b, c, offspring (larger specimens only). Scale = 1 mm; j = juvenile; figure in parentheses is the number of moults recorded.} \]
Figure 7.2. Polys illepidus abdominal shapes (dorsal), egg sac 206. a, mother; b–d, offspring (larger specimens only). Scale = 1 mm; j = juvenile; figure in parentheses is the number of moult recorded.

offspring of some females than others. This, combined with the DNA work performed on specimens from Sydney indicated that my provisional species separations based on epigyne morphology were correct. The relatively few female specimens raised from P. illepidus did not show any substantial differences in abdominal shape. Nevertheless considerable variation has also been demonstrated in what is most likely a closely related species from Japan (Ogasawara, 2000; identified as P. illepidus). Whilst the range of variation shown in Australian specimens in general is also considerable (Figs. 2.2.g–l), those with the apex extended into a central “tower” are relatively uncommon compared to those with rounded abdomens or humeral tubercles.
7.3.2.2. Confirmed pairings of NE Queensland males and females

Males of *Poltys frenchi, P. illepidus* and *P. stygius* were reared from egg sacs laid by captive females. This confirmed that the relatively small differences seen between males of *P. illepidus* and those previously suspected to be *P. stygius* were really the extent of the visible interspecific differentiation between these two species within this species group (c.f. Figs. 2.4.f and i).

![Diagram of Poltys laciniosus abdominal shapes](image)

**Figure 7.3.** *Poltys laciniosus* abdominal shapes (dorsal), egg sac 048. a, mother; b–f, offspring (larger specimens only). Scale = 1 mm; j = juvenile; figure in parentheses is the number of mouls recorded.
7.3.2.3. Epigyne variation in *P. laciniosus*

Females raised from the same mother do not necessarily have an epigyne of similar relative width to the mother (e.g., Figs 7.7). The variation found in females from this egg sac is rather less than that seen in this species overall (many Queensland specimens have a relatively large and often very broad epigyne). Nevertheless, this appears to corroborate other observations and DNA evidence that suggest that the inherent variability seen in the general morphology of these animals is also seen in the female genitalia.

![Diagram of epigyne variation](image)

**Figure 7.4.** Poltys laciniosus abdominal shapes (dorsal), egg sac 170. a, mother; b–d, offspring (larger specimens only). Scale = 1 mm; figure in parentheses is the number of moults recorded.
**Figure 7.5.** *Polys laciniatus* abdominal shapes (dorsal), egg sac 073. a, mother; b–j, offspring (larger specimens only). Scale = 1 mm; figure in parentheses is the number of mouls recorded.

### 7.3.3. Discussion

The findings arising from these primary aims of raising spiderlings are applied, or discussed, in other chapters and will not be further elaborated here. It is pertinent to note, however, that the paternity is unknown for all the above offspring but it is likely that some females mate with more than one male. Whilst this does not affect the conclusions drawn here, it makes it impossible to comment on the possible genetic basis for the observed variation.
Figure 7.6. *Polys grayi* abdominal shapes (dorsal). a, b, LHI A: a, mother (scale outline rather distorted, small sketch shows original shape); b, offspring. c, d, LHI B: details as a, b. Scale = 1 mm; figure in parentheses is the number of moults recorded. Figure reproduced from Smith (2003) courtesy of the British Arachnological Society.

7.4. AN EXAMINATION OF LIFE HISTORY THROUGH REARING DATA

The data amassed whilst rearing spiders permits an examination of aspects of fecundity, growth, moulting and maturation times. In particular, an obvious question arising from extreme sexual dimorphism in size concerns the life history strategies of species and coordination of maturation between the sexes. Amongst the many araneoid taxa that exhibit pronounced sexual dimorphism are the bolas spiders (including *Mastophora* Holmberg and *Orgdarius* Keyserling), *Celaenia, Kaira, Arachnura* and *Nephila*. In the most extreme cases, exemplified in some species in both of these genera of bolas spiders, males emerge mature (McKeown, 1952; Mascord, 1980; Gertsch, 1955 cited in Yeargan, 1994). McKeown reports that emergence is after two moults
Figure 7.7. *Poltys laciniosus* epigyne shapes (anterior then posterior views), egg sac 048. a, b mother; c–h, offspring. Scale = 0.5 mm.

whilst still inside the egg sac (McKeown, 1952). In other *Mastophora* species the males go through about two moults after emerging (reports from various sources, summarised in Levi, 2003). Details are not recorded for the other araneid genera, except that the emerging spiderlings of a *Celaenia* species in New Zealand were all rather smaller than
mature males (Forster and Forster, 1999). *Mastophora cornigera* (Hentz) is known to be active all year, which may allow continuously overlapping generations. In some species of *Mastophora* males are known to actively catch prey as adults, which would help sustain them if there is a delay before females mature (Yeargan and Quate, 1997, cited in Levi, 2003). It is not known whether the males of *Celaenia* feed as adults, but this may not be a problem due to the extended period over which egg sacs are laid and the spiderlings emerge (Hickman, 1967; McKeown, 1952); this would ensure that males are maturing over much of the year. Still amongst strongly dimorphic species, but with males usually requiring a few more moults than those discussed above, the genus *Nephila* has been studied in some detail. In *Nephila clavipes*, it was found that the peaks of numbers of maturing males and females from the same egg sac overlap, but the females grow more rapidly and so attain a larger size (Vollrath, 1980). Males can mature at any of about three instars and at a range of sizes. Moving on to a slightly less sexually dimorphic taxon, *Araneus diadematus* males have a similar leg-span to females, but males are typically still only a fifth of the mass of a female and mature after fewer moults. Ramousse (1973) examined the maturation rates from two egg sacs of *A. diadematus*. He found that spiders grew at two distinct rates from each of the egg sacs and males grew as fast as females in the same cohort. Hence, the faster-growing siblings could never interbreed as males mature earlier than females, but slower growing males could mate with their own sisters from the fast-growing group.

These examples indicate that life-history strategies that permit sexual dimorphism include a combination of differential rates of growth, intake of sustenance by mature males, and temporally dispersed generations. The data gathered whilst rearing *Polystis* enable a comparison against these other taxa to be made. Other data
collected include the number of offspring per egg sac and egg development time, as well as the more in-depth measurements of certain growth parameters. These data are presented for spiderlings from selected egg sacs, but due to the lack of control of environmental conditions and the limited scope of this part of the project, no attempt is made to discuss these in the wider context provided by other studies (a useful overview is provided by Schaefer, 1987). As an adjunct to the original aim of examining the abdominal shapes arising from one egg sac, it was also possible to trace the change in abdominal shape of spiderlings as they grew. I have not attempted to source comparable information for other species.

7.4.1. Methods

Egg sac statistics

Several methods were used to estimate the number of potential offspring per egg sac. The eggs in some egg sacs were killed by freezing or immersion in alcohol others were infertile and did not hatch. For all of these the potential number of offspring per egg sac was counted directly from eggs. Numbers from egg sacs that were allowed to hatch were mostly counted from the number of recorded spiderlings. These may be an underestimate, as some eggs may have been infertile and some spiderlings may have been eaten by siblings or may have failed to emerge from the egg case. Egg sacs known to have been parasitised or partly eaten by a predator were not included. Some laboratory-hatched egg sacs, and all those that had already hatched prior to collection, were opened and the number of pre-emergence moult skins was counted. Hatching time is presented only for species that responded well to the rearing
conditions. Times for species that in general did not grow well are unlikely to be representative due to the strong temperature effect on emergence time.

Measuring growth

All moulted skins were retained for the spiderlings of *P. illepidus* and *P. laciniosus* (unfortunately, this was not done for *P. grayi*, the first species raised). Growth stages were measured from the cast skins for the egg sacs from which the most spiders were successfully reared. Carapace length (CL) and the patella plus tibia of leg I (P+T I) were measured for each skin and for the adult animal. Leg measurements were found to provide the most complete set of data and were used to plot growth against time; the relationship between the two measurements was also plotted and this plot can be used to estimate the missing carapace lengths if required in future. For *P. grayi*, notes of moults dates were kept and these can be used to calculate the likely maturation time in this species.

The development of abdominal shape

Several specimens of *P. grayi* were drawn at intervals over the first few moults until the abdominal shape appeared to be more or less fully developed. Disturbance of specimens was minimised by only attempting to draw them during daylight and then only when they were found in a suitable position so that the twig or lid they were sitting on could be held under the microscope using “Blu Tack”. The two individual spiders that produced the best sequences of drawings are shown here.
7.4.2. Results

The full data for this section are included in Appendices 14–17.

7.4.2.1. Egg sac statistics

Table 7.1 summarises the data for numbers of offspring, or potential offspring per egg sac. The *illepidus*-group species egg sacs contained more offspring than from any other species; notable amongst the other records is the difference in numbers of eggs laid by wild-caught *P. laciniosus* specimens and those laid by captive raised individuals (but due to the differences in the method of counting, it is possible that some or all of this difference could be made up by spiderlings feeding on infertile eggs, see below). Table 7.2 shows emergence times. The temperature effect can be seen clearly by comparing the emergence time of the *P. illepidus* egg sacs that hatched in spring compared to the one that hatched in early winter. It is notable that the spiderlings in some egg sacs have developed at similar rates, and all emerge within a few days, whilst others develop at markedly different rates. In these latter the first spiderlings may emerge several weeks ahead of the slower ones. The skins left in at least one egg sac (Table 7.1) also indicated that some spiderlings may undergo the first proper moult (M1 in my terminology, but see Valerio, 1974) whilst still in the egg sac; this usually occurs after emergence. Valerio (1974) reported that if spiderlings of *Achaearanea tepidariorum* (C.L. Koch) (Theridiidae) are separated after ecdysis, but before the moult that normally occurs before the spiderlings leave the egg sac (M0 in my terminology), survival is markedly less than if spiderlings are separated after emergence from the egg sac (i.e. after M0). He attributed this to spiderlings feeding on infertile eggs, which may also allow them to proceed through a further moult (my M1)
Table 7.1. Summary of numbers of eggs or hatchlings by species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Maximum per egg sac</th>
<th>Minimum per egg sac</th>
<th>Mean</th>
<th>N</th>
<th>Notes (methods of counting)</th>
</tr>
</thead>
<tbody>
<tr>
<td>frenchi</td>
<td>302</td>
<td>147</td>
<td>224.5</td>
<td>2</td>
<td>p-e</td>
</tr>
<tr>
<td>grayi</td>
<td>299</td>
<td>231</td>
<td>265</td>
<td>2</td>
<td>eggs &amp; p-e</td>
</tr>
<tr>
<td>illepidus</td>
<td>846</td>
<td>313</td>
<td>558</td>
<td>4</td>
<td>htc &amp; eggs</td>
</tr>
<tr>
<td>jujorum</td>
<td>154</td>
<td>115</td>
<td>129</td>
<td>3</td>
<td>htc &amp; eggs</td>
</tr>
<tr>
<td>milledgei</td>
<td>122</td>
<td>95</td>
<td>108.5</td>
<td>2</td>
<td>p-e</td>
</tr>
<tr>
<td>laciniosus (raised)</td>
<td>407</td>
<td>152</td>
<td>260</td>
<td>7</td>
<td>eggs; (3 spiders involved, producing 1, 2 and 4 egg sacs)</td>
</tr>
<tr>
<td>laciniosus (wild caught)</td>
<td>150</td>
<td>56</td>
<td>102.8</td>
<td>14</td>
<td>htc</td>
</tr>
<tr>
<td>noblei</td>
<td>165</td>
<td>41</td>
<td>135</td>
<td>6</td>
<td>p-e &amp; eggs; NB one egg sac contained both M0 and M1 skins</td>
</tr>
<tr>
<td>stygius</td>
<td>981</td>
<td>981</td>
<td>981</td>
<td>1</td>
<td>htc</td>
</tr>
</tbody>
</table>

Methods of counting: p-e = from pre-emergence moult skins in egg sac; eggs = from eggs or spiderlings in egg sac; htc = from preserved and raised hatchlings.

without additional food. Since I have records both of spiderlings actually undergoing M1 before leaving the egg sac, and of considerable variation in the abdominal sizes of newly emerged spiderlings, it would appear likely that this consumption of eggs is also occurring in at least the Poltys laciniosus and P. illepidus species groups. Egg sacs that were opened after emergence were rarely found to contain unhatched eggs, but I never attempted to count the egg membrane remains (which would have been extremely
Table 7.2. Summary of egg sac emergence times, by species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Days to first emergence (days)</th>
<th>Duration of emergence</th>
<th>Reference ID</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>grayi</td>
<td>22</td>
<td>ca 2</td>
<td>KS90968</td>
<td>“LHI A”; mid summer</td>
</tr>
<tr>
<td>grayi</td>
<td>24</td>
<td>ca 4</td>
<td>KS90953</td>
<td>&quot;LHI B&quot;; mid summer</td>
</tr>
<tr>
<td>illepidus</td>
<td>24</td>
<td>n/a</td>
<td>KS86257</td>
<td>hatched late spring (Sydney); only start of emergence recorded</td>
</tr>
<tr>
<td>illepidus</td>
<td>18</td>
<td>5 to 10</td>
<td>KS86259</td>
<td>hatched late spring (Sydney)</td>
</tr>
<tr>
<td>illepidus</td>
<td>22</td>
<td>see notes</td>
<td>KS86258</td>
<td>egg sac frozen after emergence commenced. 3 spiderlings emerged; in eggsac: 38 done or completing pre-emergence moult; 67 close to or starting pre-emergence moult; 359 eggs plus developed embryos up to previous.</td>
</tr>
<tr>
<td>illepidus</td>
<td>44</td>
<td>8 or more</td>
<td>KS58036</td>
<td>hatched in early winter (in Sydney), limited heating</td>
</tr>
</tbody>
</table>

All the following *P. laciniosus* were laid in autumn (South Australia and Sydney) and emerged from late autumn through to early winter

| laciniosus | 19                             | 11 or more             | KS78296      | first few spiderlings had not undergone the pre-emergence moult |
| laciniosus | 35                             | 1                     | KS78299      | |
| laciniosus | 22                             | 11 or more             | KS78300      | first few spiderlings had not undergone the pre-emergence moult |
(Table 7.2. continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Days to first emergence</th>
<th>Duration of emergence (days)</th>
<th>Reference ID</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>lacinius</em></td>
<td>21</td>
<td>42</td>
<td>KS78301</td>
<td>first few spiderlings had not undergone the pre-emergence moult</td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>33</td>
<td>4</td>
<td>KS78307</td>
<td>see start of section (previous page)</td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>28</td>
<td>14</td>
<td>KS78310</td>
<td></td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>23</td>
<td>23</td>
<td>KS78312</td>
<td></td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>35</td>
<td>8</td>
<td>KS78313</td>
<td></td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>33</td>
<td>3</td>
<td>KS78314</td>
<td></td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>35</td>
<td>2</td>
<td>KS78315</td>
<td></td>
</tr>
<tr>
<td><em>lacinius</em></td>
<td>32</td>
<td>5</td>
<td>KS78318</td>
<td></td>
</tr>
<tr>
<td><em>nobilei</em></td>
<td>16</td>
<td>13</td>
<td>KS55689</td>
<td>mid summer (Sydney)</td>
</tr>
<tr>
<td><em>nobilei</em></td>
<td>11</td>
<td>n/a</td>
<td>KS55686</td>
<td>mid summer (Sydney)</td>
</tr>
</tbody>
</table>

Only species which responded to the rearing conditions are included. Times for species which in general did not grow well are unlikely to be representative.

difficult, if not impossible). It would seem likely that at least part of the observed variance in numbers of potential offspring between wild-caught females and captive reared females is due to this ‘oviphagy’, because the egg sacs of captive reared females were preserved as (or counted from) eggs whilst the egg sacs of wild caught specimens were, on the whole, allowed to hatch.
Figure 7.8. Relationship between carapace length and (patella + tibia I) length. a, Pollys illepidus females (offspring of both egg sacs combined); b, ditto for males; c, P. laciniosus females (egg sacs separate); d, ditto for males.

7.4.2.2. Measuring growth

Pollys illepidus specimens were pooled from two egg sacs as the results did not separate out when plotted on a per egg sac basis. Figures 7.8a, b show the relationships between carapace length and patella + tibia I length for this species. For males (Fig. 7.9b) the data suggest that the *P. illepidus* males that matured in three mouls tended to grow more rapidly than the single male from these egg sacs that matured after four mouls; but this may be an artefact of insufficient data—also see the *P. laciniosus* results below. Males matured between 55 and 96 days post emergence (but note a male from an egg sac from Rockhampton, reared in Sydney through the winter, only took 45 days—data in Appendix 16). The growth rates of *Pollys illepidus* females appear to
spread out evenly (Fig. 7.9.a): of the females that actually matured, the fastest took 136
days from emergence and eight moults, the slowest 210 days and ten or eleven moults.
Of the two subadult females for which the potential maturation dates are projected onto
Fig. 7.9.a, one would have most likely taken around 220 days (11 moults). Many
slower growing juvenile females were terminated and it is likely that some could have
taken much longer, as seen in the P. laciniosus data examined below. The records for
the first one or two moults for many P. illepidus specimens are rather sparse, as there
was a delay between hatching and separating out the spiderlings and many had already
moulted. Hence some of the points in the lower end of the data are rather tentative in
Figs. 7.9.a, b, as some dates are estimated (put mid way between emergence and the
first recorded moult post separation (two females), or the day before separation (two
males)). Male growth rate is compared to that of females in Figure 7.12.a (no estimated
records are included here, which reduces the first two moults to a single datum point
each for females and excludes three males from M1). Even these rather sparse data
clearly show that females on the whole both moult sooner and have grown more
between moults, i.e. their overall growth rate is more rapid. Whilst it would be most
unlikely that mature males and females from the same egg sac could overlap and thus
interbreed, it is possible that siblings from egg sacs laid a month apart could do so.

Graphs of the equivalent data for the two P. laciniosus egg sacs show similar
trends overall. These egg sacs were laid in the autumn and hatched in the late autumn
and early winter, so there was little activity for the first 3 months or so until the weather
began to warm in spring. In these data, males that matured in four moults did not
necessarily grow any faster or slower than three-moult specimens (Figs. 7.11.a, b). The
Figure 7.9. Growth of *Poltys illepidus*, envelopes indicate moult series. a, females (separated by individual); b, males (separated into three and four moult-to-maturity individuals).
Figure 7.10. Growth of *Pollys laciniatus* females (separated by individual), envelopes indicate moult series; a, ex egg sac 048; b, ex egg sac 073.
Figure 7.11. Growth of *Poltys laciniosus* males (separated into three and four moult-to-maturity individuals), envelopes indicate moult series; a, ex egg sac 048; b, ex egg sac 073.

Figure 7.12. (next page). Comparison of male and female growth rates during first four moults, separated by moult number and sex. a, *Poltys illepidus* (egg sacs combined); b, *P. laciniosus* ex egg sac 048; c, *P. laciniosus* ex egg sac 073. Axis titles and key are shared for all figures.
Figure 7.12. (Caption on previous page).
number of days to maturity ranged from 186 to 222 for egg sac 048 (Fig. 7.11.a) and from 162 to 213 days for egg sac 073 (Fig. 7.11.b). Female growth rate was well spread out (Figs 7.10.a, b), with females maturing in 564–664 days from egg sac 048 (Fig. 7.10.a) and 321–564 days from egg sac 073 (Fig. 7.10.b). In egg sac 048 and also from other egg sacs not detailed here, there were subadult females that were terminated in May 2004 when it became no longer possible to keep them alive due to other commitments. These would probably have matured in spring or early summer 2004 (September to December). If maturing on the 1st of September 2004, for example, this would have been 851 days post emergence and hence egg sacs would probably have been laid around 2.5 years after emergence. The shortest maturation period for this species, 321 days, would have approximately corresponded to an annual cycle. The comparison of male and female growth rates (Figs 7.12.b, c) again suggests that females on average grow faster than males from the same egg sac. The relationship between CL and P+II is illustrated in Figures 7.8.c, d. As already seen in P. illepidus, the relationships between males and females are slightly different. In these graphs the two egg sacs are also separated, and it can be seen that individuals from one egg sac consistently have slightly longer legs compared to carapace than the other. Specimens from both P. laciniosus egg sacs and of both sexes, tend to have longer legs than P. illepidus of a similar carapace size. Unlike P. illepidus, it would be impossible for sibling males and females to interbreed, unless a significantly broader spread of maturation rates than was found here is actually possible.

Polystis grayi moult data (summarised in Table 7.3) show that males in this species matured in only two or three moults, ranging over 28–53 days. No females matured from these egg sacs (all the offspring shown in Fig. 7.6 are juveniles). These
Table 7.3. Summary of *P. grayi* data for raised specimens

<table>
<thead>
<tr>
<th>Males</th>
<th>Days to maturity</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Mean</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>2 mouls (egg sac A)</td>
<td>29</td>
<td>38</td>
<td>31</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2 mouls (egg sac B)</td>
<td>28</td>
<td>43</td>
<td>35.3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3 mouls (egg sac A)</td>
<td>44</td>
<td>53</td>
<td>48</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>3 mouls (egg sac B)</td>
<td>31</td>
<td>52</td>
<td>44.5</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

**Survival post maturity** (days)  
6  51  17  8

**Sex ratio**

**Total number** of males and juvenile males (of 40 separated)  
(egg sac A+ egg sac B) = X  
19+17 = 36

**Total number** of juvenile females (of 40 separated)  
(A+B) = X  
18+18 = 36

(Other 8 specimens died too young to establish sex)

egg sacs were laid in mid December so that the spiderlings were growing at the warmest time of year. It is probable that males from summer laid egg sacs of *P. laciniostus* could also mature faster and after fewer mouls; over-wintering is costly in terms of stored energy that could otherwise be put into growth. The raised spiderlings of *P. illepidus* were also potentially compromised by less than optimal conditions, although they were kept as close as possible to tropical temperatures throughout. The Rockhampton male, referred to previously, may have been less affected by cooler temperatures, as Rockhampton is 7° further south than Cairns, where the other
specimens were obtained. Females from one of the *P. grayi* egg sacs appeared to grow slightly faster than males from the same sac (as measured by frequency of moulting), but those from the other egg sac (which were all smaller spiderlings in terms of abdominal size) seemed to have a similar growth rate to males (but the growth rate in terms of increase in size is unknown due to the lack of moult skins). Additional information that is available from the *P. grayi* spiderlings, but not from the other species for which survival was far lower, are the sex ratio and survivorship for some adult males. The majority of spiderlings survived long enough to enable their sex to be determinable and there was an approximately 1:1 ratio in each egg sac (Table 7.3). Most males were put into alcohol once they became adult, but eight were left alive to test how long they survived post maturity. Most of these specimens were given food, but there was no evidence of feeding. The shortest survival time was 6 days, the longest 51 days. This latter was over twice as long as the next longest at 21 days and most were around 10 days.

### 7.4.2.3. The development of abdominal shape

The two spiderlings used as exemplars from one of the *P. grayi* egg sacs each developed to a different abdominal shape (Fig. 7.13). The shape appears to be fully developed around moult 5 to 6. Although the two spiders shown were not figured beyond this point, some other spiderlings, which were drawn over a longer period but more sporadically, confirmed that the majority of cuticular details were constant after this point.
Figure 7.13. Development of abdominal shape in two Poltys grayi spiderlings ex LHI A. Pre-moult 1 spiderlings are drawn from alcohol-preserved exemplars; all post moult 1 figures are from live animals. For each spider the dorsal abdomen view is above and the lateral view below. Lateral views include patellae, patellar sizes confirm abdominal growth between moult. M1–M6 indicates occurrence of moult 1–6. Scale = 1 mm.
7.4.3. Discussion

The results presented above can be used to begin building a picture of the life history of *Poltys* spiders. In summary:

(i) mature males were never found to overlap with mature females from the same egg sac, as seen in *Nephila*, but males do grow slightly more slowly in most instances;

(ii) *Poltys* females, even those from the same egg sac, do not show similar rates of growth or age to maturatity;

(iii) compared to *Mastophora* species, most *Poltys* males have a short life once adult.

This information suggests the primary life-history strategy for Australian *Poltys* is to be almost continuously brooded, with spiders over-wintering at almost any stage of growth except as mature males. This continuity is facilitated in the rather unpredictable Australian environment by quite extreme plasticity in growth rates, which allows individual female life cycles to vary over one to three years (in *P. laciniosis*) and possibly more widely. The requirement for continuity could imply that seasonality may be the limiting factor controlling the distribution of the genus. This might explain the apparent absence of *Poltys* from Tasmania and New Zealand, where the warm weather for rapid growth is of shorter duration and winters are far harsher. Although all these results were gained from spiders raised in an artificial environment temperature and day length were manipulated to some extent in an attempt to keep these influences within natural boundaries (see section 7.2.1.1). Probably the most notable limitations were the lack of frost for *P. laciniosis* in winter (indoor temperatures rarely fell below about 8°C), and the difficulty of keeping humidity and
temperature higher than ambient for *P. illepidus* at most times. The other major limitation of these results is that any one species was only raised starting at a particular time of year (after mated females were collected on trips to different areas) and it is likely that average rates of growth, at least through the first few instars, are seasonal to some extent. Nevertheless, when compared to the data gained from tracking *P. noblei* along a transect in northern Sydney (Chapter 6; Appendix 11), the results appear to be quite realistic. This wild population was monitored during a time of drought and individual spiders appeared to grow at rates that would probably have resulted in generation times of one to two years. This innate variation in growth rates is also supported by the variation in development seen in some egg sacs and reflected in a prolonged emergence period (Table 7.2).
CHAPTER 8

GENERAL BIOLOGY AND BEHAVIOUR

[Plates, in whole or in part, in this chapter are reproduced from Smith (2006) courtesy of The Records of the Australian Museum]

8.1. INTRODUCTION AND AIMS

During the course of fieldwork, both the collection of specimens and of behavioural data, an immense number of general observations were made which largely fall outside the scope of the two previous chapters. These observations range from courting behaviour to the kind of habitat apparently favoured by each different species. The aim of this chapter is to present as much of this data as possible. Firstly, the known published sources of information on Poltys are reviewed; next the new information is presented and drawn together under subject headings as far as possible. Only brief comparisons are drawn to available information from other genera. Descriptions of web structures use the terminology suggested by Zschokke (1999).

8.2. A REVIEW OF THE PUBLISHED INFORMATION CONCERNING AUSTRALIAN POLTYS

The first reports of the biology of Australian Poltys specimens came from the notes of Mr Daemel, who collected the specimens described by Keyserling (1886) as P. laciniosus and P. mammatus. Both were reported to sit on dry branches where they were difficult to detect because the spider’s colour matched that of the branch. The specimen described as P. keyserlingi was said to have been found in leaves, as was Rainbow’s (1916) type of P. microtuberculatus. In the description of P. salebrosus from Western Australia (Rainbow, 1904) the field notes of the collector, Mr J.J.
Walker, were reported. “While beating a withered bush near Fremantle for beetles, I noticed a small and active spider in the umbrella. It ran rapidly up to a broken twig, which it clasped closely with all its legs bunched up as it were beneath the cephalothorax; then elevating the rather elongate abdomen at right angles to the twig, the resemblance to a dry bud was complete. To such a degree of perfection was this resemblance carried, that I am sure no one could have detected the spider when at rest in this position.” Rainbow (1909), on the ‘nesting habits’ of various spiders did not have any further information to add, only commenting that, “The webs and nesting habits of these spiders do not seem to have been observed.”

The first observation of an Australian Polysis found in a web was made by Rainbow (1916), when he reported a specimen of *P. multituberculatus* collected from an orb-web in forest. Earlier the type of *P. moluccum* from Amboina in the Moluccas had been described as making a long, extended web, “een langwerpig net” (Doleschall, 1859). But with no other details given it is difficult to interpret what the extent of elongation might have been.

More recently, Clyne (1969) and Mascord (1970) provided the first photographs of some Australian Polysis species as well as additional details. Both commented on the abdominal shape and resting position and Clyne stated that, “The vertical orb web is renewed every night”. Mascord commented further on the camouflage and resting position, and in a second publication (1980) he also mentioned the variability of the abdominal shape. In both publications the food of Polysis was given as “small flying insects”. Males attributed to *P. mammeatus* were also discussed (Mascord, 1970), but his description and original unpublished photographs held in the Australian Museum show that these were probably a Carepalxis species. Mascord (1970) also provided
more details of the web, saying that, “The orb webs built by Poltys are even finer and more closely spun than those made by the previous genus.” [Carepalxis]. “The webs are only built after dark, and cut down again before daylight”. Poltys was mentioned as an example of a genus that catches large numbers of moths due to the characteristics of the web (Stowe, 1986). Stowe referenced this information to Mascord (1970), but I can only find reference to the fineness of the web in the Mascord publications, and nothing about its effectiveness at catching moths. Main (1976) discussed the odd abdominal shape of the genus and commented on the distribution, saying that Poltys is a tropical genus but occurs at least as far south as Sydney. Mascord (1970) also gave the distribution (of P. mammeatus) as Queensland to at least Sydney. Main (1976) also commented that Poltys, along with Dolophones and Herudo [sic, presumably Heurodes] that have similarly cryptic habit and odd shapes, are strictly solitary species, not occurring in aggregates or clumps like, for instance, the equally bizarrely shaped Gasteracantha minax Thorell (now in Austracantha Dahl). Apart from a single photograph and brief caption in Preston-Mafham (1991), the only other published information on Australian Poltys was by Murphy and Murphy (2000). As well as some general information and reproductions of figures from other authors, an illustration of an unidentified Poltys species from Queensland was included.

8.3. OBSERVATIONS ARISING FROM THE PRESENT STUDIES

8.3.1. Habitat

As suggested by Main (1976), Poltys seems to be essentially a tropical genus. This is reflected in the Australian fauna by the preponderance of species that occur only
in tropical or subtropical areas. The endemic *P. laciniosus*-group seems to have evolved to exploit the more temperate regions of Australia.

*Poltys illepidus*: quite wide habitat tolerance, favours dry open forest but also on rainforest margins, in suburban gardens and in scrubby locations; probably tolerant of frosts (frequently occurs in the Brisbane area).

*Poltys stygius*: occurs with *P. illepidus* in north-eastern Australia but only in moister habitats.

*Poltys juorum* and *P. milledgei*: in seasonal tropical forest habitats (one such habitat in Litchfield NP is termed “monsoon forest”). Both *P. juorum* and *P. stygius* are common in Goldsborough State Forest.

*Poltys frenchi*: in Australia restricted to low-altitude, but mostly slightly scrubby, rainforest with openings.

*Poltys laciniosus*: mostly found in less humid areas than the other species but does occasionally overlap with *P. noblei* on the east coast, and probably with *P. illepidus* across parts of northern Australia. Found in bush and scrub habitats over much of the country except the arid interior. In South Australia *P. laciniosus* is locally common in bushy sand-dune habitats—both inland and close to the sea in sheltered areas (ditto for *P. grayi*, *P. noblei* and *P. frenchi*, which have all been found within a few metres of the sea or open beach, but only in sheltered environments).

*Poltys grayi* and *P. noblei*: both found in structurally similar habitats to *P. laciniosus*, but in more humid areas. *Poltys noblei* is also found in higher altitude vine forests in the tropical areas where *P. frenchi* occurs near the coast.
8.3.2. Webs

Placement and structure

Except for adult males, Australian Poltys make a new web in the evening, and ingest it around dawn. The sticky spirals and radii are closely placed (Plates 8.1.a–c). The spiders seem to prefer openings in the vegetation, which may form natural flight corridors for moths and other prey. Adult female webs have been observed at heights from the ground ranging between 0.2–4.0 m. Canopy fogging programmes in S.E. Asia have only recorded male Poltys specimens (many of these records are included in Chapter 2). This would suggest that whilst males may disperse through the canopy, females usually keep closer to the ground and that the upper height range recorded above may not just be the upper limit of a recorder’s vision. This may also suggest that important predators tend to forage more in the canopy. For several species, adult web hubs have been observed ranging from almost entire to with more than half bitten out. Juveniles leave the hub entire, but bite-out seems to increase as spiders become subadult to adult. Even then it is still variable, both between individual spiders and from night to night. The only entirely and cleanly bitten-out hub noted was for a P. frenchi female (Plate 8.1.b), but there were many occasions on which the hub structure was not examined and this total removal may occur in other species too. Juvenile webs usually have a distinct gap between the hub spiral and the start of the sticky spiral (the “free-zone” Foelix, 1996), and small spiders have been observed to move through this gap to change sides of the web. As spiders become larger, the gap is more or less filled
by the hub spiral and larger animals have not been observed to move to the upper side of the web.

Adult females of the *P. illepidus*-group spin a large and finely woven orb web ca 30–40 cm in diameter between trees or low herbage at night, in a space up to 4 m wide (Fig. 8.1.a). A strong, golden-coloured bridge thread is left in place during the day but the main web is usually taken down towards dawn (although Robinson et al., 1974 record female *Poltys* sp. in New Guinea leaving their webs in place during the day). Webs may be circular, taller than wide or wider than tall, depending on supports. Juvenile webs are usually between dead twigs as in most other Australian *Poltys* species.

Smaller species such as *P. noblei* and *P. grayi* also have finely spun webs that tend to be circular in juveniles but taller than wide in adults (but rather variable in shape to fit the available space). The extra height is gained by widening the sticky spiral spacing, especially towards the top of the web, except in *P. frenchi*, which may also add extra zigzags of sticky silk (Plate 8.1.b). The bridge threads of these species are less sturdy than those of the *P. illepidus* group (and in *P. noblei* at least, often get broken during the day), and the webs are usually in smaller gaps, up to approximately 1.5 m across.

Most web observations have been carried out on *P. noblei* (data in Appendix 13). In this species the slope of the web from vertical is variable, ranging from almost horizontal to totally vertical, but averages 30°, and especially for larger spiders is rarely more than 20° from this value. Perhaps an unwieldy abdomen and the stretchiness of web combine to make this the most ergonomically efficient range. The web may twist through more than one plane, so that one side may be guyed at 50° whilst the other is at
15", depending upon supports. The number of radii also varies; 16–33 have been recorded, but 20–30 seem to be the norm (Appendix 13). Some structural modifications have been observed to fit the webs around twigs in restricted areas, or when the wind does not allow webs in a more open direction. In these situations some radii and/or sticky spirals may pass either side of a twig and the web may even be ‘folded’ across the twig so that one part of the web is in a different plane. Alternatively, radii may be guyed to one or more twigs, so that instead of being loose and blowing against the twig and becoming damaged, the web is held either against the twig or safely away. This structural adaptability probably also applies to other species.

**Web construction**

The web construction sequence for *Poltys* was not studied in detail using recording equipment but it appears to be basically as described for other araneine spiders (Zschokke and Vollrath, 1995). The centre of the proto-hub is marked by a small tangle of silk, and this can be seen to be moved during the early stages of web construction. The auxiliary spiral is removed by the spider as it lays the sticky spiral: in *P. laciniosus* this appears to occur when the auxiliary spiral switches from being at the inside leg I to the outside leg I. Spiders putting in the auxiliary spiral have not been observed to U-turn, and except for the upper parts of *P. frenchi* webs, the sticky spiral also does not usually have any U-turns unless the web is unevenly constructed to fill an odd-shaped gap. In *P. noblei* and *P. laciniosus* the spider changes direction between the auxiliary spiral and the sticky spiral. An unusual aspect of construction that I have not seen mentioned in any published account, is that on occasion some specimens (of *P. noblei*) have been observed to lay the sticky spiral in two or three sections, with the
spider returning to the hub between spiral-laying sessions. The only break I timed lasted about 30 minutes. Silk availability has been shown to influence web design in two orb-weaving species (Eberhard, 1988). The closely-spaced sticky spiral of *Poltys* uses a large amount of silk, but *Poltys* webs are often constructed quite quickly (a small 7 cm diameter web may take 15–20 mins from frame lines to completion, 15 cm diameter ca 40 minutes) so demand may sometimes outstrip supply and this break may allow replenishment of the glands. When the spider restarts the sticky spiral after such a break or due to other disturbance, there is often a small gap left. Disturbance during auxiliary spiral construction also results in an uneven sticky spiral, so presumably the restarting of the auxiliary spiral is also non-contiguous. When the sticky spiral is complete the spider turns around in the centre, apparently testing the web tension in different directions and adjusting if necessary by adding or perhaps removing threads in the hub—presumably this is the time at which parts of the hub are eaten out, but I have not observed this directly.

One caged spider was seen to add two extra subsidiary radii (which branched off existing radii in the catching section of the web, one in the top half and one in the lower) to firm up a sagging web after the sticky spiral was complete. Spiders will also add extra supports and strengthen parts of the web after damage, but I have never noted any addition of what appears to be sticky silk after the original spiral is laid. The same spider does not necessarily make the spirals in the same direction each night with respect to the spider’s side of the web (i.e. the sticky spiral might be clockwise one night and anticlockwise the next).

An aspect of web construction that was used as a character by both Eberhard (1982) and Scharff and Coddington (1997), is which of the legs are used to locate the
current position of the sticky spiral and radii before a new section is attached from the spinnerets. Like *Nephila*, *Poltys* appear to use primarily leg IV, rather than leg I, which is more typical of araneids, and moves around the web with the head constantly pointing between the hub and the direction of travel. As discussed by the previous authors, however, use of this behaviour may be due to web architecture; the webs of both genera are closely woven with only small gaps between the sticky spirals. Other araneids have been observed to use similar behaviour in closely spaced areas of web, near the hub for example.

Web removal

Neither the “slow” nor the “fast” methods of removal discussed by Carico (1986) appear to be used for the whole web, at least not by *P. nobelii*. Instead, key supports are cut at the edge of the frame area and the lower sections are gathered in a few at a time; upper sections are gathered “curtain-style” in two separate passes; finally, if it is the end of the night the spider moves around cutting and scooping up any other remains and adds it all into the one ball. This all takes just a few minutes. Excess moisture is squeezed out of the bundle of silk and small droplets are flicked off the spiders’ front legs. The silk ball, along with small prey is taken back to the rest twig and ingested before the spider settles into its rest position. If it has caught prey late in the night the remains are also taken to the rest position and finished off, sometimes feeding continues well beyond dawn. If the web is being replaced the frame lines are left in place and reused.

Web building and removal times
Small spiders often start early—well before dark and may remain in webs until after it is fully light. I have never found one in a web later in the day but they are difficult to see in daylight because the web is so fine. Larger (and some small) specimens do not usually start moving from their day positions until it is almost dark and are usually back at the rest position before it is fully light. Old adult females that have already laid one or more egg sacs seem to take more risks and sometimes stay in the web until it is quite light. Small spiders often renew the web several times in one night but larger spiders usually keep the same web however tatty it becomes; but on one occasion I saw two large spiders (one of which was adult) make a new web halfway through the night when the weather conditions changed.

**Silk**

It has not been possible to include an analysis of the mechanical properties of *Poltys* web silks as part of this project, but some informal observations suggest that the webs are far more delicate and softer than those of many araneids such as *Eriophora* and *Argiope* Audouin species, yet are brittle when suddenly stressed. The flagelliform silk which comprises the sticky spiral, at least of the *P. laciniosus*-group species, is very fine. This, and the high density of sticky spirals, helps entangle relatively soft “flappy” prey such as Lepidoptera and Neuroptera, whilst fast flying hard insects such as Coleoptera often pass straight through, leaving a neat hole without pulling the rest of the web down. An accidental sharp knock against the branch supporting the web has sometimes resulted in the whole web disintegrating as if exploding, leaving nothing but a few frame lines. This disconcerting disappearing trick may be dependent on moisture content, but I have not noticed it happening to the webs of other taxa.
The composition and mechanical properties of silks used in catching webs have been characterised for various orb-web building spiders such as *Araneus diadematus* and *Nephila clavipes* (Gosline, *et al.*, 1999; Cunleff *et al.*, 1994, quoted in Gosline *et al.*, 1999). The differences in amino acid composition and mechanical properties between the silks were quite marked (Gosline *et al.*, 1999). Some spiders have also been shown to be able to vary the thickness, and therefore some aspects of the properties, of silk draglines at will (Garrido *et al.*, 2002, in Blackledge *et al.*, 2005). Blackledge *et al.* (2005) also found that the outermost end of the sticky spirals in webs of *Argiope argentata* (Fabricius) were consistently 30% thicker than those at the inner end. These authors comment that this control over the properties of webs could have important influences on our interpretation of spider behaviour and evolution. Given the prey specialisation of *Polyis*, the characterisation of *Polyis* flagelliform and major ampullate silks would be of considerable interest.

**Miscellaneous web-related observations on *P. laciniosus***

Gravid (caged) females a few days prior to making an egg sac would sometimes make a web (often rather clumsily) but then instead of sitting in the middle the spider would hang beneath the twig above and monitor the web with a leg resting on a line into the hub (I think this was an extended radius rather than a separate line). When fed in this position, these females also sometimes took the wrapped moth up to the twig to feed. One wild-caught female was also found in this position, but with an only partially completed web. This specimen also laid an egg sac soon after.

A spider raised in a small pot with no chance to make a web as a spiderling, can still make a web as an adult when released into a larger enclosure.
8.3.3. Diurnal concealment

Position

_Poltys illepidus_-group: smaller juvenile spiders and males usually mimic part of a dead twig during the day but large juveniles and females are more often on living or dead trees or even down in low herbage. An individual of _P. illepidus_ with a spiky abdomen was found on the trunk of a tree, another was on a broken branch (Plates 8.2.a, b), and one was taken in a sweep net when sampling low herbage. Two rounded specimens were found like knobs or galls on a branch, another was in a dead flower head on a tree. Variations of colour markings similar to the “gumnut” pattern described by Main (1999) in Carepalaxis have been found (also occasionally in the _P. laciniosus_-group). One specimen from Lae, New Guinea, was reportedly found on potato (from the specimen data) and Robinson _et al_. (1974) list _Poltys_ specimens tentatively identified as _P. illepidus_ hiding in curled leaves by day. A female of _P. stygius_ was found hiding hung beneath a dead curled leaf on a living sapling (Plate 8.2.d). The hiding positions of some specimens of _P. illepidus_ were over 3 m from the web position.

_Poltys columnaris_-group: these species have not been recorded during the day, but their shape and colouration would suggest they are almost exclusively dead-twig residents.

_Poltys frenchi_: less reliant on dead vegetation than most other Australian species, and often found on living or dead vines as well as other twigs, most often in a side position (9 side:1 end, Appendix 13) (Plate 8.3.e). The side positioning may be due
to the tendency of vines to terminate in tendrils. Their camouflage often includes green pigmentation in the cuticle and on the abdomen.

_Poltys laciniosus_-group: the spiders most frequently mimic part of a dead twig, bud or gall during the day (Plates 1.1.a, 8.3.a–d). Most observations are of _P. noblei_ (see Appendix 13); these most commonly sit on the end of a dead twig, or occasionally in a fork or off to one side (55 end:15 side:2 fork, Appendix 13), usually where there is a kink, or leaf scar on the twig. More spectacular colour patterns include the “broken twig” effect of a creamy median stripe, tapering away towards the spinnerets, and single or paired spots (the “gumnut” effect). Spiders also occasionally use the living parts of plants, especially spiky ones such as _Hakea_ and have also been recorded on barbed-wire fences. Small spiderlings sometimes sit on the tip of a flower bract or a thin leaf. One female with a rounded abdomen (similar to the shape shown in the figured specimen of _P. laciniosus_, Fig. 2.14.c) was found in a head of shrivelled _Lantana_ berries. Resting positions are usually quite close to the web-site (the greatest recorded distance in _P. noblei_ was less than a metre but not all specimens were located).

**Reaction to disturbance**

The behaviour of spiders whilst in their hiding positions is slightly different between the _P. illepidus_-group and the _P. laciniosus_ group (data for other taxa was not recorded). Whilst the spider is concealed during the day, it is attached to a silk disc on the tree or twig. _Poltys illepidus_ are usually reluctant to move if touched and carry on the pretence of being an inanimate lump of twig or a gall as far as possible, even if gently held and slowly pulled off the branch, to which they are firmly attached. Their
response to natural predators in the field has not been recorded. *Poltys laciniosus*-group animals usually shuffle around the twig in response to an initial probing finger, but often launch themselves off the twig with continued disturbance, either to the ground or to hang attached by a line. The silk from the piriform spigots forms the basal attachment disc for the dragline, and presumably contributes to the spider’s substrate attachment pad by day. The number of these spigots in the SEM examined *P. illepidus* specimen is over twice that of the two examined *P. laciniosus* specimens (225 vs. 95, see Plates 2.1.b–c. NB although individual spigots cannot be counted under a light microscope, the approximate difference in numbers is apparent in specimens other than those used for SEM). Thus the larger number of piriform spigots could be contributing to both the stronger drag-line required by these larger spiders and a much stronger attachment disc. It would be interesting to test this association when the relevant behaviour and spigot details of more species are known.

Another difference noted between the *P. illepidus*-group and both *P. frenchi* and *P. laciniosus*, was in the behaviour of small spiders when they had dropped from their rest position and were exposed on a surface (a table in this case, as this was noticed whilst rearing spiderlings). The latter two species would run across the table, but fairly slowly, and with increasing clumsiness as they grew larger. In contrast the *P. illepidus*-group animals tended to be much more energetic and quite fast, interspersing running with jumps and chaotic bounces, making them much more difficult to intercept.

**Colour and shape variation**

Colour and abdominal shape variation (Chapter 7) appear to be continuous rather than polymorphic in the southern Australian species, and probably also in the
other species. Most authors have only discussed colour, which is the more usual variable encountered, but there is no reason why similar arguments should not also apply to shape. The possibility of associations of ecological variables with colour parameters was discussed by Oxford and Gillespie (1998) in a review of some aspects of spider colouration and ecology. They suggested that continuous variation is more common in spiders of open areas and that both continuous variation and colour polymorphisms tend to occur in spiders that inhabit vegetation and flowers as against rocks or holes. They suggest that these observations might support the role of predation in maintaining colour variations. Habitat and colour of at least the *P. laciniosus*-group, which often occur in marginal forest or scrubby habitats, would fit with these associations; even in a forest habitat, the microhabitat of *Polys* tends to be on exposed parts of plants. Colour patterns that break up outlines were the focus of experiments by Bond and Kamil (1998) who used blue jays to demonstrate that apostatic selection (where predators concentrate on abundant prey types whilst ignoring less common ones) can indeed maintain prey polymorphisms in cryptic species. The variation in shape and colour in *Polys* can therefore be seen to work in two ways. Firstly, in some individuals, the unique combination may produce an animal that looks extremely like an inedible plant part. Secondly, the variations in outline and colour that may occur between neighbouring individuals make it difficult for a predator to become accustomed to finding this kind of prey item. Thus, a highly effective mechanism to transfer predator pressure on to more homogeneous species is effected. This may be what allows *Polys* to sometimes occur at unusually high densities for animals that rely on crypsis, which are more often well separated (Tinbergen, 1965).
As recorded in a number of the studies referenced by Oxford and Gillespie (1998), there is also the possibility of gradual colour changes, which might occur over several days, or even more slowly as a spider grows, as appears to occur in some *Eriophora* species (Main, 1976; Forster and Forster 1999). This may well occur in *Poltys*, as evidenced by some unusual black adult specimens recorded in burnt bushland habitats, but experimental evidence is lacking.

### 8.3.4. Prey and prey capture

Stowe (1986) appears to be correct in his assertion that moths are a major prey item of *Poltys*. Indeed, in an open tropical forest habitat in North-eastern Queensland in September 2003, of the *P. illepidus* females and juveniles surveyed on one evening, I estimated that about 50% of the spiders I saw were feeding, and all of the identifiable prey items were moths. Nonetheless, unlike some of the other moth-specialist genera discussed in Stowe’s article, *Poltys* do not feed solely on moths but take insects from a variety of orders.

*Poltys noblei* was the only species for which prey items were studied in any detail by placing nets under selected webs to catch prey remains. Unfortunately there were problems with the design of the nets, accessibility to suitable sampling areas and identification of prey remains so this part of the project was abandoned. In the absence of quantitative data, the following observations still provide a certain amount of information on prey. Moths are the main prey caught (pers. obs and information from D. Hain, pers. comm.) except possibly by spiderlings. These latter catch microlepidoptera, but also appear to eat a significant number of midges (Diptera) and also probably gain significant nutrition from pollen and other air-borne detritus when
they recycle their webs (Smith and Mommsen, 1984). For larger spiders, other types of prey recorded in the field include small flies (Diptera), lacewings (Neuroptera), a cockroach (Blattodea) and small chalcidoid wasps (Hymenoptera). Termites (Isoptera) were also seen in webs, but I am not sure that they were eaten. Poltys laciniosus in captivity also accepted leafhoppers and treehoppers (Hemiptera) but these were never seen in a web in the field. Of the various prey items fed to the captive spiders only Lepidoptera were heavily wrapped in silk, presumably to contain the messy scales. The others items were just wrapped enough to fold and/or hold the wings into a neat position. Fairly small prey (relative to the spider) of all kinds is plucked from the web and mashed into a small pellet (apparently without heavy wrapping, even for moths), so only larger prey remains are reasonably recognisable, except sometimes by the wings, which may be discarded. Extremely small prey (possibly that which is too small to handle) is apparently nipped to stop movement then left and ingested with the web silk. As far as I could tell, the spider only sometimes moves all the way to the prey; on other occasions, especially for smaller items, it pulls the web and prey in towards the hub, probably by holding on in the hub area and “walking” the spirals in towards the spider. The web springs out again after it is released, just leaving a narrow “laddered” pattern (visible in several areas of the web in Plate 8.1.b). This may help reduce damage to the catching area for relatively minor prey. This “pulling” may be the function of the hooked setae shown in Plate 2.2.e, which occur in neat rows on the front legs. Prey items that were rejected by Poltys included a mealworm, which I attempted to feed to a captive spider when no moths were available, and a native beetle. In the case of the mealworm, the spider moved away and avoided it for some time, but finally bit it and then cut it out of the web, starting at the posterior end of the mealworm’s body. The
spider’s venom appeared to quieten the mealworm but did not kill it. The beetle was approximately 5 mm long; it was found dead wrapped in a few lines of silk in a net that I had suspended below a *P. noblei* web in the field. In another case a flying insect of an unrecognised order (but not anything dangerous to the spider as far as I could tell) was seen to land on the web of a small *P. noblei* (the spider was much smaller than the insect). The spider initially moved towards it but then turned and retreated. I suspect either the potential prey was too large, or it was giving the wrong signals, as it did not seem to be caught but flew away after a few seconds.

*Poltys laciniosus* and *P. noblei* use the “bite-attack-wrap” method (Robinson, 1975, elaborated on by Stowe, 1986), which has been shown to be common for rapidly escaping, but non-dangerous prey such as Lepidoptera. The prey is grabbed using the spiny front legs (sometimes after a brief pause for assessment for a large moth), bitten, and held until it stops struggling enough to start the wrapping, which may be several minutes with a large moth. A leafhopper fed to a caged female was also bitten prior to wrapping, but I have no records for lacewings. The prey is ‘organised’ into a neat bundle and at least partly wrapped at the capture site. This initial wrapping can be brief but can also take some time if the moth is spread across a large area: the wings are slowly pulled into a neat cigar shape by adding ever tighter swathes of silk whilst progressively cutting them free from the surrounding web. During the final stages of the initial wrapping the spider sometimes uses the technique described by Robinson (1969b), where the bundle is cut free except for a point at each end and is spun around like a bobbin whilst swathes of silk are added rapidly using legs IV. The prey may also be spun whilst only attached at one end. Spinning the prey apparently takes some practice—on one caged spider’s first attempt the spider had not anchored itself
properly; as a result the spider spun around instead of the prey! The bundle is then cut free and is carried to the web centre; larger prey was carried on a line from the spinnerets, but I did not note whether smaller prey was instead carried in the jaws as discussed by Robinson (1969b). Larger prey at least is then attached to some part of the hub by a line. It may then be wrapped more thoroughly, or reeled in and eaten after a quick grooming session by the spider. If the spider is already feeding the wrapped bundle is usually left at the capture site in the web until the spider is ready for it (seen in all the species of the *P. laciniosus*-group), or occasionally attached at the edge of the hub; the first caught prey item may be discarded more quickly when there are more available, and small items may be discarded immediately if a large moth is subsequently caught. One old caged spider initially fed on the moth at the capture site without moving it to the centre and then took it up to her rest twig, but this spider was becoming rather ‘infirm’ and awkward. One captive spider was fed a large moth (abdomen ca 2 x the length of the spider’s abdomen, which was one of the longer forms). The spider bit at the base of the front wing and held on whilst the moth flapped and struggled for many minutes. The entire quietening and wrapping process took over an hour and during the proceedings she several times left the moth and ingested parts of the remaining web, possibly the silk needed to be recycled for wrapping. This was late one evening; the spider was still feeding until at least 0830 the next morning. Whilst watching several prey captures I have never noted that the spider bit the prey after the initial (long) bite, but this may be a lapse in recording and requires confirmation.

After wrapping and again after feeding the spider usually grooms itself, presumably to remove moth scales and stray silk strands. A discarded loose ball of silk and moth scales can often be found below where the spider was feeding, but it is not
clear whether this is from grooming or whether it is the area of the web that had scales stuck to it and has been discarded when the web was ingested.

One caged spider is worthy of special note. This female *P. laciniosus* matured from a juvenile collected about 8 months earlier; it was first transferred from a vial to the cage a few days prior to its final moult. The female had abnormal spinnerets and could not produce normal sticky silk; all the usual web-making motions were present, and the sticky spiral attachment points to the radii were visible as tiny flecks. Nevertheless, the “sticky” spiral itself was extremely sparse and apparently entirely absent through some sections of the web. The extent of its presence was difficult to assess because it was almost invisible as there appeared to be no glue droplets produced—moths hitting the web left no scales stuck. The spider was also slightly abnormal behaviourally—two males introduced to the cage finally managed to elicit the correct response from her in terms of taking up the mating position, but both were captured and eaten, apparently without mating. Despite the lack of a proper sticky spiral the female was able to capture moths successfully and produced 4 egg sacs (all infertile). She went on to survive through winter and in all lived for nearly 11 months as an adult—far longer than any of the mated females.

Occasionally caged females, including the abnormal female discussed above, apparently captured prey without making a web. On some evenings they would hang on a line beneath a twig with the front legs outstretched. I never observed the capture of freely flying prey from this position so whether the spider merely grabs a moth if it blunders into its grasp or whether they actively lunge for passing prey is unknown. Nevertheless, on several occasions a female that was observed in this position was later found feeding without any signs of a web; spiders would also often accept prey from
forceps when in this position, but would always retreat from proffered prey (or drop) if they were on a line but without outstretched legs. This might suggest it is a distinct alternative hunting technique, perhaps used on nights when the spider is not hungry enough to initiate web construction behaviour or possibly when the weather conditions signal that it is not worthwhile making a web. The caged observations all involved moth prey but a female in a garden situation was also observed feeding on a large lacewing with no sign that a web had been built (M. Gray, pers. comm.). Another web-making araneid spider that may also use this technique is *Dolophones*, which I have frequently observed hanging beneath a twig in an odd posture during the early part of the evening.

### 8.3.5. Dimorphism, courtship and mating

The courtship and mating behaviour of spiders was reviewed by Robinson (1982). In araneids courtship is usually initiated by the male vibrating the web of the female. The mating position is usually either at the hub of the web, or on a special line made by the male, which can be within the female’s web, or a separate structure. It is suggested that these positions may be characters of evolutionary significance. The correlation between these different tactics, sexual size dimorphism and sexual cannibalism has also been examined (Elgar, 1991). Elgar suggests that species with a relatively small difference in size between the sexes mostly use a separate mating thread and that this tactic may have evolved to maintain a large male size whilst avoiding sexual cannibalism. This would imply that a strong size dimorphism, such as seen in *Ptyris*, might be expected to correlate with no use of a mating thread. Sexual size dimorphism has also been investigated from a number of other viewpoints. Male
dwarfism in some species of *Metepaira* was suggested to be the result of a combination of reduced intrasexual competition and adaptation to favour the location of widely dispersed females in a harsh environment (Piel, 1996). This work stemmed from a model generated from work on *Nephila clavipes* (Vollrath and Parker, 1992), which also stimulated a robust discussion on whether size dimorphism between sexes is a result of females growing larger, or males becoming smaller (Coddington *et al.*, 1997, Vollrath and Parker, 1997). The summary phylogeny displayed by Coddington *et al.*, suggests that size dimorphism may have arisen separately in a number of different taxa, and thus the model presented by Vollrath and Parker (1992) may not be representative of all taxa. Nevertheless, Piel’s demonstration of support for this hypothesis in *Metepaira* could be of relevance to *Polys*; this could possibly provide part of the explanation of how an otherwise tropical spider might be able to move into the much harsher conditions present over much of southern Australia.

The courtship and mating of *Polys* have not previously been recorded. As reported in other taxa, subadult females attract males for at least a day or two before the final moult. A male of *P. laciniosus* put into a subadult female’s cage attempted to court the female as if she were adult; but she responded as though to an intruder, by shaking the web and chasing him away. A subadult female *P. noblei* found in a web in Ku-ring-gai Chase NP was surrounded by at least 6 males hanging on threads within about a 0.5 m radius. Unlike some other araneid species, the males may be too small to be able to force their attentions on a newly moulted female whilst she is helpless; a freshly moulted female *P. noblei* was seen to dissuade a male suitor by twitching and shaking her lines. Unfortunately I later lost track of the male. Whether a mating happened later on the same night or early on the subsequent evening I am not sure, but
there was no sign of the male when the female constructed a web on the following evening. In either scenario the female could not have had a web when mating occurred. Older mated females do not seem to attract males, but at what point the attraction ceases is not clear. I have also not investigated whether an old but virgin female could still be secreting pheromones.

Adult males are most often found in the wild either near a subadult female, or hanging on lines with front legs outstretched, presumably trying to detect traces of female sex pheromone on air currents. This position might suggest that the chemosensory organs are on leg I. As well as the circumstantial evidence of attraction from afar, even when close to a female, *P. laciniosus* males appear to use both airborne pheromones and those on silk lines to home in on the female’s position. When males are put into a cage containing a virgin female in a web they move around on the twigs and appear to sense where the female has been in the cage, presumably by a concentration of silk lines. But if they are having difficulty finding a web attachment point they may descend on a line with legs outstretched for a few seconds as though trying to obtain directional information before setting off again along the twigs.

Courtship has been observed for three *Poltys* species, but mating only for one, in a caged situation. In all three species the male approached the female in a web along one of her lower or middle side anchor threads. He plucked or strummed this line (see below). The female left the web centre and approached the male, to hang beneath the line the male was on (her anchor line). At this point it was unclear whether she had descended on a line of her own or one that the male had placed there for her. The female’s position was similar in each species: she faced the underside of her abdomen
towards the male, legs I and II were spread wide, leg III was tucked in (in *P. laciniosus* at least), and leg IV was out behind holding the line.

In *Polys milledgei*, the initial signal the male used to attract the female was not observed, but after the female had taken up position, the male then advanced slowly waving his first pair of legs from side to side in unison and presumably plucking or tapping the line, and hesitating frequently. Finally he advanced to meet the female, but each time he sprang away at the last minute to dangle on a line, but slightly closer each time. On this occasion the male eventually retreated so I collected them both and mating was not observed.

In *Polys laciniosus* courtships the male attracted the female by strumming on her line, at first gently, then energetically so that the whole spider vibrated with the line. After the female took up her position the male approached several times, but each time he reached her he swung away on a 4–5 cm line; after about the fifth approach he took a break for about 30 seconds, the female shuffled a bit (probably actually the encouraging “twitch” described below) and he started again. In one prolonged courtship this pattern was repeated about 20 times, with longer breaks between approaches as time progressed. The female appeared to lunge for him at least once as he approached. Also on several occasions when the male seemed to hesitate, the female appeared to entice him by moving her legs a little then flipping suddenly backwards to thrust the underside of the abdomen upwards; after this movement he always restarted his approaches immediately. Courtship sequences were observed on four occasions, but only two courtships apparently resulted in a mating. One of these was the extended sequence described above; the other (which was the only one to produce any fertile eggs) was much briefer, only involving two approaches by the male.
The final sequence that apparently led to copulation was enacted too fast, and the animals were too small, to see exactly what happened, but instead of swinging away the male ended up held to the female’s sternum by her leg III’s. Presumably one or both palpi are inserted during this time, but on the only occasion when the animals were close to the side of the cage and in view it was not obvious that the male could reach the female’s epigynum; nevertheless some eggs did develop after this mating so it seems reasonable to assume that insertion is somehow occurring. This position was maintained for approximately 8 minutes on each of the two matings observed, during which time the female was seen to occasionally manipulate the male’s body with her third legs. Finally the female “patted” the male with her pedipalps, then her posture changed and she transferred the male to her jaws and ate him (the pellets of remains were retrieved from the bottom of the cage on each occasion).

Given the way the males were handled by the females, it is difficult to see how there could commonly be any other outcome than cannibalism. On the occasion that the male was in view I could not see any movement at all, and it is possible that death occurs spontaneously as reported in *Argiope aurantia* by Foellmer and Fairbairn (2003). The two failed courtships involved the abnormal female discussed earlier; these also resulted in the male being eaten, but immediately, without the 8 minutes activity when the female held and manipulated the male using her third pair of legs. The death of males during mating would probably result in a short average life-span in the wild (and adult males did not usually survive long in captivity—see Chapter 7); this could partly explain why male *Poltys* are relatively rare in museum collections. One *P. laciniosus* with a broken embolus, among all the examined wild-caught males, is the only potential evidence that males may occasionally survive mating. I have never
noticed any females with any signs of broken embolus tips lodged in the copulatory ducts, so this damage may have arisen in some other way, or during capture.

A *Polys grayi* courtship was also observed in the wild. The male first approached along the top anchor line, then back-tracked and returned along the middle. Presumably he also strummed but this was not noted. The female took up the usual position but the male then vacillated, approaching and retreating several times. He also strummed at this point (but I have no record whether this was the side-to-side movement like *P. milledgei* or the plucking motion of *P. laciniosus*) and the female responded by twitching—a fast contraction of legs that seemed to encourage the male. He approached several times until he apparently touched the female, but then he jumped away and fell on a line. On the final approach he touched for slightly longer (but still less than a second I estimate) before dropping. On this occasion he retreated to the edge of the web and the female returned to the web centre. I could not stay longer and so collected both.

### 8.3.6. Egg sacs

Egg sacs are of similar shape within each species group, but quite distinctive between groups. Details of the number of eggs laid and the emergence times for captive-laid egg sacs are given in Chapter 7.

*Polys illepidus* egg sacs are finished with fluffy yellow silk over a cream inner layer (Plate 8.4.b). On two occasions they have been found laid in curled leaves at one end of the spanning web line. Nevertheless, several searches for egg sacs associated with females thought likely to have already laid eggs were unsuccessful, suggesting they may travel some distance to find a suitable place, or they are hiding them in some
unexpected way. Egg sacs of *P. stygius* were not seen in the wild by the author, but one laid by a captive female was a white fluffy sac overlaid with rose pink silk. The unidentified egg sac shown in figure 152 in Clyne (1969) appears to belong to this species. This egg sac is pictured on the underside of a green leaf.

The egg sacs of the two Australian *P. columnaris*-group species are similar in shape and colour. The small white ‘sac’ with overlay of cream, and sometimes grey or brown silk (Plate 8.4.c), is constructed on the underside of a dead twig.

Two *Poltys frenchi* egg sacs laid in captivity were of cream-coloured silk with lemon-yellow covering. One had a sparse outer layer and may not have been completed, the other was rather thicker and smoothly finished but with a hanging centre “tail” of silk (the similarly coloured *P. illepidus* egg sacs are more loosely finished). Placement in the wild is unknown.

In the *Poltys laciniosus* species-group the egg sacs are made along the underside of a twig, commonly where there is some other bump or a fork to disguise the shape (two *P. laciniosus* egg sacs are on the left of Plate 8.1.c, arrowed). In *P. laciniosus* they are of grey/brown silk with a sparse overlay of white, sometimes finished off with a light bobble of silk. *Poltys grayi* egg sacs are of white silk with an overlay of brown, usually finished off with a dark brown bobble of silk (Plate 8.4.d), whilst *P. noblei* egg sacs are of fawn to grey silk with an overlay of brown, usually finished off with a dark brown silk bobble (Plate 8.4.e). As in *P. illepidus*, some egg sacs of *P. noblei* have been laid close to the spider’s web-site, but others known to have been laid by females that were being observed on a regular basis, could not be found. The stimuli that might be involved in the selection of a suitable site are unknown. The unmated female *P. laciniosus* discussed earlier made four egg sacs, but I have never
found more than two close to a single female in the wild. Several captive females have remained sitting on egg sacs through the day immediately following construction, but returned to the normal position after the following night. In warm weather this behaviour could help protect the egg mass against parasitoids such as scelionid wasps. Austin (1984) reported that eggs laid by Clubiona robusta L. Koch were only suitable for egg deposition by Ceratobaeus masneri Austin (Sceleionidae) for 1.5 days when the temperature was 25°C, although the window of suitability rapidly increased with decreasing temperature. Some egg sacs were not completed until well after dawn.

8.3.7. Nocturnal predators and parasites

The major diurnal predators are probably wasps and to a lesser extent, birds, as discussed in Chapter 6. The only definite record of a nocturnal predator was of a spider. I owe this observation to David Hain, of Lane Cove in Sydney. David had been observing the medium-sized Poltys nobleri in his garden on a regular basis, but around midnight one night it was missing; below the web he found a long-legged spider with a loosely wrapped bundle. He duly brought me both the spider and its prey for examination. The spider was a subadult male Australomimetus maculosus (Rainbow) (Mimetidae); and the contents of the bundle were confirmed to be the missing Poltys. I have since observed several empty webs with the spider either missing entirely or hanging nearby to one side; sometimes there has also been another spider encroaching upon the web and some of these were mimetids or other potential araneophages. Other probable spider predators, especially of smaller Poltys, are whip-spiders (Argyrodes Simon (Theridiidae)), sac spiders (Cheiracanthium C.L. Koch (Miturgidae)) and jumping spiders (Salticidae), which are often seen hanging on lines near small Poltys at
night. If small *Poltys* are kept together they will often cannibalise their siblings during moulting when they cannot escape. This may also happen in the wild to some extent, where sometimes many spiderlings crowd into the same bush. Some bats have also been reported to be able to pluck nocturnal orb-weavers from their webs. In Australia the golden-tipped bat (*Kerivoula papuensis* Dobson) is reported to be a specialist feeder on small web-building spiders in or around rainforest areas (Law and Chidel, 2004); this species occurs locally along the east coast of Australia and in New Guinea (Richards, 1983).

The only egg sac parasites seen to have emerged from wild-collected egg sacs have been flies from the family Chloropidae (identified by David McAlpine), which emerged from a *P. jujorum* egg sac collected in Goldsborough State Forest in northern Queensland. No spiderlings were left to emerge. Most of the other egg sacs dealt with have been laid in captivity, so this is not a representative sample. There are many other recorded predators of spider egg sacs and eggs, but most of these do not usually destroy the entire brood. Nineteen species of insects and 11 species of spiders were recorded associated with the egg sacs of *Argiope aurantia* but few actually caused significant damage to either egg sacs or eggs, and many were only using an already broached egg sac as shelter (Lockley and Young, 1993). In this case the most damage was caused by birds; but as *A. aurantia* egg sacs are the over-wintering stage in the spiders’ life history these findings may not be directly relevant to *Poltys*, which usually hatch within a month (see Chapter 7). In Australia, araneid egg sacs have been recorded as parasitised or predated by insects from three orders (Hymenoptera (7 families), Diptera (3 families) and Lepidoptera (2 families) (Austin, 1985) [but note that *Nephila* was considered an araneid at that time]. About half of the eggs of a cage laid (but later
unprotected) egg sac of *P. laciniosus* in Sydney were found to have been eaten via a neat hole straight through the layers of silk by an unknown predator.

One incident involving emerging spiderlings was also recorded: a captive-laid egg sac of *P. illepidus* started hatching but then the spiderlings rapidly disappeared. I finally discovered that a *Cheiracanthium* sac spider had somehow gained entry to the container; it was living within the tangle of threads holding the egg sac on to a leaf and eating spiderlings as they emerged. Although this was in artificially constrained circumstances this could occasionally happen to emerging spiderlings in the field, especially where egg sacs are laid in rolled leaves, a favourite haunt of sac spiders.

### 8.3.8. Competition and other interactions

**Intraspecific competition**

Field observations of spider interactions around web-sites indicate that a larger *Poltys* can always displace a smaller one, which supports the reports of conspecific interactions from other studies (e.g. Heiling and Herberstein, 1999). But small juvenile *Poltys* often initiate web-building much earlier in the evening than larger conspecifics and can sometimes have a web up in a good position for an hour or more before the larger site-occupant becomes active and chases smaller animals away. The smaller spider loses this first web but makes a second web off to one side. The loss of the silk may be compensated by having access to a good web-site at dusk, when many potential prey insects become active. *Poltys*, along with many other spider species appear to readily use other spiders’ webs and lines as web supports or to move along. Possibly a slightly larger spider would actually use the web built by another spider if it suited its needs. I have observed a new occupant moving into the centre of the smaller spider’s
web and settle into a hunting posture for a few minutes before apparently deciding that it was not suitable and cutting out the old web to make space for the new one. Despite this apparent disregard for ownership, among Poltys it appears that spiders can recognise their own lines, as evidenced by their ability to return to their own rest sites even in a bush containing several interacting spiders. Sometimes there are many small spiders in one tree or bush, or a mixture of sizes. The smaller ones usually move around between web-sites—sometimes shifting several times in the same night. I also observed at least two small spiders apparently feeding consecutively on the same relatively large moth, which one of them had caught. It appeared that the first had its fill initially, the second then moved in and took over, and then finally the original spider came back for a second feeding session. This was judged by the positions of the spiders and the states of their webs over about a three hour period. The spiders were of similar sizes and I did not see the switch-over points.

Direct physical agonistic behaviour between individuals at web-sites was rarely seen. Nevertheless, two small to medium sized P. noblei were seen in direct combat. It was not possible to see the detail but a blur of movement caught my attention; this happened on a vertical frame-line of a former web from earlier that night. The situation was suddenly resolved as two apparently evenly matched spiders separated from each other onto separate lines. Neither was obviously hurt in the encounter, but one spider moved away whilst the other later moved to the hub position of the former web.

**Interspecific competition**

Other spiders (mainly Eriophora but also Phonognatha Simon) have been observed apparently taking over Poltys web-sites, and displacing the incumbent. It is
often difficult to judge the relative sizes given the different abdominal shapes, but Poltys seem to be displaced by similarly sized Eriophora. A juvenile Eriophora (?) was also observed feeding on a moth in what appeared to be a Poltys web. When first spotted, the Poltys, which was a medium–large-sized spider and, I assume, the owner of the web, was about 80 cm away on an anchor thread. The Poltys then started to move back into the web and the intruder moved off to the edge of the sticky spiral, leaving the moth in the web centre. But after a few minutes the Eriophora moved in once more and the Poltys again retreated, but this time to only about 20 cm away. At this point I had to carry on with a transect so further proceedings were not recorded.

Another observation was of a small araneid, again possibly an Eriophora, which made its web next to a small Poltys. Instead of using a solid substrate to anchor its lines the interloper attached them across the upper half of the Poltys web. By the time I first noticed it the Poltys had already retreated and was on a line nearby.

8.3.9. Brief comments on the use of light and insect repellent during field studies

In my preliminary night observations the spiders sometimes seemed affected by my presence. I was unsure whether this might be due to the torchlight or to wearing insect repellent. By trial and error I found that the spiders are not affected by the light as long as it is not shone directly into their eyes (they will quickly take the web down if this happens). I tried placing coloured filters over the torch glass as suggested in some other publications, but it proved too difficult to see any detail using these. New torch batteries are too bright, but using older batteries for monitoring these spiders there is generally no problem. The new batteries can be used to initially locate spiders (which is easiest with a strong light) until they lose some power.
The use of insect repellent, however little, definitely can affect spider behaviour and they will often move out of the web and away from the direction of the observer without any obvious cause. All later observations were made when not using insect repellent. If collecting or just locating spiders is the aim then there is no problem with the use of repellent as long as it is kept off the hands if the spiders are wanted alive.
CONCLUDING REMARKS

The scope of this project was rather broad. This breadth has inevitably resulted in some omissions and unevenness as to the depth of treatment, as well as some data that could not be fully explored. Nevertheless, the study advances our knowledge of the taxonomy, biology and behaviour of a peculiar and little studied genus of nocturnal orb web weaving spider, and hopefully paves the way for further research in several areas.

Several problems were encountered during this project, some of which influenced the scope and direction of the work. The first unexpected problem with *Poltys* was the remarkably wide intra-specific variations in abdominal shape, which made elucidation of the species boundaries rather more time-consuming than would normally be expected. A second problem arose with locating and obtaining types. Due to rebuilding work and understaffing, a considerable delay (almost 3 years) was experienced in obtaining the loan of much of Thorell’s material, housed in Genoa, Italy. Several other relevant types, including the type species of the genus, have still not been located. A further inconvenience was the drought that slowly intensified through the main field-working years of the study. Specimens, especially larger ones, became increasingly scarce in many of the areas around Sydney that were otherwise suitable for my studies. This situation was exacerbated as many potential sites became affected by fire after controlled burns were widely employed in the suburban bushland reserves to cut down the danger from wild-fires. The results for some aspects of the field studies are therefore rather provisional and ideally would benefit from further data. The final problem, which was the only one known about at the commencement of the study, was the paucity of males in collections. This problem was made more acute than expected
due to the problems of separating females but was overcome by extensive fieldwork and by raising males from eggs.

Despite these problems and shortcomings, the project has achieved results in several areas. In systematics:

- the taxonomy of Australian Pollys has been revised: of the 10 original species originally described from Australia only two remain valid; two already described species from outside Australia account for all the rest; another four species are newly described;

  - one new species is described from New Caledonia and the Loyalty Islands; this is the only species outside Australia in which males and females could be matched;

  - the relationships of Pollys are still uncertain, but it is extremely unlikely that the tribe Poltyini is a monophyletic grouping;

  - data on Pollys, and on other genera from the tribe Poltyini, are now published and available for wider use;

  - a preliminary phylogeny of the Australian species using morphological and DNA data supports the informal species groups used in the taxonomic section (at least in an Australian context);

  - the phylogeny indicates that Pollys frenchi is most closely related to the endemic P. laciniosus-group. Relationships higher than this clade and the species groups are unresolved due to inadequate coverage of the SE Asian taxa.

The considerable volume of data recorded has also revealed or confirmed various aspects of the biology and behaviour of Pollys. In particular:

- variability of abdominal shape is quite extreme in the endemic P. laciniosus-group and in P. frenchi (already reported in the P. illepidus-group);
- different abdominal shapes can develop amongst spiders from the same egg sac. (Also already reported in the *P. illepidus*-group);

- *Poltys* males and females mature at different times hence overlapping generations are required for continuity;

- continuity is facilitated by plasticity in growth rates of females; female generation times of between one and three years were seen in reared *P. laciniosus; P. noblei* in the wild appeared to grow at rates that would have resulted in one to two year generations;

- *Poltys noblei* show a far higher period of web-site tenacity than would be expected for a spider without significant material relocation costs;

- *Poltys noblei* are unevenly distributed in terms of their aspect and position on bushes when hiding during the day.

- The points listed above are suggested to be facets of adaptation to avoid predation and thereby exploit the open and exposed microhabitat of dead twigs, which are in abundance in the Australian environment.

Taxonomically, the next major challenge in *Poltys* from an Australian viewpoint should be work on the SE Asian fauna, a task that, judging by my Australian experience, will necessitate a significant amount of fieldwork. This will help place the Australian endemic fauna in a better framework for evolutionary studies. I hope the work presented here will help to stimulate and guide future endeavours in this direction.

Studies on araneid generic relationships are continuing (T. Blackledge & N. Scharff, pers. comm.) but now with the addition of fully identified *Poltys* specimens.
made available by this study. The above workers are using both morphology and DNA characters and I will be fascinated to find out what these much more comprehensive data sets reveal compared to the limited data available to me in the present study.

*Poltys* has also emerged as an interesting genus for studying aspects of cryptic behaviour. I was fortunate, in August 2000, to move into an area where I could study *Poltys* on my doorstep (I can see the gate at the head of the transect, shown in Figure 6.1.b, from where I sit typing now). Without this proximity to a suitable study site, the regular transect would have been much more difficult, especially the confirmation that some of the longer-term specimens were really the same animal dusk and dawn, night and day. I hope that the behavioural information gathered here can be built upon and that it will stimulate further studies examining the roles of nocturnal orb-weaving spiders both as predators and as prey.
REFERENCES

Adams, E.N. III. (1972). Consensus techniques and the comparison of

the survival of three species of sac spiders on natural and artificial diets.
*Journal of Arachnology* 29: 253–262.

hunting spider *Hibana velox* (Araneae: Anyphaenidae), raised on


(Araneae, Argiopidae). *Revue de Zoologie et de Botanique Africaines*
72: 79–82.

Ausserer, A. (1871). Neue Radspinnen. *Verhandlungen der Zoologisch-

Austin, A.D. (1984). The fecundity, development and host relationships of
*Ceratobaeus* spp. (Hymenoptera: Scelionidae), parasites of spider eggs.

Austin, A.D. (1985). The function of spider egg sacs in relation to parasitoids
and predators with special reference to the Australian fauna. *Journal of
Natural History* 19:359–376.


Main, B.Y. (1999). Notes on the Biogeography and natural history of the orbweaving spider *Carepalxis* (Araneae, Araneidae), including a gumnut


a discussion on phylogenetic methods. *Invertebrate Systematics* 17: 185–259.


Rambaut, A. (1996). Sequence alignment editor version 1.0 a1. The package and information is available from the WWW site:


http://www.americanarachnology.org/JoA_toc5/JOA_contents_v33n2.html


