CSIRO PUBLISHING
www.publish.csiro.au/journals/ajz *Australian Journal of Zoology*, 2008, **56**, 103–115

Evolution and maintenance of colour pattern polymorphism in *Liopholis* (Squamata : Scincidae)

David G. Chapple[A,B,G], *Mark N. Hutchinson*[C], *Brad Maryan*[D], *Mike Plivelich*[E], *Jennifer A. Moore*[F] *and J. Scott Keogh*[B]

[A]Museum Victoria, Division of Sciences, Herpetology Section, GPO Box 666, Melbourne, Vic. 3001, Australia.
[B]School of Botany and Zoology, Australian National University, Canberra, ACT 0200, Australia.
[C]South Australian Museum, Herpetology Section, Adelaide, SA 5000, Australia.
[D]Western Australian Museum, Terrestrial Vertebrates, Locked Bag 49, Welshpool DC, WA 6986, Australia.
[E]Ministry of Agriculture and Forestry, PO Box 2526, Wellington, New Zealand.
[F]Allan Wilson Centre for Molecular Ecology and Evolution, School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand.
[G]Corresponding author. Email: dchapple@museum.vic.gov.au

Abstract. We examined the evolution and maintenance of colour pattern polymorphism in an Australian lineage of scincid lizards, the genus *Liopholis. Liopholis* comprises 11 species, with representatives in both the temperate zone and arid zone. Specimens from all major Australian museums were examined to characterise colour pattern polymorphism within *Liopholis*, and investigate geographic variation in the relative abundance of morphs within polymorphic species. We used a previously published phylogeny for *Liopholis* to investigate the evolution and maintenance of colour pattern polymorphism within the group. Five species were found to exhibit colour pattern polymorphism (*L. margaretae margaretae* Storr, *L. m. personata* Storr, *L. montana* Donnellan *et al.*, *L. multiscutata* Mitchell & Behrndt, *L. pulchra* Werner, *L. whitii* Lacépède), with six species being monomorphic (*L. guthega* Donnellan *et al.*, *L. inornata* Rosén, *L. kintorei* Stirling & Zietz, *L. modesta* Storr, *L. slateri* Storr, *L. striata* Sternfeld). Three colour morphs occur in *L. whitii*, with the relative abundance of each varying significantly among latitudes. The patterned morph is most common, while the incidence of the plain-back morph decreases at latitudes higher than 35°S. The *L. whitii* patternless morph occurs only within a narrow latitudinal band (34–38°S). In *L. multiscutata*, the relative abundance of the patterned (~89–93%) and patternless morph (~7–11%) is consistent across regions, except for the Nullarbor Plain region where the patternless morph is more common (~39%). Our analyses suggest a single origin of colour pattern polymorphism in *Liopholis*, followed by the subsequent loss of polymorphism on four occasions. The secondary loss of polymorphism might be associated with climate or habitat, possibly as the result of shifts into the arid zone or alpine regions of Australia. This study provides the necessary framework for future studies of colour pattern polymorphism in *Liopholis*.

Introduction

Colour pattern polymorphism (CPP) refers to the simultaneous occurrence of two or more distinct, genetically based colour and/or pattern phenotypes in a population (Hoffman and Blouin 2000; Gray and McKinnon 2007). CPP has evolved on numerous occasions in invertebrates (e.g. Hughes and Mather 1986; Losey *et al.* 1997; Forsman *et al.* 2002), fish (e.g. Franck *et al.* 2001), amphibians (Hoffman and Blouin 2000), birds (Galeotti *et al.* 2003; Roulin 2004) and mammals (e.g. Hoekstra *et al.* 2004). However, the occurrence of CPP is particularly widespread in squamate reptiles (e.g. Capula and Luiselli 1994; Forsman and Shine 1995; Johnston 1996; Shine *et al.* 1998; Rosenblum *et al.* 2004; Forsman and Aberg 2008).

Within the *Egernia* Group, a radiation of Australasian skinks (~47 species: Gardner *et al.* 2008), at least five species exhibit CPP, all of these being members of the genus *Liopholis* (Chapple 2003; Table 1; note that Gardner *et al.* (2008) recently resurrected *Liopholis* to accommodate the members of the former *Egernia whitii* species group). *Liopholis* represents a monophyletic lineage within the *Egernia* Group, and comprises 11 species that can be characterised as either rock-dwelling species (*L. guthega* Donnellan *et al.*, *L. margaretae* Storr, *L. modesta* Storr, *L. montana* Donnellan *et al.*, *L. pulchra* Werner, *L. whitii* Lacépède) or obligate burrowing species (*L. inornata* Rosén, *L. kintorei* Stirling & Zietz, *L. multiscutata* Mitchell & Behrndt, *L. slateri* Storr, *L. striata* Sternfeld) (Chapple 2003; Chapple and Keogh 2004; Chapple *et al.* 2004, 2005; Gardner *et al.* 2008) (Table 1). CPP in *Liopholis* is generally evident in dorsal and/or lateral colour pattern. The attributes of CPP among species are remarkably similar, with three distinct colour morphs generally evident: patterned morph (i.e. dorsal and lateral patterning), plain-back morph (i.e. lateral patterning, but lacks dorsal patterning), and patternless morph (i.e. lacks dorsal and lateral patterning) (Chapple 2003). However, in some species only two of these three

10.1071/ZO08040 0004-959X/08/020103

Table 1. Characteristics of colour pattern polymorphism in *Liopholis* species (modified from Chapple 2003)
The habitat preferences of each species are indicated: RD, rock-dweller; OB, obligate burrower

Species	No. of morphs	Morph types	Most frequent morph	Reference
Liopholis guthega (RD)	1	–	–	Donnellan *et al*. (2002)
Liopholis inornata (OB)	1	–	–	This study
Liopholis kintorei (OB)	1	–	–	This study
Liopholis margaretae margaretae (RD)	2	Patterned, patternless	Patternless	Horner (1991), Donnellan *et al*. (2002)
Liopholis margaretae personata (RD)	3	Spotted, striped-back, patternless	Striped-back	This study
Liopholis modesta (RD)	1	–	–	Chapple (2003), this study
Liopholis montana (RD)	2	Patterned, plain-back	Plain-back	Donnellan *et al*. (2002)
Liopholis multiscutata (OB)	2	Patterned, patternless	Patterned	Donnellan *et al*. (2002), this study
Liopholis pulchra pulchra (RD)	2	Patterned, plain-back	Patterned	Ford (1963), Ford (1965), this study
Liopholis pulchra longicauda (RD)	1	Patterned	–	This study
Liopholis slateri slateri (OB)	1	–	–	This study
Liopholis slateri virgata (OB)	1	–	–	This study
Liopholis striata (OB)	1	–	–	This study
Liopholis whitii (RD)	3	Patterned, plain-back, patternless	Patterned	Donnellan *et al*. (2002), this study

colour morphs are present. Each colour morph represents a discrete colour pattern, without the occurrence of intermediate forms (Chapple 2003, 2005). Apart from colour pattern, colour morphs within a species do not appear to differ morphologically (e.g. Milton *et al*. 1983).

Within *Liopholis*, CPP is heritable and is present in both sexes (Milton 1990; Chapple 2003, 2005). The mechanism of inheritance is autosomal, and is largely consistent with simple Mendelian inheritance (Milton 1987, 1990; Chapple 2003, 2005). Chapple (2003) hypothesised that there was a single origin of CPP in the *Liopholis* ancestor, followed by the secondary loss of CPP on multiple occasions. Within species that exhibit CPP, there appears to be considerable geographic variation in the relative abundance of each colour morph (Chapple 2003); however, this has been examined in detail only in *L. whitii*. The relative abundance of the plain-back morph of *L. whitii* varies geographically, being completely absent from Tasmania, but extremely common in regions of South Australia (e.g. Kangaroo Island) (Milton 1990; Donnellan *et al*. 2002). Milton (1990) examined museum specimens, and using state as a proxy for latitude, suggested that the relative incidence of the plain-back morph of *L. whitii* decreased with increasing latitude (Queensland: 0.26, $n = 131$; New South Wales: 0.21, $n = 541$; Victoria: 0.10, $n = 828$). The mechanism(s) responsible for this geographic variation in relative abundance of colour morphs remains unclear, as relatively few differences in behaviour, ecology, reproductive ecology, and life history have been documented between the colour morphs of *L. whitii* (Milton *et al*. 1983; Milton 1987, 1990; Chapple 2003, 2005; Chapple and Keogh 2005, 2006).

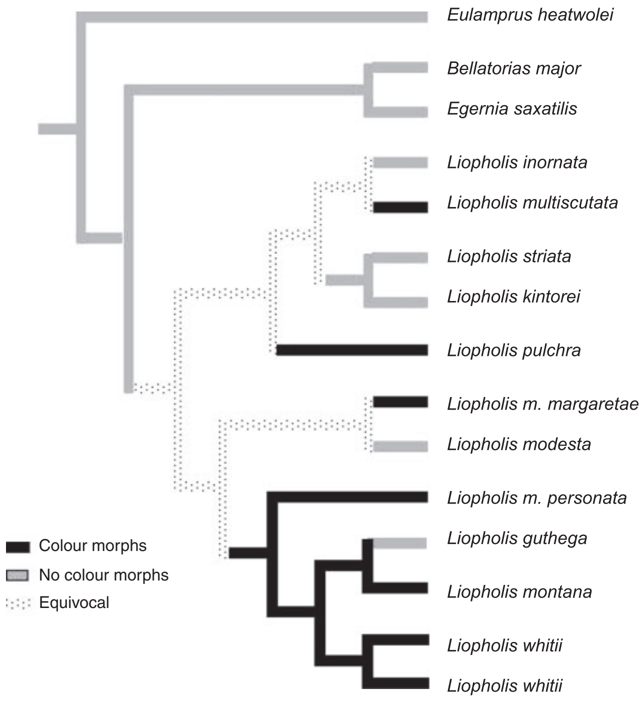

Fig. 1. Evolution of colour pattern polymorphism in *Liopholis*, indicating the origins/losses of polymorphism (phylogenetic tree adapted from Chapple and Keogh 2004).

In this study we examine museum specimens and utilise a published phylogeny for *Liopholis* (Chapple and Keogh 2004) in order to:

(1) determine which *Liopholis* species exhibit CPP, and characterise the attributes of CPP in each of these species;
(2) investigate the evolution (or loss) of CPP in *Liopholis* and test the hypothesis of Chapple (2003) that there was a single origin of CPP, followed by multiple losses of CPP; and
(3) examine the geographic variation in relative colour morph abundance within species, and specifically test the hypothesis of Milton (1990) that the relative incidence of the plain-back morph of *L. whitii* decreases with increasing latitude.

Materials and methods

Examination of museum specimens

During 2002–03, we examined specimens from all *Liopholis* species at all major Australian museums: Australian Museum (AM), Queensland Museum (QM), CSIRO Australian National Wildlife Collection (ANWC), Museum Victoria (MV),

Table 2. Overall incidence of each colour morph (patterned, plain-back, patternless) in polymorphic *Liopholis* species
Figures in parentheses indicate the sample size for each colour morph. n.a., not applicable

Species	*N*	Patterned	Plain-back	Patternless
Liopholis whitii	2145	82.84% (1777)	14.45% (310)	2.71% (58)
Liopholis multiscutata	549	90.16% (495)	n.a.	9.84% (54)
Liopholis margaretae margaretae	32	34.38% (11)	n.a.	65.62% (21)
Liopholis margaretae personata	55	30.91% (17)[A]	43.64% (24)[A]	25.45% (14)
Liopholis montana	43	9.30% (4)	90.70% (39)	n.a.
Liopholis pulchra	143	83.22% (119)	16.78% (24)	n.a.
Liopholis pulchra pulchra	111	78.38% (87)	21.62% (24)	n.a.
Liopholis pulchra longicauda	32	100% (32)	n.a.	n.a.

[A] In *L. m. personata*, the 'Patterned' column refers to the Patterned (spotted) morph, and the 'Plain-back' column refers to the Patterned (striped-back) morph.

Tasmanian Museum and Art Gallery (TMAG), Queen Victoria Museum (QVM), South Australian Museum (SAM), Western Australian Museum (WAM), and the Northern Territory Museum and Art Gallery (NTMAG). For species listed by Chapple (2003) as exhibiting CPP, we examined all available specimens at each museum. For species reported to lack CPP, we examined all available specimens at the AM, QM, ANWC and MV to confirm the absence of CPP in these species. For each specimen, we recorded: museum registration number, collection locality details (including state, latitude and longitude) and colour morph. Since CPP in *Liopholis* exhibits autosomal inheritance (i.e. polymorphism is present in both sexes: Milton 1987; Chapple 2005), the sex of each specimen was not recorded.

For *L. whitii* we examined geographic variation using 2° latitude bands, except for the northern and southern extremes of the distribution where 3° latitude bands were used (in order to attain sufficient sample sizes). Geographic variation in *L. multiscutata* was assessed using 4° longitude bands (a 8° longitude band was used in the Nullarbor Plain region to attain a sufficient sample size). Geographic variation in relative morph frequency was not examined statistically in *L. margaretae*, *L. montana* and *L. pulchra* because of their restricted distribution

Fig. 2. The three *Liopholis whitii* colour morphs: (*a*) patterned morph (Antechamber Bay, Kangaroo Island, South Australia), (*b*) plain-back morph (Kilmore Reservoir, Mt Disappointment, Victoria), and (*c*) patternless morph (Cape Hart, Kangaroo Island, South Australia).

and/or small number of museum specimens. Chi-square tests, conducted in SPSS ver. 16.0 (Chicago, IL, USA), were conducted to examine variation in the relative abundance in colour morphs among latitudes and longitudes.

We follow the taxonomy of Wilson and Swan (2008) and Gardner *et al.* (2008) for *Liopholis*. In particular, we consider the two subspecies of *L. pulchra* (*L. p. pulchra* and *L. p. longicauda*) and of *L. slateri* (*L. s. slateri* and *L. s. virgata*) separately. However, recent molecular evidence indicates that the two subspecies of *L. margaretae* (*L. m. margaretae* and *L. m. personata*) are genetically distinct (Chapple and Keogh 2004; Chapple *et al.* 2005). We therefore consider *L. m. margaretae* and *L. m. personata* separately in this study.

Evolution of colour pattern polymorphism in Liopholis

We used the molecular phylogeny from Chapple and Keogh (2004) as the basis for a simple comparative analysis of the colour pattern polymorphism in *Liopholis*. The phylogeny was simplified so that only a single representative of each taxon was

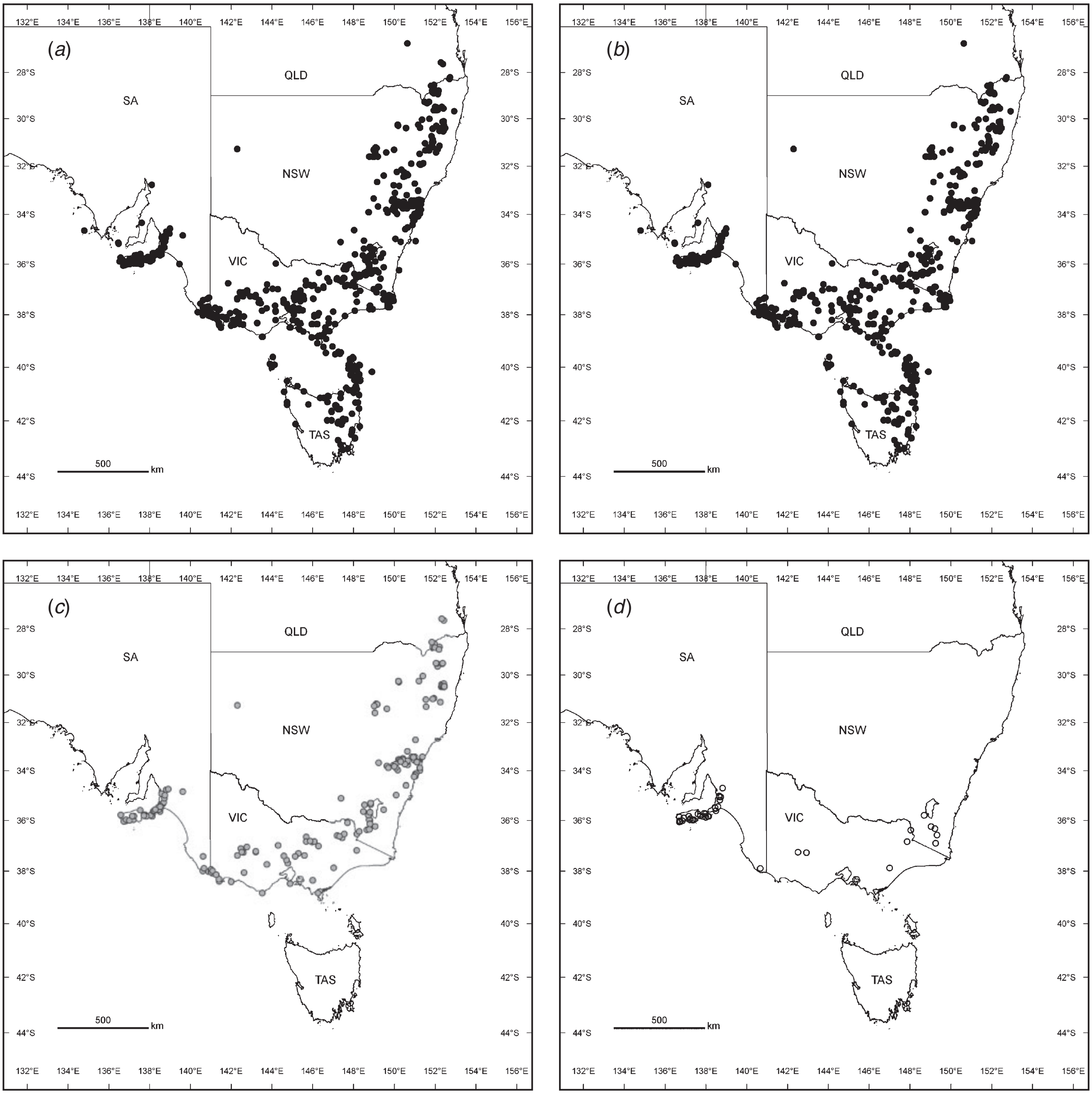

Fig. 3. Distribution of *Liopholis whitii* museum specimens: (*a*) all *L. whitii* specimens, (*b*) patterned morph, (*c*) plain-back morph, and (*d*) patternless morph.

included. We mapped the presence or absence of CPP in each taxon using MacClade 4.08 (Maddison and Maddison 2000) in order to determine the pattern of gains or losses in CPP in an evolutionary context.

Results

Incidence of colour pattern polymorphism in Liopholis

Examination of specimens from Australian museums confirmed the presence of CPP in five *Liopholis* species: *L. margaretae* (present in both *L. m. margaretae* and *L. m. personata*), *L. montana*, *L. multiscutata*, *L. pulchra* and *L. whitii* (Table 1). However, due to the limited number of specimens available of *L. montana* ($n = 43$), and the low incidence of the patterned morph, it is possible that the variation observed in *L. montana* represents continuous colour pattern rather than the presence of two discrete colour morphs. CPP was found to be absent in six species: *L. guthega*, *L. inornata*, *L. kintorei*, *L. modesta*, *L. slateri* and *L. striata* (Table 1). The incidence of CPP was higher in rock-dwelling species (four of six species) than in obligate burrowing species (one of five species) (Table 1).

Evolution of colour pattern polymorphism in Liopholis

Our phylogenetic analysis of the evolution of CPP was unable to determine the ancestral state (i.e. polymorphic or monomorphic) within *Liopholis* (Fig. 1). However, if CPP represents the ancestral state within *Liopholis*, CPP has been lost on four separate occasions: (1) *L. guthega*; (2) *L. modesta*; (3) *L. striata* and *L. kintorei*; and (4) *L. inornata* (Fig. 1). Alternatively, if CPP represents the derived state, CPP has evolved independently on four separate occasions: (1) *L. whitii*, *L. montana* and *L. m. personata*; (2) *L. m. margaretae*; (3) *L. pulchra*; and (4) *L. multiscutata* (Fig. 1). However, the latter scenario also requires the secondary loss of CPP in *L. guthega* (Fig. 1).

Colour pattern polymorphism in Liopholis whitii

Three distinct colour morphs are present in *L. whitii*: patterned morph, plain-back morph, and patternless morph (Fig. 2, Tables 1, 2). Only the patterned form was present in Tasmania (Fig. 2). There is substantial latitudinal variation in the relative abundance of each colour morph ($\chi^2 = 304.4$, d.f. = 14, $P < 0.001$; Table 3; note that 105 museum specimens lacked specific collection localities). The relative abundance of the patterned colour morph increases with latitude, while the incidence of the plain-back morph decreases (Table 3, Fig. 3). However, since colour morph frequency remains constant across the first four latitude bands (26–28°S to 33–34°S; $\chi^2 = 3.6$, d.f. = 6, $P = 0.726$), the incidence of the plain-back colour morph does not begin to decline significantly until latitudes higher than 35°S (Table 3, Fig. 3). The patternless morph occurs only in a narrow latitudinal band (34–38°S), although its incidence is significantly greater in the 35–36°S latitudinal band compared with the other two latitudinal bands in which it occurs ($\chi^2 = 54.7$, d.f. = 1, $P < 0.001$; Table 3, Fig. 3).

Fig. 4. The two *Liopholis multiscutata* colour morphs: (*a*) patterned morph (Boyagin Rock, Western Australia), and (*b*) patternless morph (Boyagin Rock, Western Australia).

Table 3. Latitudinal variation in the incidence of each *Liopholis whitii* colour morph (patterned, plain-back, patternless) ($n = 2040$)

Figures in parentheses indicate the sample size for each colour morph

Latitude (°S)	*N*	Patterned	Plain-back	Patternless
26–28	73	72.60% (53)	27.40% (20)	0% (0)
29–30	201	71.14% (143)	28.85% (58)	0% (0)
31–32	93	78.49% (73)	21.51% (20)	0% (0)
33–34	222	71.17% (158)	28.38% (63)	0.45% (1)
35–36	532	75.19% (400)	14.85% (79)	9.96% (53)
37–38	446	88.12% (393)	10.98% (49)	0.90% (4)
39–40	385	100% (385)	0% (0)	0% (0)
41–43	88	100% (88)	0% (0)	0% (0)

Table 4. Longitudinal variation in the incidence of each *Liopholis multiscutata* colour morph (patterned and patternless) ($n = 537$)

Figures in parentheses indicate the sample size for each colour morph

Longitude (°E)	*N*	Patterned	Patternless
113–116	85	92.94% (79)	7.06% (6)
117–120	87	88.51% (77)	11.49% (10)
121–124	57	91.23% (52)	8.77% (5)
125–132	28	60.71% (17)	39.29% (11)
133–136	236	93.22% (220)	6.78% (16)
137–141	44	88.64% (39)	11.36% (5)

Colour pattern polymorphism in Liopholis multiscutata

Two distinct colour morphs occur in *L. multiscutata*: patterned morph and patternless morph (Fig. 4, Tables 1, 2). There is significant regional variation (as measured by ~4° longitudinal bands) in the relative abundance of each *L. multiscutata* colour morph ($\chi^2 = 31.0$, d.f. = 5, $P < 0.001$) (note that 12 museum specimens lacked specific collecting localities), although this is entirely due to the increased frequency of the patternless morph in the Nullarbor Plain region (longitude 125–132°E) (excluding longitudes 125–132°E: $\chi^2 = 2.6$, d.f. = 4, $P = 0.622$; Table 4, Fig. 5). The patternless morph occurs in relatively low abundances (6.78–11.49%) across the range of *L. multiscutata*, except for the Nullarbor Plain region (longitude 125–132°E), where it is substantially more common (39.29%) ($\chi^2 = 28.74$, d.f. = 1, $P < 0.001$; Table 4, Fig. 5).

Colour pattern polymorphism in Liopholis margaretae

Two distinct colour morphs are present in *L. margaretae margaretae*: patterned (spotted) morph and patternless morph (Fig. 6, Table 1). The patternless colour morph is the more abundant of the two morphs, although both have relatively similar distributions (Fig. 7, Table 2).

Three distinct colour morphs occur in *L. margaretae personata*: patterned (spotted) morph, patterned (striped-back)

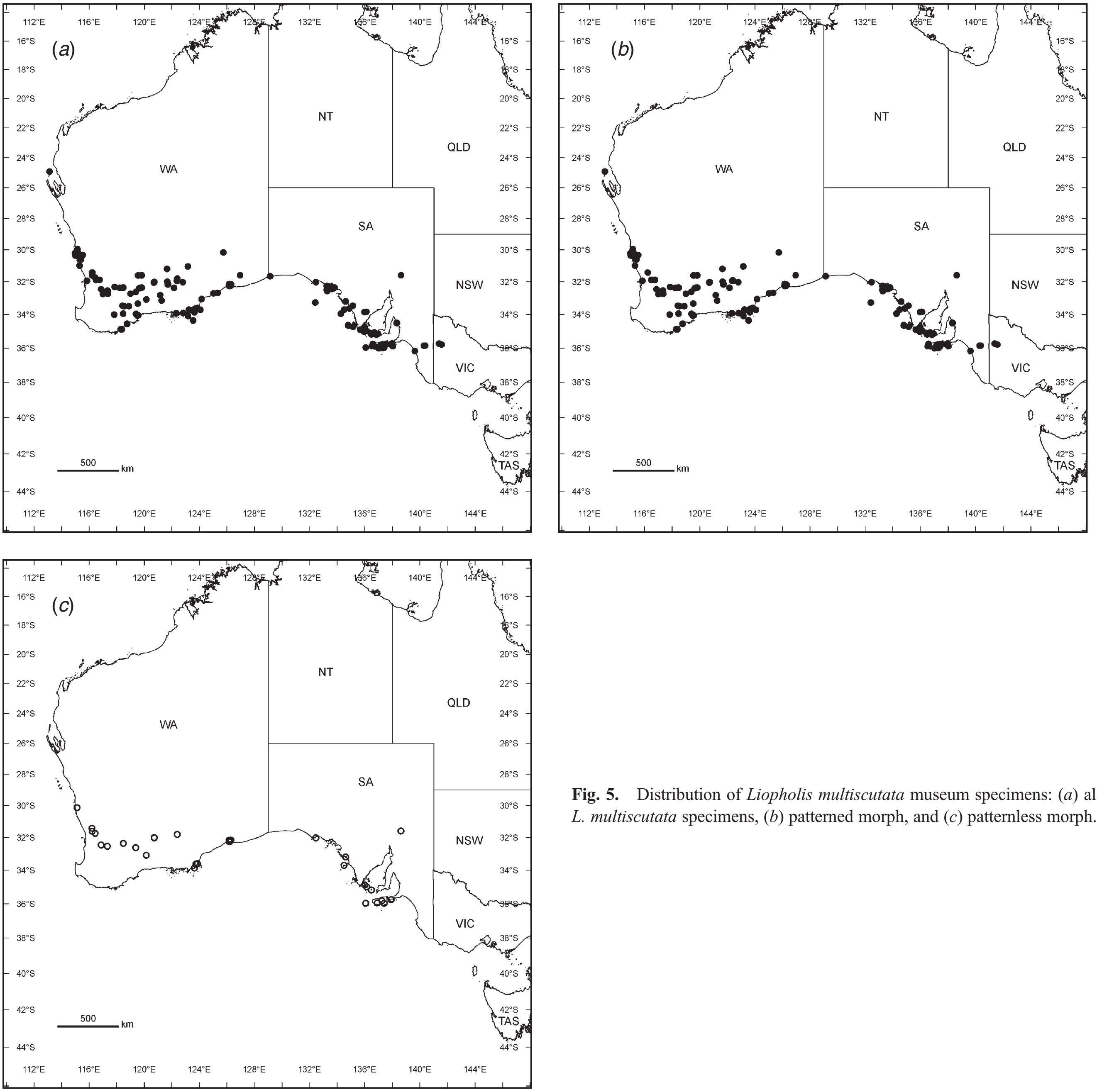

Fig. 5. Distribution of *Liopholis multiscutata* museum specimens: (*a*) all *L. multiscutata* specimens, (*b*) patterned morph, and (*c*) patternless morph.

morph, and patternless morph (Fig. 8, Table 1). The patterned (striped-back) morph is the most common colour morph and is present across the entire range of *L. m. personata* (Fig. 7, Table 2). However, both the patterned (spotted) morph and patternless morph appear to be absent from the southern end of the range (southern Flinders Ranges) (Fig. 7).

Colour pattern polymorphism in Liopholis pulchra

Two distinct colour morphs are evident in *L. pulchra*: patterned morph and plain-back morph (Fig. 9, Table 1). Only the patterned form is present in *L. pulchra longicauda* (Table 2). In *L. pulchra pulchra*, the patterned morph has a higher relative abundance than the plain-back morph, although both occur across the entire distribution of *L. p. pulchra* (Fig. 10, Table 2).

Colour pattern polymorphism in Liopholis montana

Two distinct colour morphs occur in *L. montana*: patterned morph and plain-back morph (Fig. 11, Table 1). The plain-back morph is substantially more abundant and widespread than the patterned colour morph (Fig. 12, Table 2).

Discussion

This study represents the most comprehensive examination of CPP in *Liopholis* conducted to date. Our examination of museum specimens has confirmed that five species, and six taxa (*L. m. margaretae*, *L. m. personata*, *L. montana*, *L. multiscutata*, *L. pulchra*, *L. whitii*), within *Liopholis* exhibit CPP, while the remaining six species (*L. guthega*, *L. inornata*, *L. kintorei*, *L. modesta*, *L. slateri*, *L. striata*) do not exhibit CPP. Here we discuss the geographic variation in the relative colour morph abundance within polymorphic species, and examine the maintenance and secondary loss of CPP within *Liopholis*.

Geographic variation in relative colour morph abundance in L. whitii, L. multiscutata *and* L. m. personata

The existence of geographic variation in relative colour morph abundance in *L. whitii* has been known for several decades (Henzell 1972; Milton 1990; Donnellan *et al*. 2002). Milton (1990), using Australian states as a proxy for latitude, suggested that the relative incidence of the plain-back colour morph decreases with increasing latitude (i.e. relative abundance decreases from Queensland to Tasmania). However, there are several potential problems with Milton's (1990) examination of colour polymorphism in *L. whitii*. First, it is unclear whether using arbitrary Australian state borders is an effective method for inferring latitudinal patterns in relative abundance of colour morphs. Second, Milton (1990) did not consider South Australian populations of *L. whitii*. Third, two recently described species (*L. guthega*, *L. montana*: Donnellan *et al*. 2002), previously part of *L. whitii* (as *E. whitii*), are likely to have been included in Milton's (1990) study. Finally, Milton (1990) appears to have overlooked the presence of a third, patternless colour morph in *L. whitii* (Donnellan *et al*. 2002).

Our analyses confirm Milton's (1990) suggestion that there is significant geographic variation in relative abundance of colour morphs in *L. whitii*. However, our latitudinal analyses reveal more fine-scale patterns in the relative abundance of colour morphs. The patternless morph is extremely rare, and is restricted to a very narrow latitudinal band (34–38°S, but predominately 35–36°S). The distribution of the plain-back colour morph is also more complex than indicated by Milton (1990). Although our analyses indicate that there is a significant negative relationship between latitude and the relative incidence of the plain-back colour morph, this pattern is restricted to the southern portion of the *L. whitii* range. Relative abundance of colour morphs is consistent (~72% patterned morphs, ~28% plain-back morphs) across the northern end of the *L. whitii* range (~26–34°S), with the significant decline in plain-back colour morph abundance evident only at latitudes higher than 35°S (Table 3).

What mechanisms drive this geographic variation in relative colour morph abundance? It is currently unclear why the incidence of the plain-back morph decreases with latitude and what restricts the patternless morph to such a narrow latitudinal band. However, similar geographic patterns are evident in other squamate reptile species in Australia (e.g. Forsman and Shine 1995; Johnston 1996). For instance, Forsman and Shine (1995) demonstrated that there are two colour morphs (striped and unstriped morphs) evident in the delicate skink (*Lampropholis delicata* De Vis), with the incidence of the striped morph decreasing with increasing latitude. A parallel pattern of increasing prevalence of previous tail autotomy with increasing latitude also exists in *La. delicata*, indicating that the striped morph might experience higher predation rates at higher latitudes (Forsman and Shine 1995). Forsman and Shine (1995) suggested that climate and geographic variation in predation intensity might

Fig. 6. The two *Liopholis margaretae margaretae* colour morphs: (*a*) patterned (spotted) morph (Simpson's Gap, Northern Territory, Photo: G. Fyfe), and (*b*) patternless morph (Areyonga Valley, Northern Territory, Photo: G. Fyfe) (Both photos reproduced from Horner 1991; with permission of P. Horner).

result in spatial variation in the relative fitness of the striped and unstriped morphs of *La. delicata*. However, the fitness consequences of the colour morphs of *La. delicata* also appeared to be influenced by sex, with striped females having significantly higher reproductive output than unstriped females, but striped males having lower survival rates than unstriped males (Forsman and Shine 1995). It is therefore clear that differences between morphs (e.g. morphological, behavioural, ecological, life history, physiological) need to be considered when examining the geographic variation in relative abundance of colour morphs. Since the relative advantage of each morph might vary spatially or temporally, climate and habitat might also influence the distribution and relative abundance of colour morphs.

Relatively few differences have been detected between the colour morphs of *L. whitii*, although only the patterned morph and plain-back morph have been compared directly (Milton *et al*. 1983; Milton and Hughes 1986; Milton 1987, 1990; Donnellan *et al*. 2002; Chapple 2003, 2005; Chapple and Keogh 2006). The

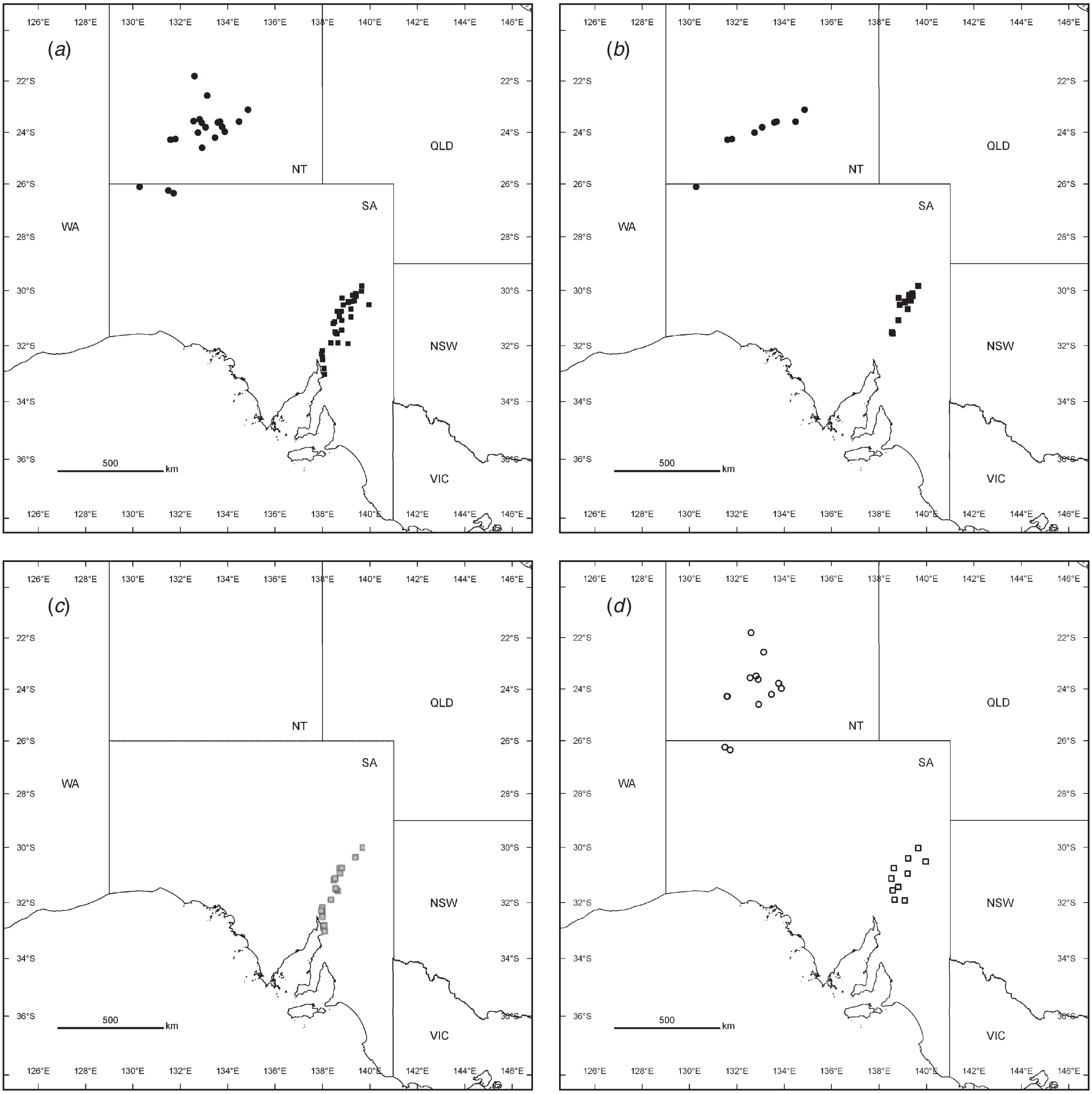

Fig. 7. Distribution of *Liopholis margaretae* museum specimens: (*a*) all *L. m. margaretae* (solid black circles) and *L. m. personata* specimens (solid black squares), (*b*) patterned (spotted) morph (solid black circles: *L. m. margaretae*; solid black squares: *L. m. personata*), (*c*) patterned (striped-back) morph (solid grey squares: *L. m. personata*), and (*d*) patternless morph (hollow black circles: *L. m. margaretae*; hollow black squares: *L. m. personata*).

colour morphs of *L. whitii* appear to be morphologically indistinguishable (apart from colour pattern) (Milton *et al*. 1983; Donnellan *et al*. 2002), show no differences in habitat use (Milton and Hughes 1986; Chapple 2003), and only subtle differences are evident in life history and reproductive ecology (Milton 1987; Chapple 2003, 2005). Although Milton (1987) suggested that there was segregation according to colour morph in social groups in *L. whitii*, such morph-based segregation is not evident in all *L. whitii* populations (Chapple 2003; Chapple and Keogh 2005, 2006). Similarly, there is evidence both supporting (Milton 1990) and rejecting (Donnellan *et al*. 2002) the possibility of non-random mating between colour morphs. Such results might simply indicate spatial variation in the relative advantage of each colour morph. The lack of differences between colour morphs in *L. whitii* might be a consequence of all studies being completed in Queensland, New South Wales and the Australian Capital Territory (across which relative colour morph frequencies remain consistent), and not at higher latitudes where the abundance of the plain-back morph begins to decline.

There are two main processes that result in the loss of polymorphism within populations (i.e. where polymorphic populations become monomorphic through the fixation of one colour morph): (1) the net selective advantage of one colour morph (through the colour morph differences discussed above); and (2) random genetic processes (e.g. founder effects, genetic drift, bottlenecks) (Roulin 2004). For *L. whitii*, it is possible that the fixation of the patterned colour morph in Tasmania was the result of random genetic processes (e.g. founder effect or genetic drift on an isolated island). However, since the incidence of the plain-back colour morph declines at latitudes higher than ~35°S, the absence of the plain-back colour morph in Tasmania might be due to a net advantage of the patterned morph at higher latitudes. Interestingly, both the plain-back and patternless morph occur across multiple genetic lineages in *L. whitii* (Chapple *et al*. 2005), and therefore must have been lost (or evolved) on multiple occasions within the species. Thus, it is clear that further study is needed to determine the processes that influence the geographic

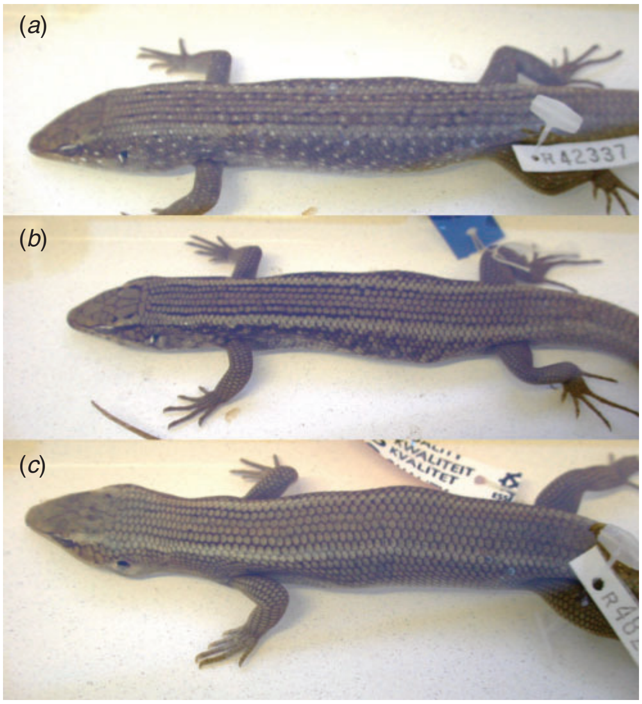

Fig. 8. The three *Liopholis margaretae personata* colour morphs: (*a*) patterned (spotted) morph (Moro Gorge, South Australia), (*b*) patterned (striped-back) morph, and (*c*) patternless morph (6 km NW Baratta, South Australia).

Fig. 9. The colour pattern morphs in *Liopholis pulchra*: (*a*) *L. pulchra pulchra* patterned morph (24 km NE Walpole, Western Australia), (*b*) *L. pulchra pulchra* plain-back morph (7 km E Hamel, Western Australia), and (*c*) *L. pulchra longicauda* (patterned) (Favourite Island, Western Australia).

variation in relative colour morph abundance in *L. whitii*. It is recommended that future studies that compare colour morphs in *L. whitii* include a spatial component in order to account for climatic and habitat variation in the relative advantage of each colour morph.

Geographic variation in the relative abundance of colour morphs is also evident in *L. multiscutata* and *L. m. personata*. In *L. m. personata*, all three colour morphs occur in the northern Flinders Ranges, but the patterned (spotted) and patternless colour morphs appear to be absent from the southern Flinders Ranges. In contrast, there is no variation in the relative abundance of the two *L. multiscutata* morphs between states, with the patternless morph occurring in low densities (~7–11%) across the entire distribution. However, the Nullarbor Plain region appears to represent a local 'hotspot' (~39%) for the patternless morph. Interestingly, there is also a distributional gap and substantial genetic break in this region in *L. multiscutata* (Chapple *et al.* 2004). It is unclear why the patternless morph is relatively more common in the Nullarbor Plain region. It could simply be an artefact of lower sample size in this region, or could potentially be due to the climate and habitat present in the Nullarbor Plain. No studies have examined potential differences between colour morphs in these two species (or in the other three polymorphic species), so there is much scope for future studies to examine the

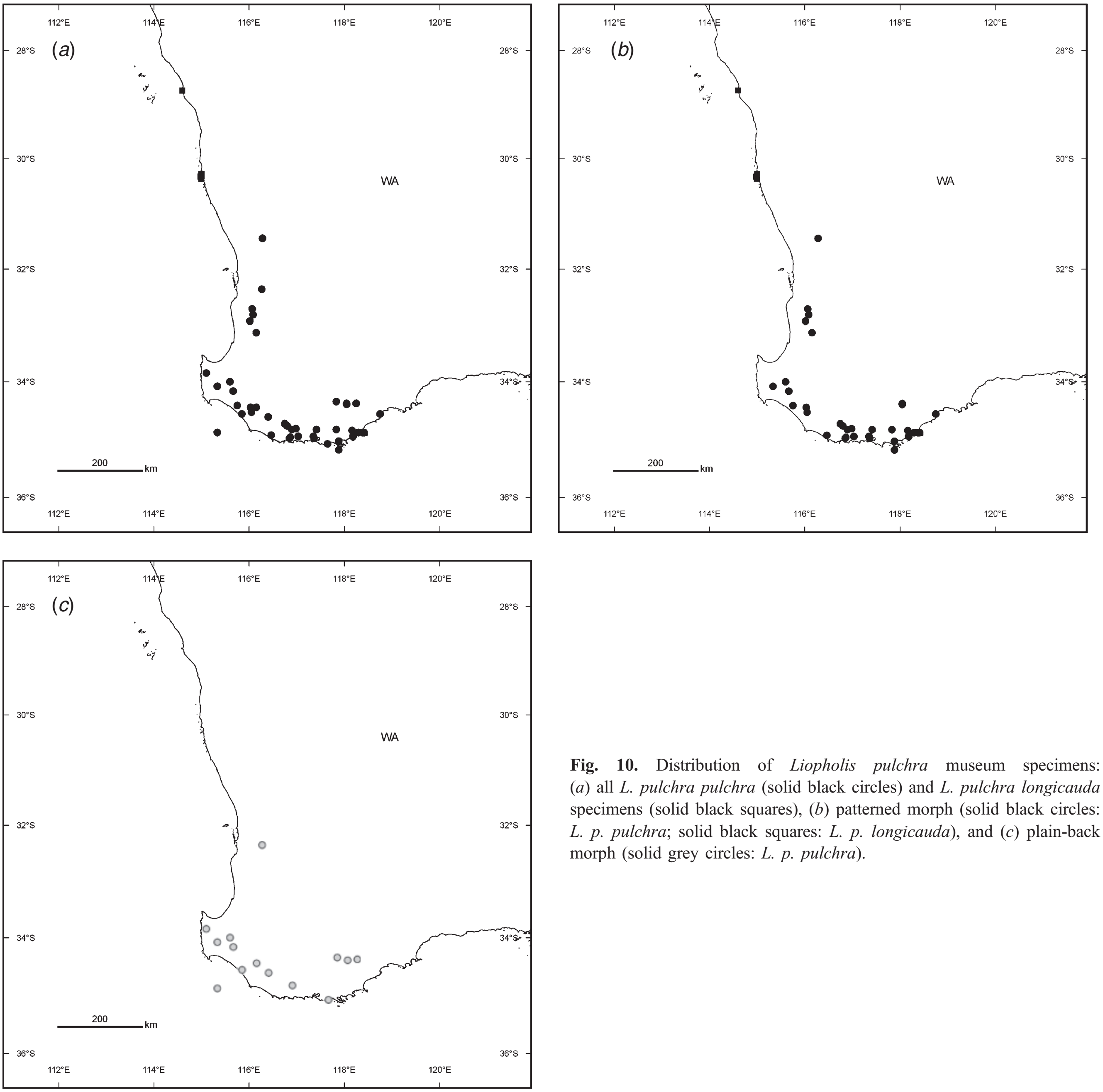

Fig. 10. Distribution of *Liopholis pulchra* museum specimens: (*a*) all *L. pulchra pulchra* (solid black circles) and *L. pulchra longicauda* specimens (solid black squares), (*b*) patterned morph (solid black circles: *L. p. pulchra*; solid black squares: *L. p. longicauda*), and (*c*) plain-back morph (solid grey circles: *L. p. pulchra*).

differentiation between colour morphs in species in the *L. whitii* species group.

Maintenance and secondary loss of colour pattern polymorphism in Liopholis

Our analyses of the evolutionary history of CPP within *Liopholis* were inhibited by the inability to determine the ancestral character state for the group. The most parsimonious scenario suggests that CPP was the ancestral state within the *L. whitii* species group, with four separate losses of CPP occurring during the evolution of the group (Fig. 1). The alternative scenario, whereby CPP represents the derived character state, requires four separate evolutions of CPP followed by one subsequent loss of CPP. Once CPP has originated in a species, the maintenance of polymorphism requires one of the following specific situations: (1) neutral polymorphism; (2) disruptive or divergent selection (including temporal or spatial variation in selection regimes); (3) balancing selection; (4) frequency-dependent selection; or (5) heterozygote advantage (heterosis) (reviewed in Galeotti *et al*. 2003; Roulin 2004; Gray and McKinnon 2007). In contrast, the breakdown of CPP within a species (i.e. the transition from polymorphism to monomorphism) only requires there to be a net selective advantage to one morph, or random genetic processes (where the morph that persists is not necessarily the ancestral one) (reviewed in Roulin 2004). Our analyses indicate that the breakdown of CPP (at the population level) appears to have occurred repeatedly in *L. whitii*, *L. multiscutata* and *L. m. personata*. This evidence supports the scenario whereby CPP represents the ancestral state within *Liopholis*, with the evolutionary history of the group characterised by the repeated secondary loss of CPP. This scenario corresponds to the hypothesis proposed by Chapple (2003).

Fig. 11. The two *Liopholis montana* colour morphs: (*a*) patterned morph, and (*b*) plain-back morph (Photo: Nick Clemann).

The ‘secondary loss’ scenario is further supported by the remarkable similarity in the characteristics of CPP among *Liopholis* species (Table 1), which might be indicative of single origin of CPP. Thus, since CPP might have arisen on only a single occasion within *Liopholis*, it is necessary to focus on the factors responsible for the maintenance or breakdown of CPP within the group. The incidence of polymorphism is higher in rock-dwelling species that inhabit the temperate zone (four of six species) than in obligate burrowing species that inhabit the Australian arid zone (one of five species). This might indicate that climatic or ecological factors could be correlated with the secondary loss of CPP in *Liopholis*. For instance, the species that lack CPP occur in either the arid zone (*L. inornata*, *L. kintorei*, *L. slateri*, *L. striata*) or alpine zone (*L. guthega*). Polymorphism is most likely to be maintained in species that occur in a heterogenous environment, where differential selection for morphs between microhabitats has a higher probability of occurring (e.g. Galeotti *et al*. 2003). Thus, it might be likely that the temperate zone offers more microhabitat heterogeneity than the Australian arid zone. However, all arid-zone *Liopholis* species (*L. inornata*, *L. kintorei*, *L. slateri*, *L. striata*) exhibit some degree of background matching to the various soil colours on which they live (Chapple 2003); polymorphism might therefore have been lost in these species as a result of selective pressures to avoid predators. However, the only obligate burrowing species to exhibit CPP occurs predominately in coastal sand dues, rather than the arid zone, and still displays some degree of background matching to the soil/sand on which it occurs (Chapple 2003; Chapple and Keogh 2004). Predictions concerning the influence of climate and habitat on the maintenance of CPP could be tested experimentally or in the field, using widespread species such as *L. multiscutata* and *L. whitii*.

It is clear that numerous questions remain regarding the evolution, maintenance and secondary loss of CPP in *Liopholis*. Our study has characterised CPP within *Liopholis* and provided some initial insight into the maintenance, geographic variation and secondary loss of CPP within the group. The six polymorphic taxa within the group provide an excellent opportunity to complete ‘replicated’ studies. Thus, the present study provides an ideal framework for future studies to examine hypotheses relating to CPP within *Liopholis*.

Acknowledgements

We thank S. Blomberg, N. Clemann, J. Cole, G. Fyfe, R. Henzell, P. Horner, D. Matthews, D. Milton, C. Pavey, G. Shea and A. Moussalli for providing information and/or useful discussions that greatly improved the manuscript. J. Melville, D. Bray, R. Sadlier, A. Amey, P. Couper, J. Wombey and R. Palmer provided access to museum collections. We thank N. Clemann (plain-back *L. montana*), P. Horner and G. Fyfe (patterned and patternless *L. m. margaretae*) for providing photos of colour morphs. Funding was provided to DGC from the following sources: Australian Geographic, Australian Society of Herpetologists (ASH), Student Research Grant, American Society of Ichthyologists and Herpetologists (ASIH) Gaige Fund Award, Society for Systematic Biologists (SSB) Award for Graduate Student Research, Joyce W. Vickery Scientific Research Fund (Linnean Society

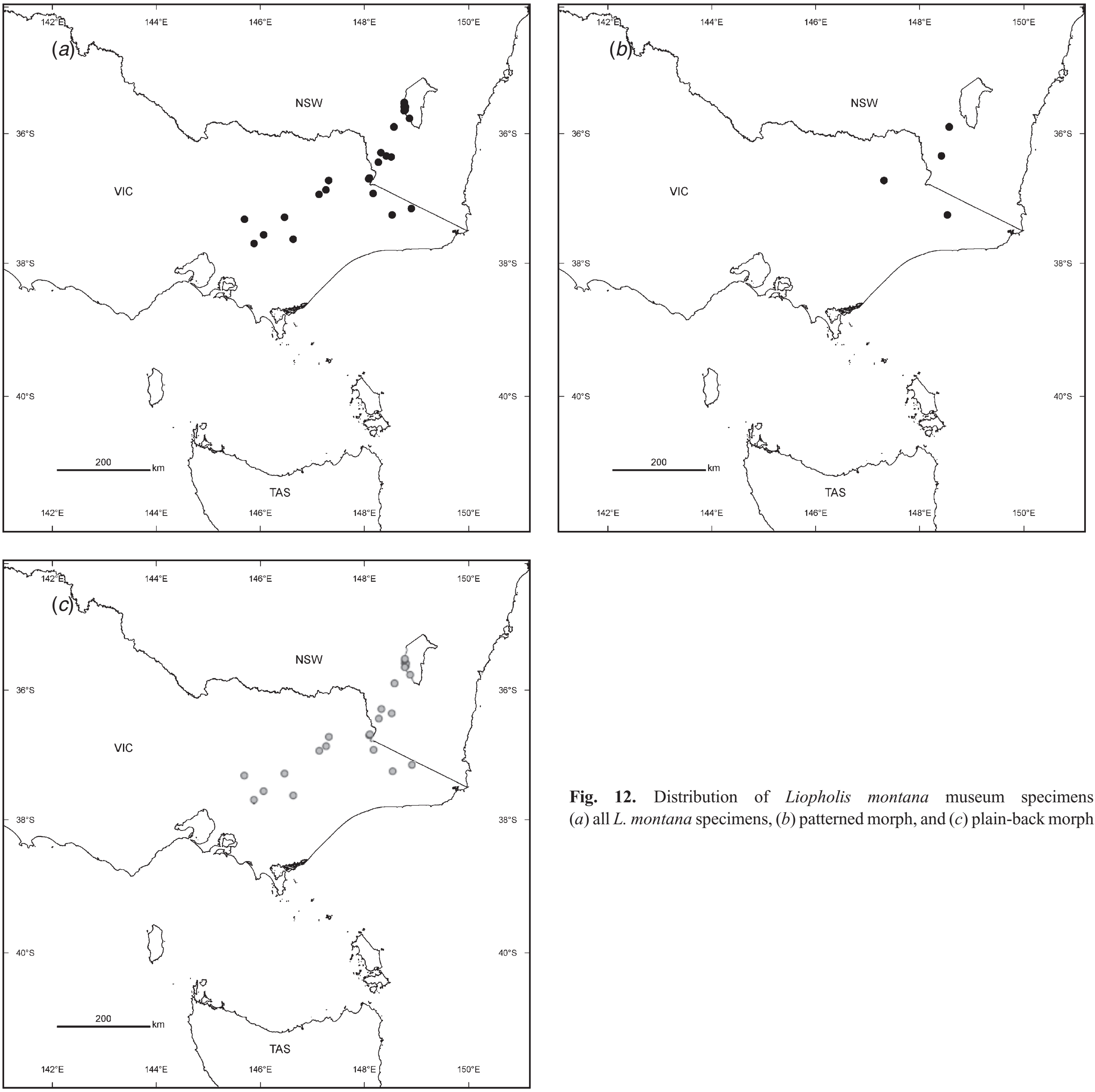

Fig. 12. Distribution of *Liopholis montana* museum specimens: (*a*) all *L. montana* specimens, (*b*) patterned morph, and (*c*) plain-back morph.

of NSW), Peter Rankin Trust Fund for Herpetology, and the Ecological Society of Australia (ESA) Student Research Grant. Financial support was also provided by a research grant from the Australian Research Council (ARC) to JSK.

References

Capula, M., and Luiselli, L. (1994). Reproductive strategies in alpine adders, *Vipera berus*. The black females bear more often. *Acta Oecologica* **15**, 207–214.

Chapple, D. G. (2003). Ecology, life-history, and behavior in the Australian scincid genus *Egernia*, with comments on the evolution of complex sociality in lizards. *Herpetological Monograph* **17**, 145–180. doi: 10.1655/0733-1347(2003)017[0145:ELABIT]2.0.CO;2

Chapple, D. G. (2005). Life-history and reproductive ecology of White's skink, *Egernia whitii*. *Australian Journal of Zoology* **53**, 353–360. doi: 10.1071/ZO05030

Chapple, D. G., and Keogh, J. S. (2004). Parallel adaptive radiations in arid and temperate Australia: molecular phylogeography and systematics of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Biological Journal of the Linnean Society* **83**, 157–173. doi: 10.1111/j.1095-8312.2004.00378.x

Chapple, D. G., and Keogh, J. S. (2005). Complex mating system and dispersal patterns in a social lizard, *Egernia whitii*. *Molecular Ecology* **14**, 1215–1227. doi: 10.1111/j.1365-294X.2005.02486.x

Chapple, D. G., and Keogh, J. S. (2006). Group structure and stability in social aggregations of White's skink, *Egernia whitii*. *Ethology* **112**, 247–257. doi: 10.1111/j.1439-0310.2006.01153.x

Chapple, D. G., Keogh, J. S., and Hutchinson, M. N. (2004). Molecular phylogeography and systematics of the arid zone members of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Molecular Phylogenetics and Evolution* **33**, 549–561. doi: 10.1016/j.ympev.2004.08.010

Chapple, D. G., Keogh, J. S., and Hutchinson, M. N. (2005). Substantial genetic substructuring in south-eastern and alpine Australia revealed by molecular phylogeography of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Molecular Ecology* **14**, 1279–1292. doi: 10.1111/j.1365-294X.2005.02463.x

Donnellan, S. C., Hutchinson, M. N., Dempsey, P., and Osbourne, W. (2002). Systematics of the *Egernia whitii* species group (Lacertilia: Scincidae) in south-eastern Australia. *Australian Journal of Zoology* **50**, 439–459. doi: 10.1071/ZO01065

Ford, J. (1963). The distribution and variation of the skinks *Egernia pulchra* and *E. bos* in Western Australia. *Western Australian Naturalist* **9**, 25–29.

Ford, J. (1965). The skink *Egernia pulchra* in the Stirling range. *Western Australian Naturalist* **8**, 172–173.

Forsman, A., and Aberg, V. (2008). Associations of variable coloration with niche breadth and conservation status among Australian reptiles. *Ecology* **89**, 1201–1207. doi: 10.1890/07-1670.1

Forsman, A., and Shine, R. (1995). The adaptive significance of colour pattern polymorphism in the Australian scincid lizard *Lampropholis delicata. Biological Journal of the Linnean Society* **55**, 273–291.

Forsman, A., Ringblom, R., Civantos, E., and Ahnesjo, J. (2002). Coevolution of color pattern and thermoregulatory behavior in polymorphic pygmy grasshoppers *Tetrix undulata. Evolution* **56**, 349–360.

Franck, D., Dikomey, M., and Schartl, M. (2001). Selection and the maintenance of a colour pattern polymorphism in the green swordtail (*Xiphophorus helleri*). *Behaviour* **138**, 467–486. doi: 10.1163/156853901750382115

Galeotti, P., Rubolini, D., Dunn, P. O., and Fasola, M. (2003). Colour polymorphism in birds: causes and functions. *Journal of Evolutionary Biology* **16**, 635–646. doi: 10.1046/j.1420-9101.2003.00569.x

Gardner, M. G., Hugall, A. F., Donnellan, S. C., Hutchinson, M. N., and Foster, R. (2008). Molecular systematics of social skinks: phylogeny and taxonomy of the *Egernia* group (Reptilia: Scincidae). *Zoological Journal of the Linnean Society*, in press.

Gray, S. M., and McKinnon, J. S. (2007). Linking color polymorphism maintenance and speciation. *Trends in Ecology & Evolution* **22**, 71–79. doi: 10.1016/j.tree.2006.10.005

Henzell, R. P. (1972). Adaptation to aridity in lizards of the *Egernia whitei* species-group. Ph.D. Thesis, University of Adelaide.

Hoekstra, H. E., Drumm, K. E., and Nachman, M. W. (2004). Ecological genetics of adaptive color polymorphism in pocket mice: geographic variation in selected and neutral genes. *Evolution* **58**, 1329–1341.

Hoffman, E. A., and Blouin, M. S. (2000). A review of colour and pattern polymorphisms in anurans. *Biological Journal of the Linnean Society* **70**, 633–665.

Horner, P. (1991). 'Skinks of the Northern Territory.' (Northern Territory Museum of Arts and Sciences: Darwin.)

Hughes, J. M., and Mather, P. B. (1986). Evidence for predation as a factor in determining shell color frequencies in a mangrove snail *Littorina* spp. (Prosobranchia: Littorinidae). *Evolution* **40**, 68–77. doi: 10.2307/2408604

Johnston, G. (1996). Genetic and seasonal variation in body colour of the Australian death adder, *Acanthophis antarcticus* (Squamata: Elapidae). *Journal of Zoology* **239**, 187–196.

Losey, J. E., Ives, A. R., Harmon, J., Ballantyne, F., and Brown, C. (1997). A polymorphism maintained by opposite patterns of parasitism and predation. *Nature* **388**, 269–272. doi: 10.1038/40849

Maddison, D. R., and Maddison, W. P. (2000). 'MacClade 4: Analysis of Phylogeny and Character Evolution.' (Sinauer Associates: Sunderland, MA.)

Milton, D. (1990). Genetic evidence for sympatric differentiation between two colour morphs of the skink *Egernia whitii. Australian Journal of Zoology* **38**, 117–130. doi: 10.1071/ZO9900117

Milton, D. A. (1987). Reproduction of two closely related skinks, *Egernia modesta* and *E. whitii* (Lacertilia: Scincidae) in south-east Queensland. *Australian Journal of Zoology* **35**, 35–41. doi: 10.1071/ZO9870035

Milton, D. A., and Hughes, J. M. (1986). Habitat selection by two closely related skinks, *Egernia modesta* Storr and *Egernia whitii* Lacepede (Lacertilia: Scincidae). *Australian Wildlife Research* **13**, 295–300. doi: 10.1071/WR9860295

Milton, D. A., Hughes, J. M., and Mather, P. B. (1983). Electrophoretic evidence for the specific distinctness of *Egernia modesta* and *E. whitii* (Lacertilia: Scincidae). *Herpetologica* **39**, 100–105.

Rosenblum, E. B., Hoekstra, H. E., and Nachman, M. W. (2004). Adaptive reptile color variation and the evolution of the MCIR gene. *Evolution* **58**, 1794–1808.

Roulin, A. (2004). The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews of the Cambridge Philosophical Society* **79**, 1–34. doi: 10.1017/S1464793103006183

Shine, R., Ambariyanto, Harlow, P. S., and Mumpuni. (1998). Ecological divergence among sympatric colour morphs in blood pythons, *Python brogersmai. Oecologia* **116**, 113–119. doi: 10.1007/s004420050569

Wilson, S., and Swan, G. (2008). 'A Complete Guide to Reptiles of Australia.' 2nd edn. (Reed New Holland: Sydney.)

Manuscript received 18 April 2008, accepted 25 July 2008