Gene flow in highly variable environments: Population structure of an Australian freshwater turtle, *Emydura macquarii*.

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Declaration

This thesis is my original work and has not been submitted, in whole or in part, for a degree at this or any other university. Nor does it contain, to the best of my knowledge and belief, any material published or written by another person, except as acknowledged in the text.

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Above all, I would like to thank my husband, Cris, who has fed me, kept me in clean clothes, taken responsibility of all the housework, put up with my moodiness, looked after the cats and raised my spirits when all seemed lost. I don't think I can ever really express my gratitude for his overwhelming loyalty, love and support, but it might be enough to say that I regard this work as being our success rather than my own personal accomplishment.

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Abstract

Flow in Australia's dryland rivers is dependant on variable rainfall patterns that result in episodic flooding events. The gentle gradient and low topographic relief that is typical of the arid zone result in complex river channels and vast floodplain networks of over 100,000 km^2 during high flows (Walker *et al.*, 1997). Connectivity of the aquatic populations that are resident in this river habitat range from being highly connected by floodplain, to being reduced in isolated waterholes during dry periods (Ward *et al.*, 1999). Permanent waterholes form areas of *refugia*; regions that are less disturbed by drought and flood, where organisms have a higher chance of survival and are available to recruit and later recolonise new territory.

This study investigated the levels of genetic divergence and gene flow of turtle populations of southwest Queensland in Cooper Creek and the Warrego River. Analysis of seven highly variable microsatellite loci was used to investigate the population structure of *E. macquarii*. The level of genetic divergence among populations within and between the Cooper and Warrego catchments was calculated with F_{ST} and R_{ST} estimates. Patterns of migration were analysed through the assignment of individuals to populations using their multilocus genotypes.

Freshwater turtles were found to have a high level of divergence among populations between Cooper Creek and Warrego River. Furthermore, populations within each catchment demonstrated a moderate level of population differentiation, suggesting that these turtles contain a strong population structure within and between catchments. Similarly,

there was no migration detected between Cooper and Warrego catchments, although gene flow was clearly present among populations within these catchments. In addition to this, the Welford site in Cooper Creek was found to contain a distinctive genetic character, suggesting this site is poorly connected with the rest of the catchment.

These unique dryland rivers, characterised by variation in flow and ranging in aquatic connectivity, have resulted in the development of highly structured turtle populations contained within a network of waterholes. It is clear that protection of the natural flow of dryland rivers is required in order to conserve the unusual population genetics of *E. macquarii*. An understanding of these processes will serve to better manage dryland rivers and the populations of freshwater turtles they sustain.

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1.0 Introduction

1.1 River Systems of Arid Australia

1.1.1 The Character of Dryland Rivers

Approximately 70% of Australia is classified as arid and receives less than 500 mm of rainfall a year (Smith and Morton, 1990). Furthermore, rainfall in arid and semi-arid zones are extremely unpredictable on a temporal and spatial scale (Kingsford *et al.*, 1999). This is reflected in the flow of dryland rivers that are distinguished by long periods without flow and punctuated by episodic flooding (Knighton and Nanson, 2001). Variations in flow are influenced by monsoonal discharges that respond to the El Nino-Southern Oscillation (ENSO), and high transmission losses that are a result of aridity (Sheldon *et al.*, 2002).

The dryland rivers of Australia are characterised by a braiding system of anastomosing channels that are uncommon elsewhere in the world and are capable of transporting a high sediment load (Knighton and Nanson, 1994). The channel systems cut into a continuous floodplain and are a product of the gentle topographic gradients present in the interior arid zones (Knighton and Nanson, 2000). Permanent water in dryland rivers is retained in turbid waterholes that serve as refugia for aquatic organisms (Morton *et al.*, 1995). Refugia are spatially discrete areas that are less disturbed by drought and flood, where organisms have a higher chance of survival and are able to recruit and later recolonise new territory (Lancaster and Belyea, 1997). Refugia are defined in the context of the biota and what may represent a refugium for one species may not be so for

another. During adverse conditions, such as times of drought, dependant species may be spatially and temporally restricted to permanent waterholes as regions of refugia (Choy *et al.*, 2002).

Waterholes also vary in size and depth and these characteristics influence waterhole permanency, which ranges from being permanent (having no history of drying since European settlement), to semipermanent (drying every 15-20 years) or ephemeral (drying every 5-10 years) (White, 2002). Rivers in dryland regions usually represent the only source of water available for both human exploitation and biological refuge, and it is therefore a resource that is often placed under far greater stress than in more temperate areas (Davies *et al.*, 1993). In order to conserve dryland river refugia, it is important to further our understanding of the biota contained in these areas and their patterns of connectivity within a highly variable environment.

1.1.2 Connectivity and Dispersal

During episodic flooding events, flow from dryland rivers exceeds waterhole and channel banks and expands onto the low-lying floodplain, covering up to 100,000 km^2 (Walker *et al.*, 1997). However, during periods of drought, waterholes are significantly reduced and the less permanent ones dry out altogether, leaving aquatic fauna restricted to deeper pools (Douglas, 2001). This highly variable aquatic habitat can therefore be viewed as having a number of spatial and temporal dimensions that may change quite abruptly (Sedell, 1990). Connectivity of populations in a river will range widely from being connected by floodplain, to being isolated in waterholes (Figure 1.1) (Ward *et al.*, 1999).

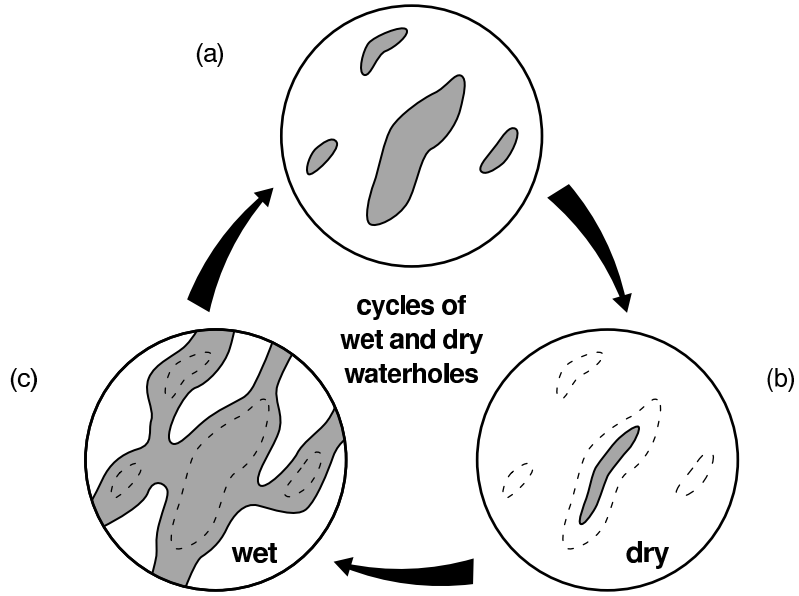

Figure 1.1 Patterns of connectivity are highly variable in dryland river environments. For much of the time (a), the river system exists as a cluster of permanent and ephemeral waterholes; during dry periods (b) permanent waterholes are reduced and ephemeral waterholes may dry up altogether; episodic flooding (c) connects waterholes through flowing channels and expanses of floodplain.

In this environment, the dry channel beds between waterholes represent a barrier to migration and gene flow for much of the time. However, during floods the gates to these environmental barriers are opened and a temporally limited opportunity exists for aquatic organisms to disperse. The question that arises from this highly variable river environment is, how do patterns of alternating isolation and connectivity influence the gene flow and population structure of aquatic organisms?

Connectivity is a measure of the ease with which organisms can disperse through ecotones to adjacent ecological units (Ward *et al.*, 1999). Connectivity also refers to gene flow between populations because estimates of gene flow provide a measure of dispersal and therefore of connectivity (Ward *et al.*, 1999). If connectivity is high, organisms can freely disperse and if it is low, there is a barrier to dispersal that will lead to genetic isolation. High gene flow between populations leads to a continuous population structure that is panmictic in nature, where individuals and their genetic components interchange freely (Slatkin, 1985). This may occur when barriers do not inhibit dispersal, as in a marine environment, or if an organism will, over time, surmount the barrier, as in the case of long-lived species (Doherty *et al.*, 1996).

Alternatively, natural populations may contain two or more geographically and genetically discrete subpopulations. Levins (1969) first used the term *metapopulation* to describe a "population of populations", where a number of discrete populations occupy patches of idealised habitat in a matrix of unsuitable habitat. There are at least four recognised theoretical models for patterns of gene flow and migration within

Table 1.1 Four recognised models of gene flow indicating migration between populations that vary in structure (from Futuyma, 1986).

1)	**"island" model**	migration occurs at random among a group of subpopulations.
2)	**"mainland-island" model**	migration is one-way from a large source population to a smaller, isolated sink population.
3)	**"stepping-stone" model**	each population receives migrants only from neighbouring populations.
4)	**"isolation-by-distance" model**	gene flow occurs among neighbouring populations in a continuous distribution.

metapopulation structures (Table 1.1) (Neigel, 1997; Rousset, 1997). Of these, the isolation by distance model defines gene flow as a continuous gradient (Futuyma, 1986) and is perhaps more representative of the linear population structure that occurs in riverine environments. The challenge for ecologists is to understand the divergence and connectivity in natural populations and determine if these patterns are reflective of theoretical metapopulation models.

1.2 The Population Structure of Aquatic Organisms

1.2.1 Studies of Aquatic Populations in Dryland Rivers

The varying connectivity of Australia's dryland rivers provide an excellent opportunity to investigate the relationship between gene flow and genetic divergence in natural populations. Cooper Creek is recognised as the longest and probably one of the most ecologically important dryland river systems in Australia (Kingsford *et al.*, 1999). Recent studies have used estimates of species diversity and population genetics to establish the connectivity and structure of organisms from Cooper Creek. Community composition of invertebrates in the Coongie Lakes of the lower Cooper were not found to have a strong association with hydrological history and connectivity between sampling sites, although the similarity may have been a result of recent flooding (Sheldon, 2002). Douglas (2001) used allozymes and mitochondrial DNA (mtDNA) to assess the dispersal and recolonisation of *Macrobrachium australiense* in the Cooper, Bulloo, Paroo and Warrego catchments. Genetic variation of populations within these catchments was not significant, suggesting a high dispersal among populations of this freshwater prawn (Douglas, 2001). However, significant population subdivision was found among

catchments and it could be therefore concluded that, for *M. australiense*, population subdivision between catchments is more important in generating genetic patterns than dispersal among subpopulations (Douglas, 2001).

In both studies, a high level of migration and gene flow was present among invertebrate populations within a single catchment. However, both studies investigated a short-lived invertebrate species and patterns of connectivity may be dissimilar for a longer-lived species. The dryland rivers of western Queensland are also inhabited by a long-lived species of short-necked turtle, *Emydura macquarii*. This species is slow growing, reaching an age of more than 80 years and becoming sexually mature at 15 years (White, 2002). *E. macquarii* is unable to travel far overland, thus restricting this species to an aquatic environment where migration depends on the flooding events that occur sporadically in dryland rivers.

1.2.2 Predictions for a Long-lived Turtle

The patterns of gene flow that occur among populations of *E. macquarii* in Australia's dryland rivers are difficult to predict. There have been a number of genetic studies conducted abroad that analysed freshwater turtle populations within and between drainages. Walker *et al.* (1995) and Walker *et al.* (1997) investigated two species of musk turtle using mtDNA and found genetic differences in populations from separate drainages or separate major tributaries within the same drainage. Similarly, Souza *et al.* (2002) used random amplified polymorphic DNA (RAPDs) to reveal genetic structure between populations from different river reaches within a catchment and strong

divergence between separate catchments in South America. Gray (1995) used DNA fingerprinting to study the western pond turtle and found no genetic divergence among neighbouring populations except for one site, whereas regions that were geographically separated were found to be significantly different from each other. In contrast, Scribner *et al.* (1984) and Scribner *et al.* (1986) detected little divergence in allelic variation from mtDNA and suggested a panmictic structure for populations of the yellow-bellied slider turtle. Roman *et al.* (1999) used mtDNA to investigate alligator snapping turtles and also found no genetic divergence within catchments but recognised strong separation in populations between drainages. Similarly, a microsatellite study conducted by Sites *et al.* (1999) on the giant Amazon River turtle detected strong genetic structure between river systems with no significant divergence among populations of the same catchment.

This evidence suggests that turtles are unlikely to be genetically distinct among populations within the same catchment, although there is some possibility that differences may occur in separated river reaches. However, a strong genetic divergence is likely to occur between populations from adjacent catchment drainages. Notably, the river systems studied in the previous examples are located in temperate climates where flow is continuous. Although the river systems abroad lack the ecological extremes and variability of Australia's dryland rivers, invertebrate studies from Cooper Creek seem to suggest similar patterns of high gene flow within a catchment and genetic separation between catchments. It is therefore anticipated that gene flow in populations of *E. macquarii* will be high within a single catchment, promoting low genetic divergence among

populations. Furthermore, migration of *E. macquarii* between catchments is unlikely, producing a high genetic diversity among populations from adjacent catchments. This study will test these hypotheses using populations of the freshwater turtle *E. macquarii* in Cooper Creek and the Warrego River, two neighbouring dryland river catchments located in southwest Queensland. Gene flow and genetic divergence among populations will be estimated using molecular markers.

1.3 Selected Molecular Techniques

1.3.1 Choosing a Genetic Marker

The studies previously mentioned have used a range of molecular techniques to gather genetic data from populations, including allozymes, mtDNA, RAPDs and microsatellites. Allozymes were among the earliest molecular techniques from which allele frequencies could be used to quantify genetic variation in a range of organisms (Parker *et al.*, 1998). In more recent times, technological advances have allowed researchers to examine variation in greater detail within nucleic acid sequences (Parker *et al.*, 1998). However, as observed mutation rates may not be equal in all parts of the genome, the optimal DNA segment for use in a particular study will depend on the degree of relationship among individuals (Parker *et al.*, 1998; Sunnucks, 2000).

To study population level processes, the use of microsatellites as a genetic marker is now widely accepted because they are highly polymorphic in natural populations and have heterozygosity values that are above average (Jarne and Lagoda, 1996). Microsatellites are regions of tandemly repeated DNA sequences between 1-6 base pairs in size

(Hancock, 1999). These repeat motifs occur in all organisms, although they are longer in vertebrates than in invertebrates and ectothermic species, such as turtles, seem to have the longest repeat arrays (Chambers and MacAvoy, 2000). Microsatellites are considered to be a powerful genetic marker for population genetics, owing to their exceptional variability and relative ease of scoring (Goldstein and Pollock, 1997). For these reasons, microsatellites were chosen as the molecular marker for this study.

1.3.2 Highly Variable Microsatellites

High mutation rates in microsatellites are a result of the addition or subtraction of repeat units (Goldstein and Pollock, 1997). Variability in the number of repeat units is believed to be mostly due to 'slipped-strand mispairing' whereby a DNA strand slips out of phase during replication and produces errors in the number of repeat units (Hancock, 1999). Mutations tend toward positive asymmetry, or the addition rather than deletion of repeat units, although there must also be a process which restricts or acts as a ceiling to this trend (Goldstein and Pollock, 1997). High mutation rates lead to a complex evolutionary history in microsatellites and it is possible to overestimate the true relationship among taxa because of size homoplasy (Chambers and MacAvoy, 2000). Homoplasy occurs when two alleles converge to the same size, that is they are identical in state, but do not share the same history of mutation and are not identical by descent (Estoup and Cornuet, 1999). The recent development of *R*-statistics, however, take into account the high homoplasy present in microsatellite markers when estimating levels of genetic divergence between populations (Balloux *et al.*, 2000).

1.3.3 Detection of Genetic Divergence with F_{ST} and R_{ST} Estimates

Estimations of F_{ST} and R_{ST} are an indication of the amount of genetic subdivision between populations, where values close to 1 imply that populations are highly separated, and values close to 0 imply that minimal separation exists (Hartl and Clark, 1997). For the interpretation of F_{ST}, it has been suggested that values within the range of 0-0.05 indicate little genetic differentiation; values between 0.05-0.15 show moderate differentiation; values between 0.15-0.25 demonstrate great differentiation and values above 0.25 show very great genetic differentiation (Hartl and Clark, 1997).

1.3.4 Assumptions of F_{ST} and R_{ST}

Although F_{ST} and R_{ST} both estimate genetic divergence between populations, they may vary in value because the underlying assumptions of the accepted models of mutation are very different. F_{ST}, from which R_{ST} is derived, is based on the probability that populations are variable for two alleles, which is calculated from Wright's (1951) inbreeding coefficient, *F*, the probability that two alleles of a gene are identical by descent (Weir and Cockerham, 1984). For F_{ST}, an infinite allele model (IAM) is assumed, whereby each mutation creates a novel allele at random by the given rate *u* (Hartl and Clark, 1997). In contrast, R_{ST} was designed to predict the special mutation processes of microsatellites and therefore assumes mutation adheres to a step-wise mutation model (SMM). In the SMM, a novel allele is created either by adding or deleting a single repeated unit with equal probability in both directions (Rousset, 1996). In this case, the SMM retains a memory of allele size because very different sized alleles are more distantly related than similar sized alleles (Balloux and Lugon-Moulin, 2002).

Empirical work on microsatellite mutations has shown that they generally mutate under complex models, with frequent addition or deletion of several repeat units (Schlötterer *et al.*, 1998). The SMM is thought to better reflect the way microsatellites mutate, however neither the IAM or the SMM appear to adhere strictly to the observed mutation processes of microsatellites (Gaggiotti *et al.*, 1999). The true mutation pattern of microsatellites probably lies somewhere in between these two extreme models (Balloux and Lugon-Moulin, 2002). R_{ST} takes into account the higher probability of homoplasy that occurs in microsatellites and retains a memory of mutation. These factors will improve performance of R_{ST} over that of F_{ST} as the level of population subdivision increases and the effects of mutation become more important than that of migration (Balloux *et al.*, 2000) Therefore, R_{ST} is probably a better indicator of interspecific divergences that occur during longer historical separations (Forbes *et al.*, 1995). On the other hand, R_{ST} may be less accurate at estimating recently separated populations because of its high associated variance (Balloux and Lugon-Moulin, 2002). This would indicate that F_{ST} is more efficient than R_{ST} at detecting intraspecific differentiation between closer populations (Forbes *et al.*, 1995).

1.3.5 Detecting Patterns of Migration and Gene Flow

Assignment tests can identify migrants by assigning the genotype of each individual to the closest genetic population from which it has the highest likelihood of occurrence (Paetkau *et al.*, 1995). There are a number of different approaches that have been used to assign individuals to their population of origin, but most are based on either a distance or frequency method. The distance method uses genetic distance to assign

individuals to their closest population, whereas frequency methods assign an individual to the population in which that individual's genotype is most likely to occur (Cornuet, 1999). Recent modelling suggests that distance based methods do not perform as well as the frequency based methods, among which the most efficient involve the use of Bayesian simulation (Cornuet, 1999). However, correct assignment of individuals is dependant on the assumptions that loci are at Hardy-Weinberg equilibrium and that each locus is independent (Cornuet, 1999). It is important to note that unlike measures of interpopulational differentiation such as F_{ST} and R_{ST} estimates, assignment tests are based on individual genotypes. Thus, assignment tests have the power and conceptual simplicity of an individual based genetic descriptor that can be applied to large numbers of highly variable loci (Waser and Strobeck, 1998).

1.4 Aims and Objectives

1.4.1 Overall Project Aims

This study aims to determine the extent of genetic divergence, population structure and migration patterns of *Emydura macquarii* populations using genetic markers. This freshwater turtle species inhabits the highly variable dryland rivers of southwest Queensland including Cooper Creek and the Warrego River. Here they form discrete populations in isolated waterholes that are connected by episodic flooding events which create a continuous aquatic environment and allow infrequent migration events. The genetic structure of these turtle populations will be investigated among waterhole populations within a single catchment and among waterhole populations between different

catchments. The level of genetic divergence and patterns of gene flow between turtle populations will be analysed using seven highly variable microsatellite loci.

This study is part of the Dryland Refugia Project, a long-term joint collaboration between the University of Canberra and a range of other contributors to the CRC for Freshwater Ecology. The Dryland Refugia Project is conducting a number of studies on the biological and physical processes of waterholes and their role as refugia in Australia's dryland rivers. Together, these studies will identify the biological components and diversity of waterholes, determine the connectivity of refugia and patterns of dispersal of organisms and investigate the biophysical processes of these river systems. An understanding of these processes will guide future management plans toward better conservation of dryland river refugia and of the organisms they sustain.

1.4.2 Specific Research Objectives

The objectives of this research are:

- To determine the level of genetic divergence among populations of turtles inhabiting waterholes in two dryland river catchments.

- To determine if metapopulation models, such as isolation by distance, explain patterns of population divergence.

- To determine the migratory patterns and gene flow of turtles among different breeding populations.

- To determine if migration is sex-biased.

- To analyse both the physical waterhole characteristics and genetic characteristics of turtle populations to determine whether any correlations exist.

- To identify management priorities for the conservation of *Emydura macquarii*.

2.0 Materials and Methods

2.1 Study Area

2.1.1 The Cooper Creek Catchment

Cooper Creek flows in a southwesterly direction from Queensland through to South Australia, finally emptying into Lake Eyre (Figure 2.1). As the Cooper Creek runs southwest, woodland and grasslands become increasingly arid and near South Australia it reaches the dune fields of the Strzelecki Desert (Walker *et al.*, 1997). The catchment is estimated to be 306,000 km^2 and is classified as arid or semi-arid with a median rainfall of less than 200 mm per year (Kingsford *et al.*, 1999). However, a combination of monsoonal discharge, aridity and high transmission losses makes the Cooper one of the most hydrologically variable rivers in the world (Sheldon *et al.*, 2002). The middle reaches that form the channel country of Cooper Creek consist of narrow anastomosing channel systems that transport sand and mud during high flows (Knighton and Nanson, 1994). In this region, waterholes or self-maintaining scour features that are generally expansions of the braiding channels, are a distinctive feature of the river system (Knighton and Nanson, 2000). For much of the time, river channels remain dry and only waterholes retain water, although during episodic flooding events, channels and waterholes overflow into an expansive floodplain. These complex river channels and vast floodplain networks cover over 100,000 km^2 and are a result of the gentle gradient and low topographic relief that is characteristic of the Cooper catchment (Walker *et al.*, 1995; Walker *et al.*, 1997).

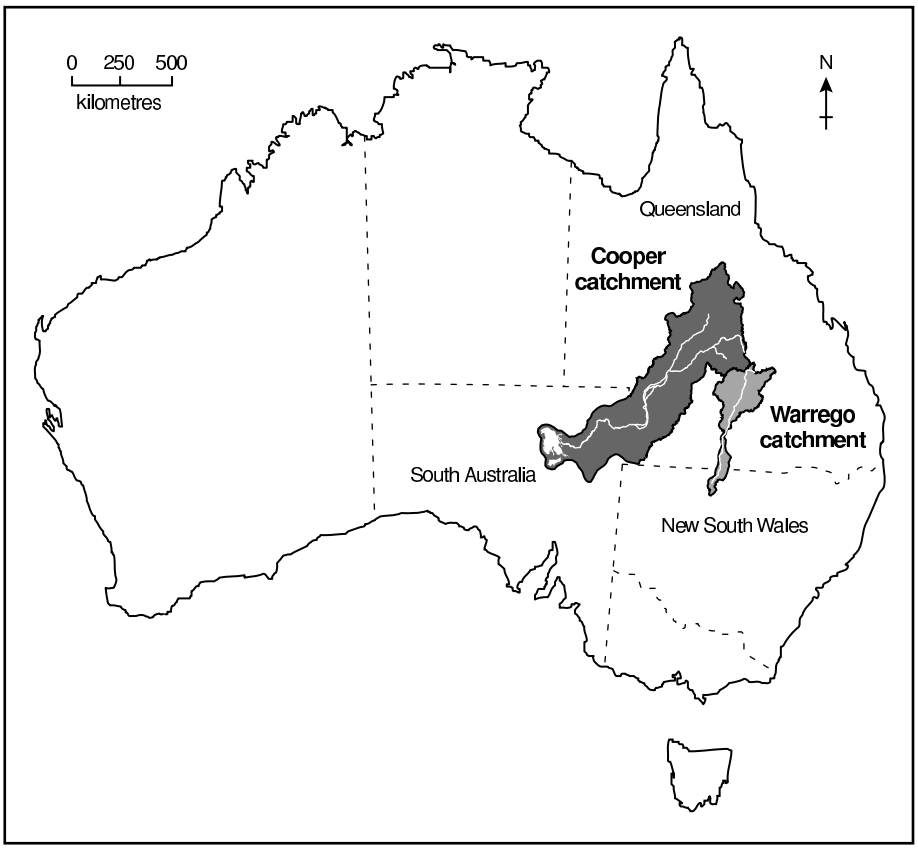

Figure 2.1 Geographical location of the Cooper and Warrego catchments.

2.1.2 The Warrego Catchment

The headwaters of the Warrego River form the most northerly point of the Murray-Darling Basin and extend in a southerly direction through Queensland to just inside the New South Wales border (Figure 2.1). The entire catchment is contained within a semi-arid area of 78,400 km^2 and rainfall is higher than the Cooper, averaging around 400 mm per year (Queensland Government, 2000). Similar to the Cooper catchment, river flow in the Warrego is both variable and seasonal although episodic flooding events are more common (Queensland Government, 2000). River character is also similar to the Cooper and consists of braided anastomosing channels that form a vast floodplain punctuated by turbid waterholes. In addition to the increased rainfall, development and flow regulation of the Warrego is a fundamental difference between the two catchments. The primary industry of this area is cattle and sheep grazing, although smaller areas of irrigated crops also exist and are generally provided for by the Cunnamulla Wier (Cottingham, 1999). The development of surface water resources for dams and other flow diversions has had major ecological impacts in other areas of the Murray Darling Basin, and although such development in the Warrego is considered minor, it is a distinguishing environmental component of this catchment (Cottingham, 1999).

2.2 Study Animal

2.2.1 Taxonomy of the Cooper Creek Turtle

A detailed taxonomy of the Cooper Creek turtle is, as yet, unresolved and this study will follow Georges (1996) in considering the species as *Emydura macquarii*. It is grouped under the family Chelidae, a group of freshwater turtles confined to Australia,

New Guinea and South America. In Australia, the family gives rise to five genera; *Chelodina, Pseudemydura, Rheodytes, Elseya* and *Emydura*. Within the genus *Emydura*, there are currently three recognised species including *E. macquarii*, *E. subglosa* and *E. kreftii* (Cogger, 2000). Some authors have suggested the Cooper Creek turtle may be a potentially new and undescribed species (Legler, 1981), while others have found very little genetic variability throughout the entire genus (Georges, 1996). Regardless of their taxonomy, the turtle populations used in this study are widely dispersed throughout the Murray Darling and Cooper catchments and are easily identifiable from other turtle species present.

2.2.2 Description

E. macquarii is a large short-necked freshwater turtle that can reach an average carapace length of 302 mm for females and 240 mm for males (White, 2002). The species is sexually dimorphic, with females maturing to a larger size than males and macrocephaly (enlargement of the head) becoming common in larger adults (Figure 2.2). *E. macquarii* has been found to be the slowest growing freshwater turtle in Australia, reaching maturity at approximately 15 years of age and the oldest individuals thought to be around 100 years old (White, 2002). *E. macquarii* looks similar to other turtles in this genera (Figure 2.2), with a dull olive-green carapace and lighter cream plastron, although this turtle has a distinctive yellow-cream stripe that extends behind the eye that tends to fade in older turtles (Cann, 1998). Other distinctive features include two small barbels present on the underside of the jaw and tubercules covering the skin of the upper neck (Cann, 1998).

Figure 2.2 Large female and smaller juvenile *Emydura macquarii* caught during the April 2002 field trip. This large female exhibits macrocephaly and the usually distinctive stripe behind the eyes is almost completely faded.

2.2.3 Breeding Biology

Clutch sizes of turtles in the Cooper and Warrego are unstudied, however Cann (1998) reports finding clutches of between 7 and 16 eggs. In conjunction with their large body size, the eggs of *E.macquarii* are the largest known in the *Emydura* family, ranging between 13 to 16 grams in weight (Cann, 1998). Nesting season varies in different catchments and may begin as early as September or as late as December and continues until early January (Judge, 2001). Nests are dug into the soil on dry waterhole banks above the waterline and are susceptible to predation, particularly by foxes (Cann, 1998).

2.3 Sampling Strategy

2.3.1 Sampling Design

This study uses a nested hierarchical sampling design with six waterhole sites from the Cooper Creek catchment and four waterhole sites from the Warrego catchment. Selected waterholes from the Cooper provide six replicates of varying distance from each other (Figure 2.3) and likewise for the four waterholes selected within the Warrego catchment (Figure 2.4). Waterholes can be compared within a catchment or combined to compare entire catchments.

2.3.2 Waterhole Selection

Waterholes were located within the Queensland quarters of Cooper Creek and the Warrego River via satellite images. These images provided specific features of waterhole size and channel structure from which detailed maps could be constructed. Each waterhole selected was considered to be long-term permanent, drying only every 100 years.

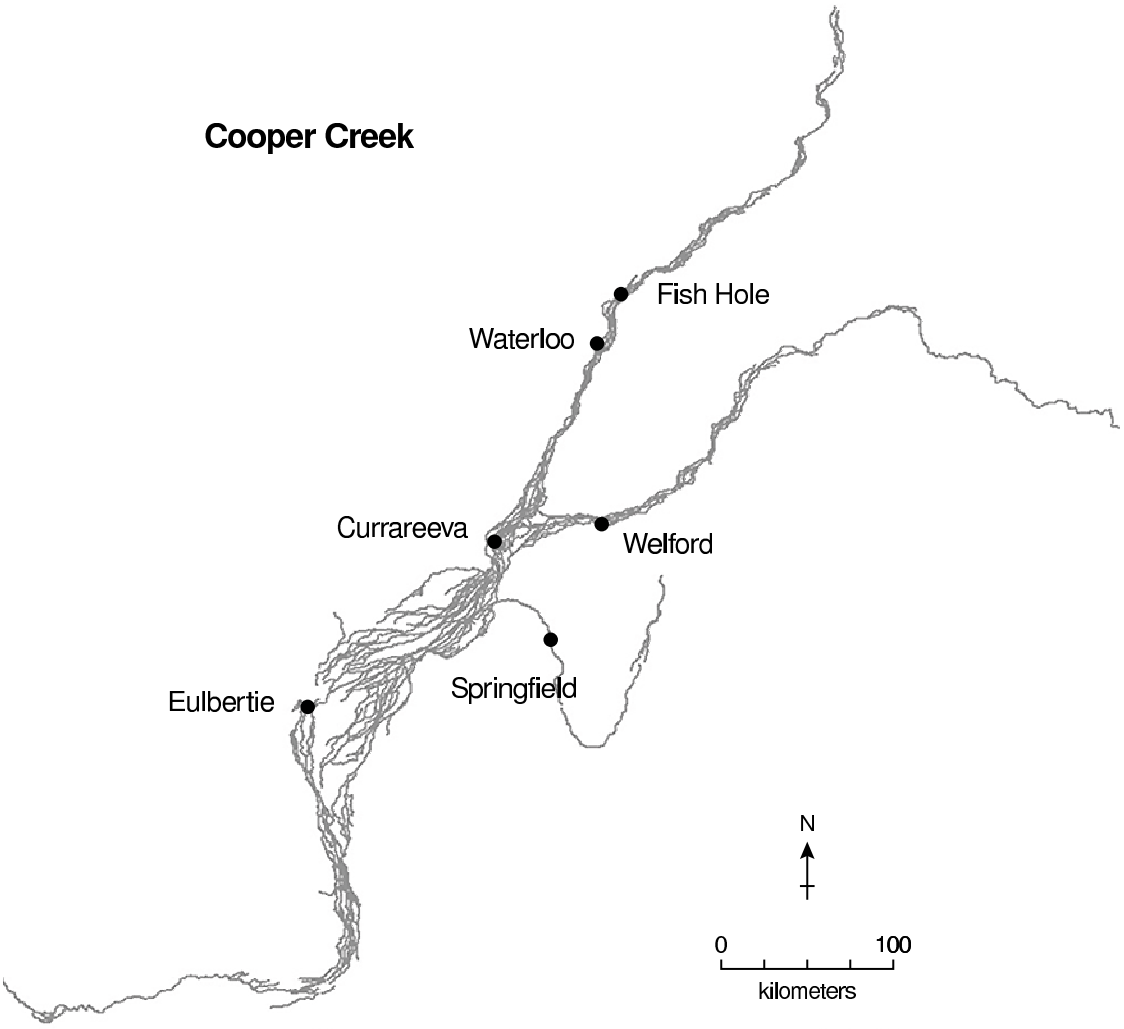

Figure 2.3 Channel country in the Cooper drainage. The six permanent waterhole sites selected for this study are indicated with black circles.

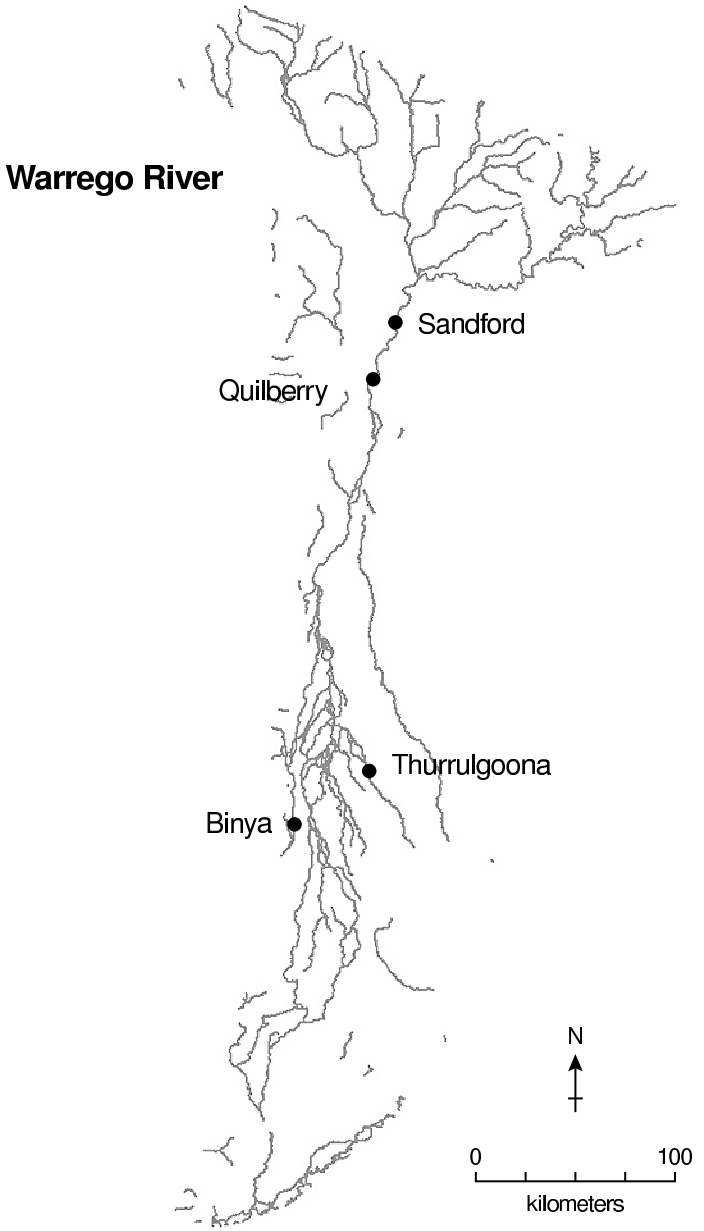

Figure 2.4 Warrego river drainage. The four permanent waterhole sites selected for this study are indicated with black circles.

From fifteen sites visited in Cooper Creek, six major waterholes that achieved a high sampling success were chosen for this study. Similarly, four sites from thirteen visited waterholes in Warrego River were chosen (Table 2.1). Samples from each waterhole were treated separately, except for those from the Welford site where samples from two nearby waterholes were combined to increase the sample size.

2.3.3 Sample Selection

Sample numbers from the waterholes in this study were large and it was therefore possible to select an equal ratio of males and females for genetic analysis. To reduce bias from an unequal sex ratio or through selection of multiple generations, the fifteen largest males and fifteen largest females from each waterhole were used.

2.4 Field Procedures

2.4.1 Trapping

Traps were made of nylon netting and opened into a box shape with a side sleeve that turtles could enter. In addition, most traps were fitted with a snorkel device made from wire rings inside a net that opens into a cylindrical shape and is attached to the top of the trap. When tied above water level, a snorkel allowed turtles in a trap below water to swim up for air, thus ensuring the safety of trapped turtles and reducing the urgency of checking traps every few hours. Bait consisting of canned catfood and fresh meat was enclosed in a mesh bag and suspended from the roof of the trap. Trap sites were located by boat so that snorkel traps could be tied up to a tree branch near shore, and non-snorkel traps could be released into the deeper middle channel with a line and float as

Table 2.1 Numbers of turtles caught from each waterhole selected for this study. Where possible, the largest fifteen adult males and largest fifteen adult females were selected for analysis.

Catchment	Waterhole	Female	Male	Juvenile	Total
Cooper	Fish Hole	48	67	71	186
	Waterloo	37	46	30	113
	Welford	10	21	1	32
	Currareeva	45	74	32	151
	Springfield	105	65	20	190
	Eulbertie	85	103	0	188
Warrego	Quilberry	37	32	81	150
	Sandford	32	27	60	119
	Binya	22	13	8	43
	Thurulgoona	32	19	12	63

marker. Traps without snorkels were left under water for no longer than three hours, although snorkel traps could be left longer. Turtles were removed from traps and transported back to camp in large tubs for processing.

2.4.2 Processing and Tissue Collection

Each turtle was given an individual number and was marked by cutting notches, approximately 2 cm^2 in size, from the marginal scutes. A small tissue sample from each individual, approximately 1 cm^2 in size, was taken from the webbing of corner of the back foot. Sharp scissors were used to collect the tissue and all utensils were cleaned thoroughly with 6% H_2O_2 between each turtle to prevent disease transmission and sample contamination. As many turtles already had naturally damaged and scarred webbing and recaptured turtles had healed very well, it does not appear that this process caused ill effects. All tissue samples were stored in labelled 1.5 ml sample tubes that were filled with 70% ethanol and refrigerated. Turtles were released as soon as possible to the waterhole from where they were captured.

2.5 Laboratory Procedures

2.5.1 DNA Extraction

DNA was extracted from tissue samples using a salting-out protocol (Sunnucks and Hales, 1996; Fetzner, 1999). A small section of each tissue sample, approximately 1 mm^2 in size, was rinsed in deionised water, finely sliced with a clean, sterilised scalpel and placed into a 1.5 ml centrifuge tube with 300 µl of tissue extraction buffer (40 mM Tris, 20 mM EDTA, 100 mM NaCl, pH7.2) 10 µl of 20% SDS and 10 µl of proteinase

K (10 mg/ml). Samples were left rotating overnight at 55°C to break up cell membranes and digest proteins. After incubation, 150 µl of 7.5M NH_4 acetate was added and samples were mixed by inversion then set to chill at -80°C for 30 minutes. Cellular debris was pelleted by spinning samples in a centrifuge at 13,000 rpm for 20 minutes. The supernatant was transferred into new 1.5 ml centrifuge tubes, 1 ml of chilled 100% ethanol was added and samples were left to chill for 25 minutes at -80°C to precipitate DNA. Samples were again spun at 13,000 rpm for 20 minutes and the supernatant was poured off and discarded, leaving the DNA pellet in the tube. 600 ml of 70% ethanol was added for final rinsing and samples were spun for a further 10 minutes. Ethanol was poured off and samples were given a quick spin to collect the remaining ethanol which was carefully pipetted off. The samples were left open to dry at room temperature in a closed box for at least 30 minutes. DNA was resuspended with 55 µl of 1x TE to make a stock solution. Samples (5 µl) were run through a 1.2% agarose gel and stained with ethidium bromide to determine the quality and quantity of DNA collected and then diluted with 1x TE accordingly for a working solution concentration.

2.5.2 Polymerase Chain Reaction

The polymerase chain reaction (PCR; Saiki *et al.*, 1988) is a method used to amplify a selected segment of DNA from small quantities of starting material to an amount necessary for analysis (Page and Holmes, 1998). The PCR process involves stages of denaturation, annealing and extension that are achieved chemically at specific temperatures over short periods of time (Palumbi, 1996). Amplification of a targeted sequence of DNA requires the use of two designed segments of oligonucleotides (primers) that

attach to a complimentary piece of the DNA and trigger the DNA polymerase to copy that segment (Li and Graur, 1991). This study used PCR to amplify seven loci, requiring the use of seven sets of primers that are listed in Table 2.2. All primers were selected from a microsatellite library that was established in Jane Hughes laboratory at Griffith University. PCR amplification was performed in 12.5 µl reaction volumes containing 1 µl of DNA working solution and measured amounts of nanopure H_20, 10x NH_4 reaction buffer (Bioline), 50mM $MgCl_2$ (Bioline), 10mM dNTP, 10mM forward and reverse primer and 5 units/µl BioTaq DNA polymerase as detailed in Table 2.3. DNA working solution (1 µl) was pipetted into a 96-well PCR reaction tray and kept on ice while a master mix of PCR reagents was combined for one set of primers, thoroughly mixed and then added to the DNA. Samples and a negative control were loaded into a programmable BioRad Thermal Cycler and set to a PCR cycle described in Table 2.4. Samples remained refrigerated after removal.

2.5.3 Fragment Analysis

PCR products were run through 12.5% denaturing acrylamide gels that allowed for high resolution viewing. The acrylamide gel solution was made by adding 21 g urea, 3 ml of 10x TBE buffer, 6.25 ml of acrylamide and then topping with nanopure H_2O to make 50ml of solution. Approximately 15 ml of acrylamide solution was drawn into a syringe and the solution was agitated to remove gas. Immediately prior to pouring the gel, 8.5 µl of Tmed and 85 µl of 10% APS were added to the acrylamide solution. The gel solution was carefully poured between two clean glass plates laid out horizontally

Table 2.2 Microsatellite loci designed for *Emydura macquarii* including; name of primer, the primer sequences, the amplified microsatellite units and the size of clone sequences.

Name	Sequence (5' - 3')	Repeat Units	Size of clone
TLE 2.1 F	5' ATG AAC TTT CCC GTG GTG CTC	$(GT)_{6T}(TG)_{2TT}TC)_2AA(TG)_{19}$	167 bp
TLE 2.1 R	5' GTT CCG ATA CAG AGC TTC ACC		
TLE 6.2 F	5' TC AAT CTA ACG TAA TTG TGC C	$(GT)_{13}$	121 bp
TLE 6.2 R	5' GTT TAC AGY YCA CCT CTT CAG		
TLE 7.2 F	5' ACA GCC ATC ACG TTT AGC CAC	$(CA)_{13}$	137 bp
TLE 7.2 R	5'GCC AAT TTG TTT ACA TAT CCC		
TLE 9.2 F	5' CAA ATG TTC AGC AGC ACC CTG	$(TG)_{13}$	118 bp
TLE 9.2 R	5' TGT GTT CGT GCG ATG CAA CTC		
TLE 10 F	5' TTC TGC TTC TGT GGT TCC ACC	$(AC)_{11}$	147 bp
TLE 10 R	5' TGA GTT TCA GGC ATC TCC TCG		
TLE 13.1 F	5' TGG GTC TAA TTC AGT GAA GAG	$(TG)_{13}$	199 bp
TLE 13.1 R	5'TGA GTT TCA GGC ATC TCC TCG		
TLE 13.3 F	5' GT GTC AGC CCCT CCA GAA TGT C	$(TG)_{13}$	134 bp
TLE 13.3 R	5' TCA ACG AGA AGC AAA TTG AAG		

Table 2.3 Reaction volumes used for each locus in a 12.5 µl PCR reaction solution (volumes in µl units).

Reagent	TLE 2.1	TLE 6.2	TLE 7.2	TLE 9.2	TLE 10	TLE 13.1	TLE 13.3
H_20	8.60	8.45	8.60	8.45	8.59	8.54	8.45
10x buffer	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$MgCl_2$ (50mM)	0.30	0.50	0.30	0.50	0.36	0.36	0.50
dNTP (10mM)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Primer F (10µM)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Primer R (10µM)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Taq (5 units/µl)	0.10	0.05	0.10	0.05	0.05	0.10	0.05
DNA	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 2.4 All primers required one of two PCR programs which were identical except in temperature during the second annealing phase.

Phase	Time	Temp.	Repeats	Primer used
Initial denature:	3 minutes	@ 94°C	x1	
Denature Phase 1:	30 seconds	@ 93°C	x15	
Anneal Phase 1:	30 seconds	@ 50 °C		
Extension Phase 1:	30 seconds	@ 72°C		
Denature Phase 2:	30 seconds	@ 93°C	x25	
Anneal Phase 2:	30 seconds	@ 54 °C		TLE 2.1, 7.2, 9.2, 10, 13.3
		or		
		@ 56°C		TLE 6.2, 13.3
Extension Phase 2:	30 seconds	@ 72°C		
Final Extension:	7 minutes	@ 72°C	x1	

on a tray that was then clamped. A comb was added at the top of the plates to form 52 wells and the gel was left to set for approximately 1 hour.

PCR products were denatured for 10 minutes at 95°C after adding 25 µl of deionised formamide (99.5%) and bromophenol blue loading dye. Samples were placed on ice for at least 10 minutes prior to loading. Gels were run using a Corbett Research GelScan 2000, a PCR fragment analyser. The gel was run vertically in 0.6x TBE buffer. 1µl of denatured PCR product from each sample was loaded into the wells using a pipette. A size ladder, with a band at 86bp and at every 50bp thereafter, was loaded after every eight samples for allele size calibration. Gels were run at 1200 V at 40°C for approximately one hour.

The image output was viewed with the program OneDScan. This software detects the number and location of lanes in a gel and the brightness of each band. The resulting high resolution image clearly differentiates bands to one basepair (bp) apart and identifies alleles that have gained or lost a microsatellite repeat unit (Figure 2.5). The size ladder was used to determine the exact length of the DNA fragments. The basepair length of samples was compared to an estimated size for each loci (Table 2.2) and bands within this range were marked and scored.

2.6 Data Analysis

2.6.1 Analysis of Allele Frequencies

Once bands had been marked and scored from an acrylamide gel, a database of allele

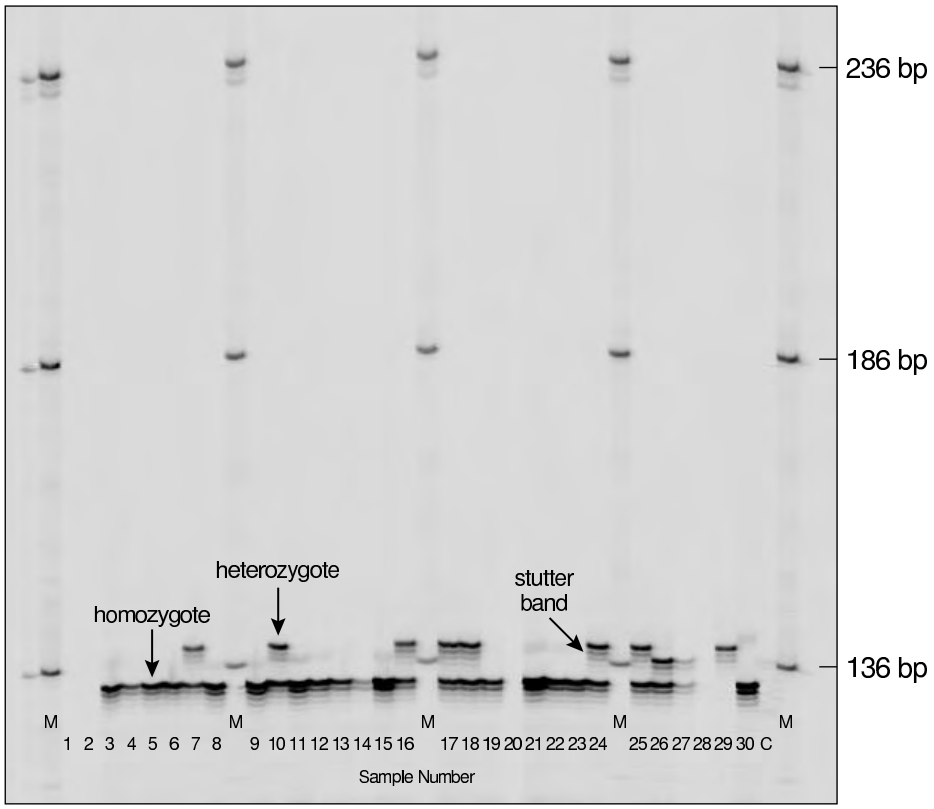

Figure 2.5 Acrylamide gel image of 30 individuals from Fish Hole for locus TLE 7.2. Size marker lanes are labelled "M" and a negative control lane is labelled "C". Examples of a homozygote (single band), heterozygote (double bands) and stutter band are indicated with arrows.

sizes was created using Microsoft EXCEL. Significant deviation from zero for Hardy-Weinberg equilibrium of observed and expected heterozygosity and F_{IS} values were tested using FSTAT software. GENEPOP was used to test for linkage disequilibrium between pairwise loci combinations (Raymond and Roussett, 1995). Differentiation among allele frequencies of populations was assessed by pairwise tests using GENEPOP.

2.6.2 Population Subdivision

Population subdivision was analysed under two models of mutation: F_{ST} estimates that assume an IAM (infinite allele model) and R_{ST} estimates that assume a SMM (stepwise mutation model). F_{ST} values were calculated for separate and combined catchments using the program FSTAT (Weir and Cockerham, 1984). R_{ST} values were calculated for separate and combined catchments using the program RSTCALC (Goodman, 1997). Genetic differentation among populations was given by Fisher's exact test of allele frequency using a Markov chain method in GENEPOP (Raymond and Rousset, 1995).

F_{ST} estimates were spatially scaled within a two dimensional area with the statistical program SPSS using the Bray and Curtis (1957) distance matrix method. F_{ST} and R_{ST} estimates were correlated in Microsoft EXCEL.

2.6.3 Assignment Tests

Individuals were assigned to populations based on their multi-locus genotypes using a Bayesian method (Rannala and Mountain, 1997) with GENECLASS software. The

program was run using the simulation option and a threshold of 0.01 applied to assignments (Cornuet *et al.*, 1999). This method can assign individuals to a single site, but individulas can also be assigned to multiple sites if the probability of assignment is high for more than one population. To produce pie charts, individuals that were assigned to a single site were given the value 1 and individuals assigned to multiple sites were given the value of 1 divided evenly by the number of sites assigned. Interpretation of the data for Figures 3.10, 3.11 and Table 3.4 does not imply a distinct representation for each individual, however this study is more focused on the overall patterns of gene flow and dispersal and these factors remain clearly detectable.

Evidence of sex-biased dispersal was tested using the methods described by Favre *et al.* (1997). Calculations were performed by log-transforming the assignment index value and then subtracting the population mean to obtain a corrected assignment index value.

2.6.4 Correlations Between Genetic Character and Geomorphology

Channel distance between sites was estimated using satellite imagery. FSTAT was used to test for an association between channel distance (log distance) and genetic divergence (F_{ST} values).

2.6.5 Adjustment of Significance Levels

All significance levels for tests involving multiple comparisons were adjusted following the Bonferoni method; a' = a/k, where a = predetermined significance level and k = number of tests performed (Rice, 1989).

3.0 Results

3.1 Validation of the Data

3.1.1 Amplification of Loci

All loci were amplified and scored successfully, with the exception of locus TLE 9.2. PCR conditions and reaction solutions were varied for TLE 9.2, particularly PCR annealing temperature and volume of $MgCl_2$ or DNA polymerase taq. The resulting acrylamide gel showed monomorphic allele fragments near the expected allele size. If these fragments are identified as the correct locus, there was no variation in alleles found and the locus is monomorphic. However, there is also a chance that these fragments were nonspecific and amplification was unsuccessful. If this is the case, there may have been an error in primer or design of the microsatellite library for this one locus. To rectify this, the original clone sequences from which the microsatellite library was created would need to be re-evaluated. It was decided, in this case, to leave TLE 9.2 out of this study and continue with the evaluation of six loci, TLE 2.1, TLE 6.2, TLE 7.2, TLE 10, TLE 13.1 and TLE 13.3.

3.1.2 Linkage Disequilibrium

Tests for linkage disequilibrium between paired loci indicated no significant deviation from zero ($p < 0.002$, after correction for multiple comparisons). Deviations from a random combination of allele frequencies at different loci would imply that the fate of an allele is correlated with that of a neighbouring loci. The results from this test imply that all loci were independent and could therefore be considered for further analysis.

3.1.3 Hardy-Weinberg Equilibrium

Six loci from ten populations were tested and all data conformed to the expected Hardy-Weinberg equilibrium (Table 3.1), with the exception of one locus. The ratios of expected and observed heterozygosity deviated significantly away from zero at the locus TLE 2.1 for Welford and Currareeva populations. Both populations exhibited a deficiency of heterozygosity.

Compared to other loci, TLE 2.1 was the most difficult to score and had the highest proportion of nonspecific fragments. There is a chance that the two populations did not conform to expectations as a result of an error in scoring. However, scored alleles were more carefully checked and compared at this locus than any other, both within populations and between populations. Even with a higher number of nonspecific fragments, the allele range seemed clear.

The combined data per site remains consistent between ratios of observed and expected heterozygosity, according to Hardy-Weinberg equilibrium (Table 3.1). While two sites may have a statistically significant decrease of heterozygosity at one locus, there appears to be no biological significance attached to this. These results indicate that sampled populations do not violate the underlying assumptions of Hardy-Weinberg equilibrium and that populations do not exhibit significant levels of non-random mating, migration or selection (Frankham *et al.*, 2002). Conformation to the expected Hardy-Weinberg ratios also suggest that populations do not show signs of inbreeding and that gene flow is not restricted within fragmented populations (Frankham *et al.*, 2002).

Table 3.1 Average number of alleles observed per loci (A_o / loci) and standard error for alleles observed per loci (S.E.). Expected heterozygosity (H_e), observed heterozygosity (H_o) and values for the identity in state (F_{IS}) for all loci. Populations abbreviated as follows: in Cooper Creek, Fish Hole (FH), Waterloo (WO), Welford (WE), Currareeva (CU), Springfield (SP), Eulbertie (EB); in the Warrego River, Sandford (SF), Quilberry (QB), Thurrulgoona (TH) and Binya (BY).

	Cooper catchment						Warrego catchment			
	FH	WO	WE	CU	SP	EB	SF	QB	TH	BY
A_o / loci	5.000	5.333	5.000	4.500	4.000	4.500	8.500	7.167	5.833	6.333
S.E.	0.483	0.491	0.298	0.361	0.211	0.376	0.935	0.865	0.552	0.534
H_e	0.569	0.618	0.609	0.594	0.542	0.602	0.670	0.650	0.633	0.663
H_o	0.511	0.561	0.539	0.511	0.496	0.567	0.588	0.593	0.622	0.644
F_{IS}	0.103	0.093	0.117	0.141	0.087	0.059	0.125	0.089	0.018	0.030

Estimations of F_{IS} are an indication of the proportion of the total heterozygosity within a population due to heterozygosity within subpopulations (Frankham *et al.*, 2002), and thus have resulted in a similar outcome to the Hardy-Weinberg ratios of expected and observed heterozygosity. Again, Welford and Currareeva show significant deviations from zero at loci TLE 2.1 ($p < 0.00083$, after correction for multiple comparisons), although the combined data from six loci conform to expected values of F_{IS} for each population. Estimations of F_{IS} therefore indicate that no further subdivision exists within designated populations, and that combining samples from two waterholes at the Welford site has not resulted in any bias.

3.2 Allele Frequency and Diversity

3.2.1 Variation in Allele Size Between Populations

Variation in allele sizes was detected at each locus and was clearly shown in a histogram of the alleles detected and their frequency. Allele frequencies displayed differences in the number of alleles and allele size between Cooper populations (Fish Hole to Eulbertie) and Warrego populations (Sandford to Binya) (Figure 3.1). In particular, Warrego River populations demonstrated a broader range of allele sizes compared to those of Cooper Creek. Populations within each catchment also varied in allele size, although these differences were smaller in magnitude than those between catchments.

3.2.2 Variation in Number of Alleles Between Populations

There was strong variation in the number of alleles detected in populations within each catchment. The number and diversity of alleles was clearly distinct between the Cooper

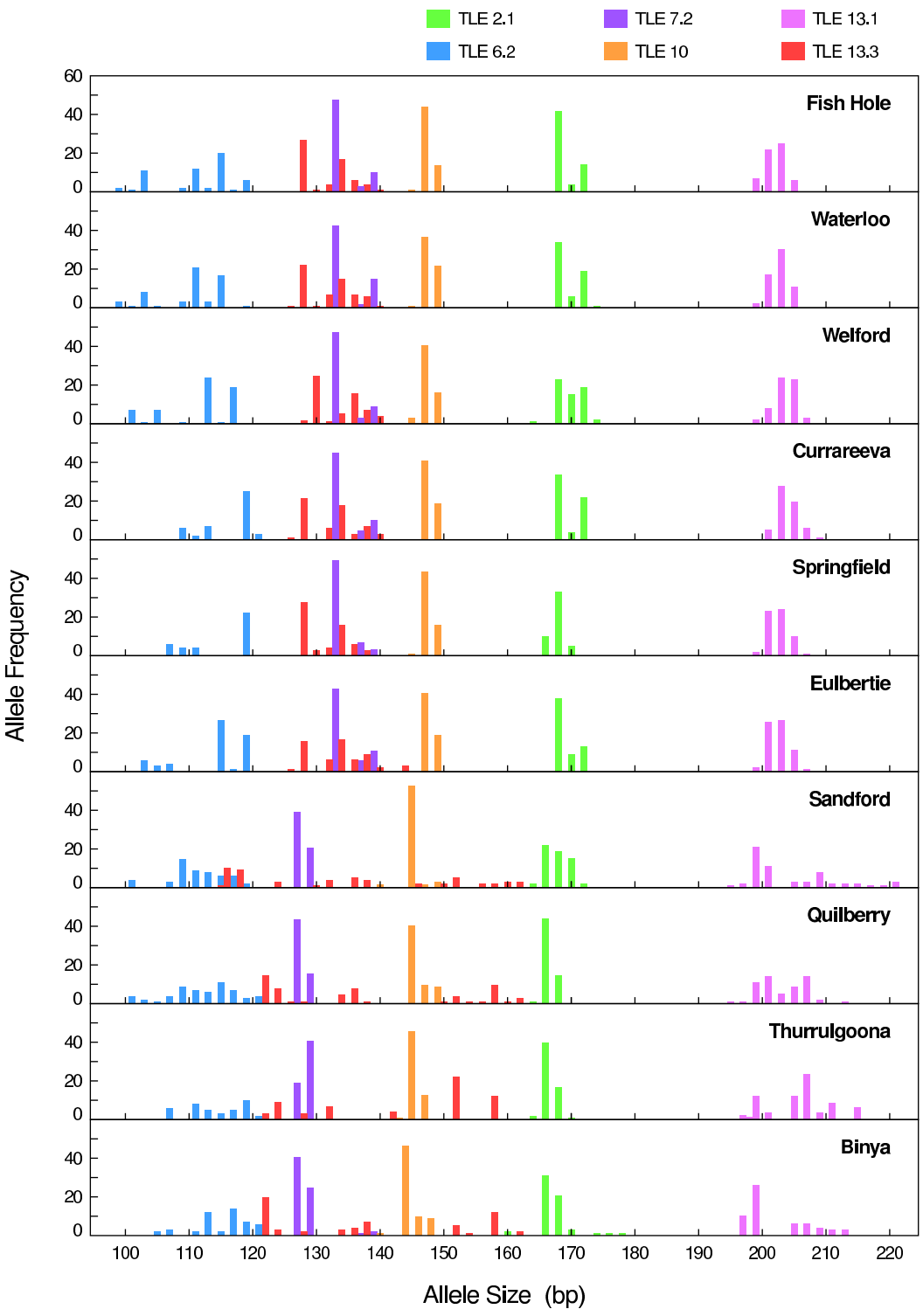

Figure 3.1 Diversity of allele sizes and frequencies at each locus for Cooper Creek and Warrego River populations. Each loci is shown in a different colour with ranges in size and frequency along the X and Y axis.

and Warrego catchments, although this variation was weaker within each catchment (Figures 3.2-3.7). Interestingly, Warrego River contained higher allele diversity compared to Cooper Creek, especially where high allele numbers were present, for example at locus TLE 6.2, TLE 13.1 and TLE 13.3 (Figures 3.3, 3.6 and 3.7). This difference was evident in the mean number of alleles observed, a total mean of 4.72 alleles per locus in the Cooper and an average of 6.96 alleles per locus in the Warrego (Table 3.1).

The Welford population was compromised of some unusual alleles compared to other sites within the Cooper catchment, particularly for loci TLE 6.2, TLE 10 and TLE 13.3 (Figures 3.3, 3.5 and 3.7). Some of these alleles were also present in the Warrego (Figures 3.3 and 3.5) while another seemed to be unique to Welford (Figure 3.7). The Springfield site in Cooper Creek also contained one allele at 166 bp that was common to the Warrego, but unique elsewhere in the Cooper at locus TLE 2.1 (Figure 3.2).

3.2.3 Pairwise Tests for Allele Frequency Differences

Pairwise tests of allele frequencies across all loci between Cooper and Warrego catchments were all highly significant. Similarly, pairwise tests of allele frequencies within each catchment were also highly significant ($p < 0.001$, after correction for multiple comparisons), with the exception of Fish Hole and Waterloo in Cooper Creek ($p = 0.51$). This indicates strong subdivision among populations between Cooper and Warrego catchments and moderate subdivision among each population within separate catchments, except for the Fish Hole and Waterloo populations.

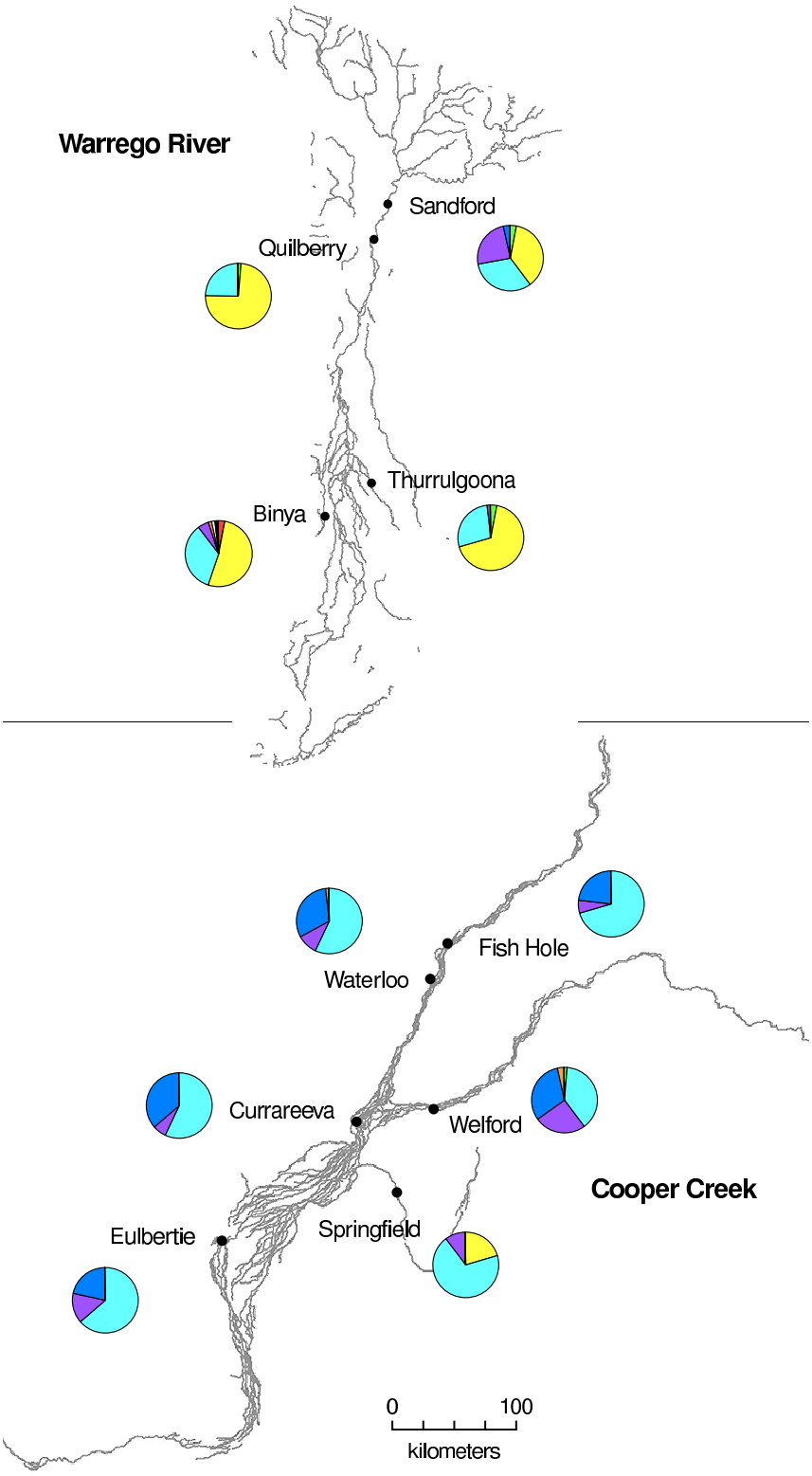

Figure 3.2 Number of alleles and their frequency at locus TLE 2.1 for the Warrego River (above) and Cooper Creek (below). Each allele is shown in a different colour as a portion of the total allelic variation for each site.

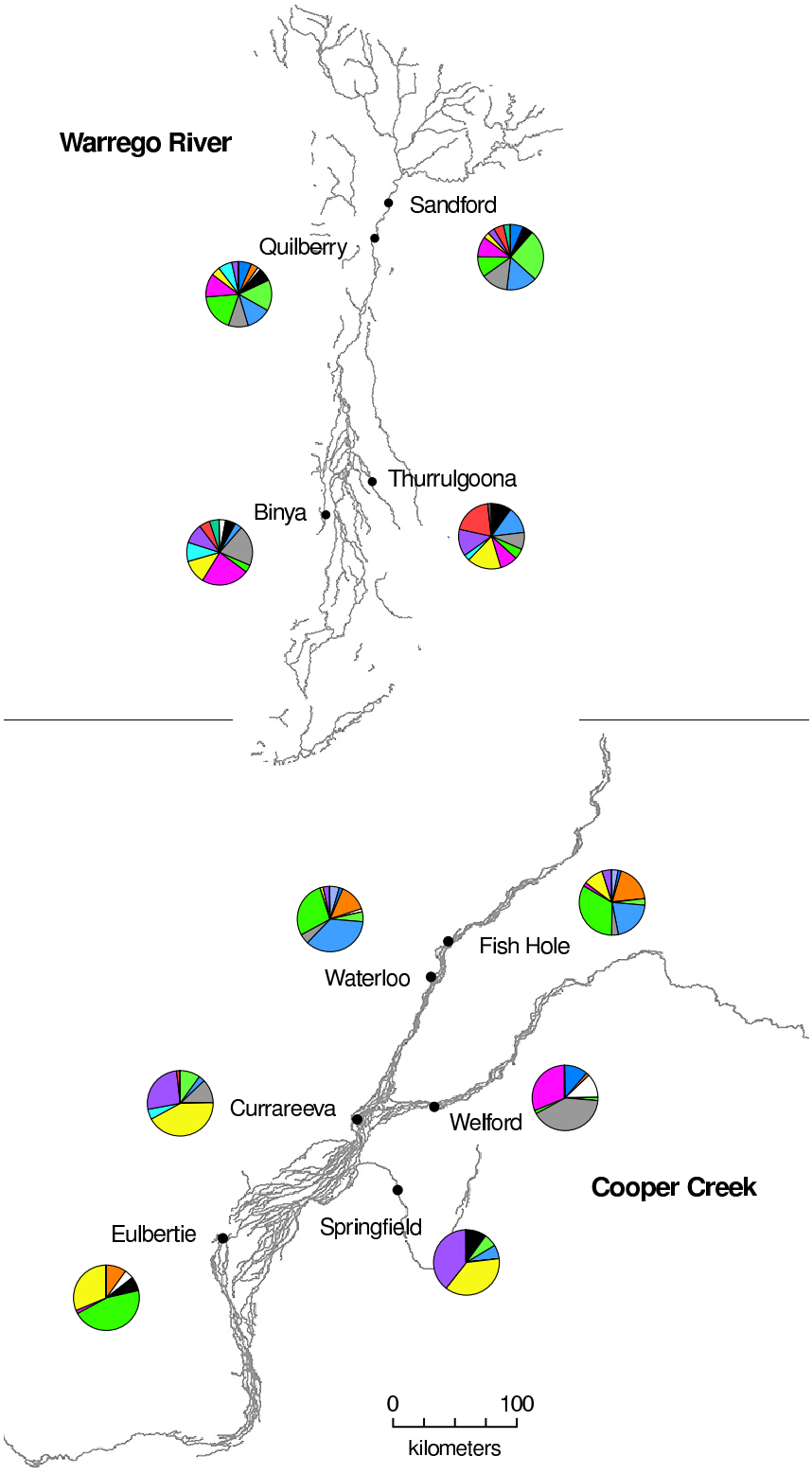

Figure 3.3 Number of alleles and their frequency at locus TLE 6.2 for the Warrego River (above) and Cooper Creek (below). Each allele is shown in a different colour as a portion of the total allelic variation for each site.

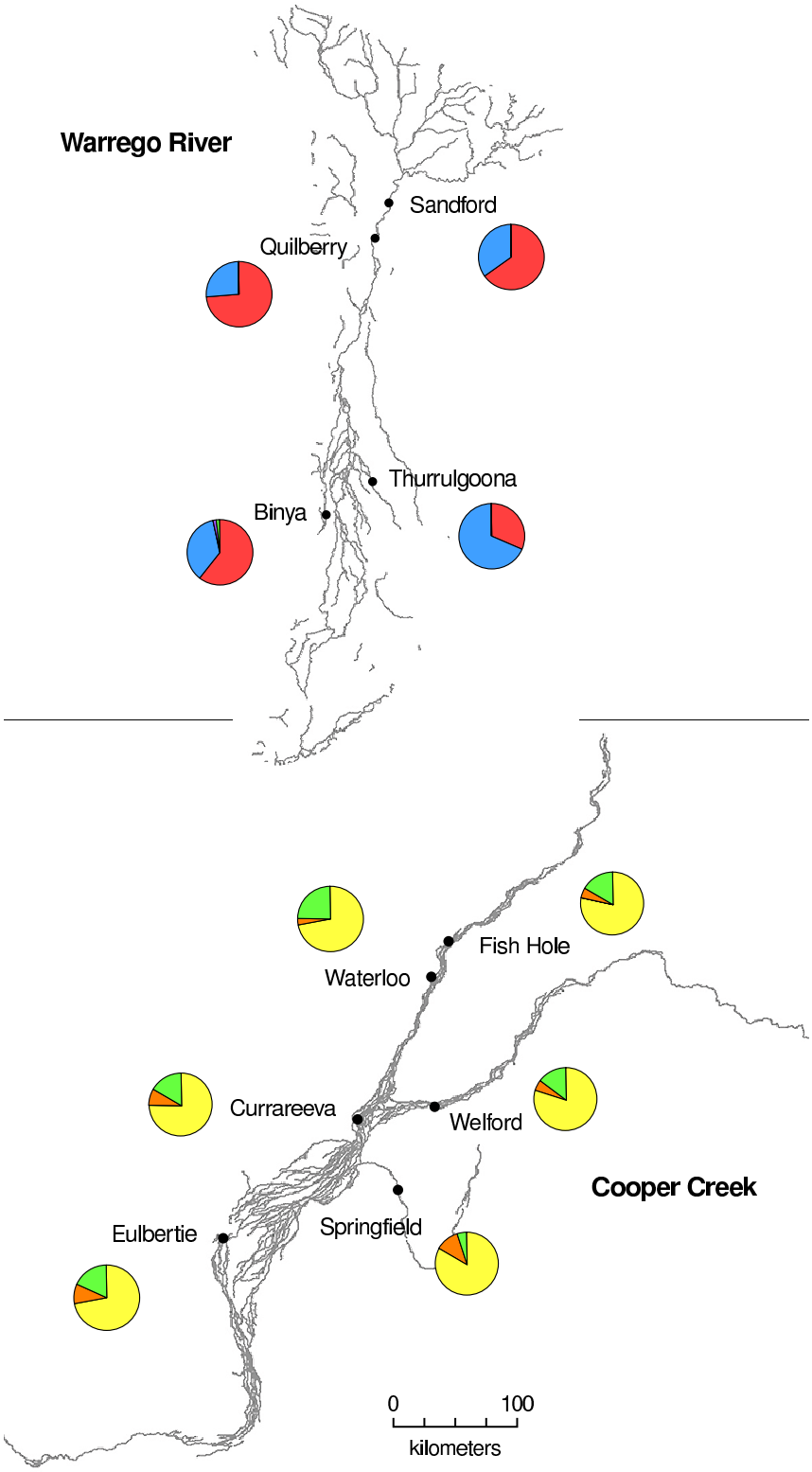

Figure 3.4 Number of alleles and their frequency at locus TLE 7.2 for the Warrego River (above) and Cooper Creek (below). Each allele is shown in a different colour as a portion of the total allelic variation for each site.

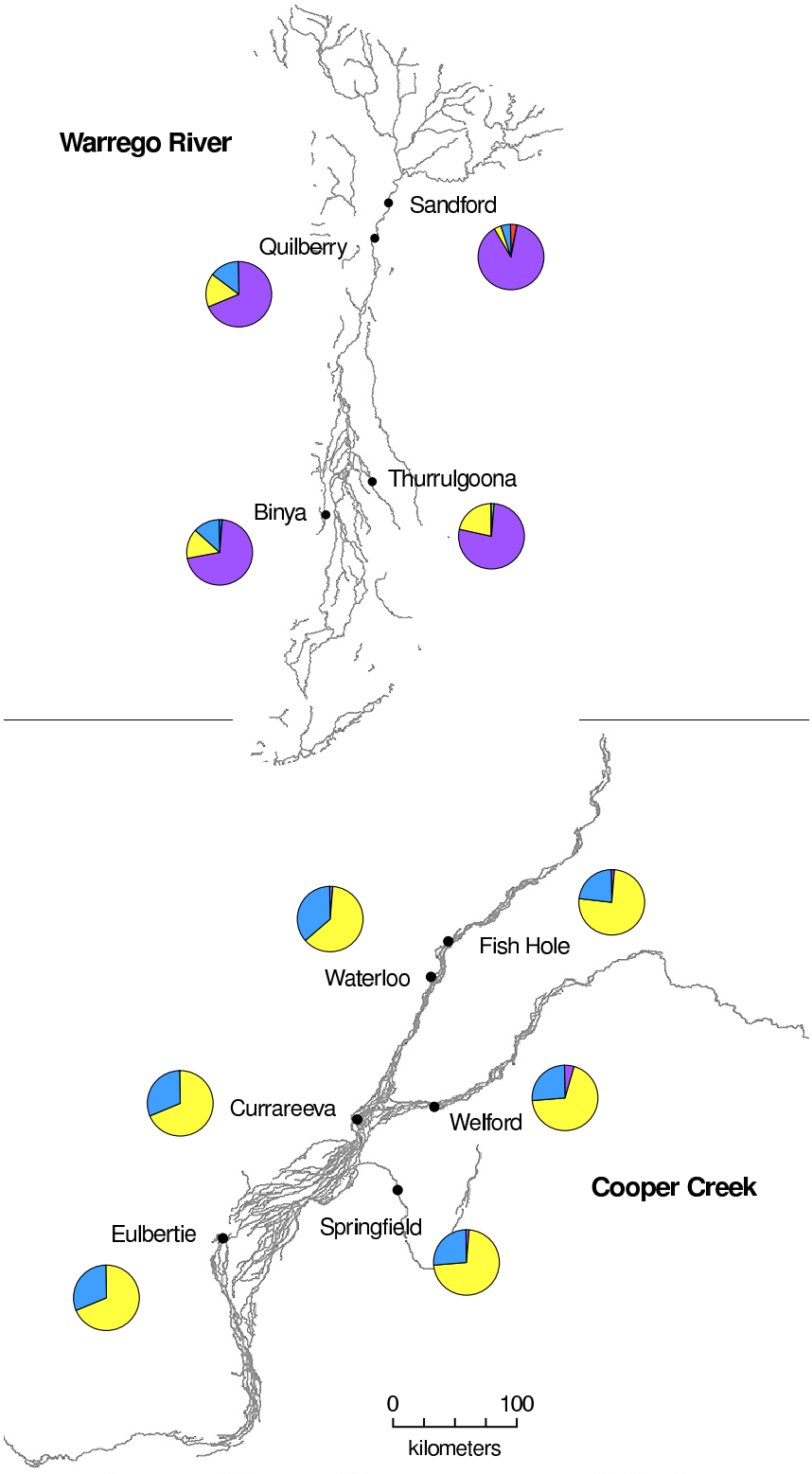

Figure 3.5 Number of alleles and their frequency at locus TLE 10 for the Warrego River (above) and Cooper Creek (below). Each allele is shown in a different colour as a portion of the total allelic variation for each site.

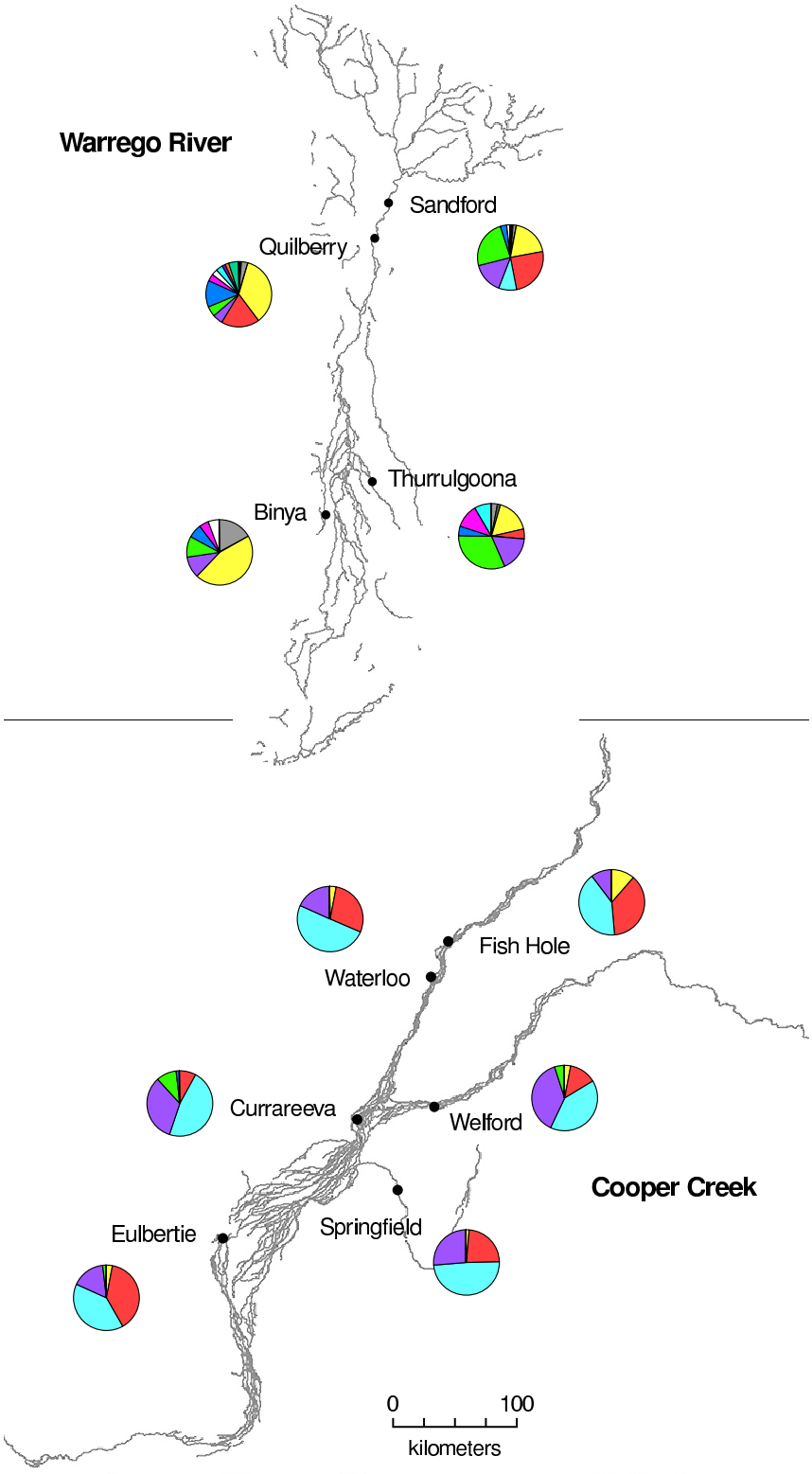

Figure 3.6 Number of alleles and their frequency at locus TLE 13.1 for the Warrego River (above) and Cooper Creek (below). Each allele is shown in a different colour as a portion of the total allelic variation for each site.

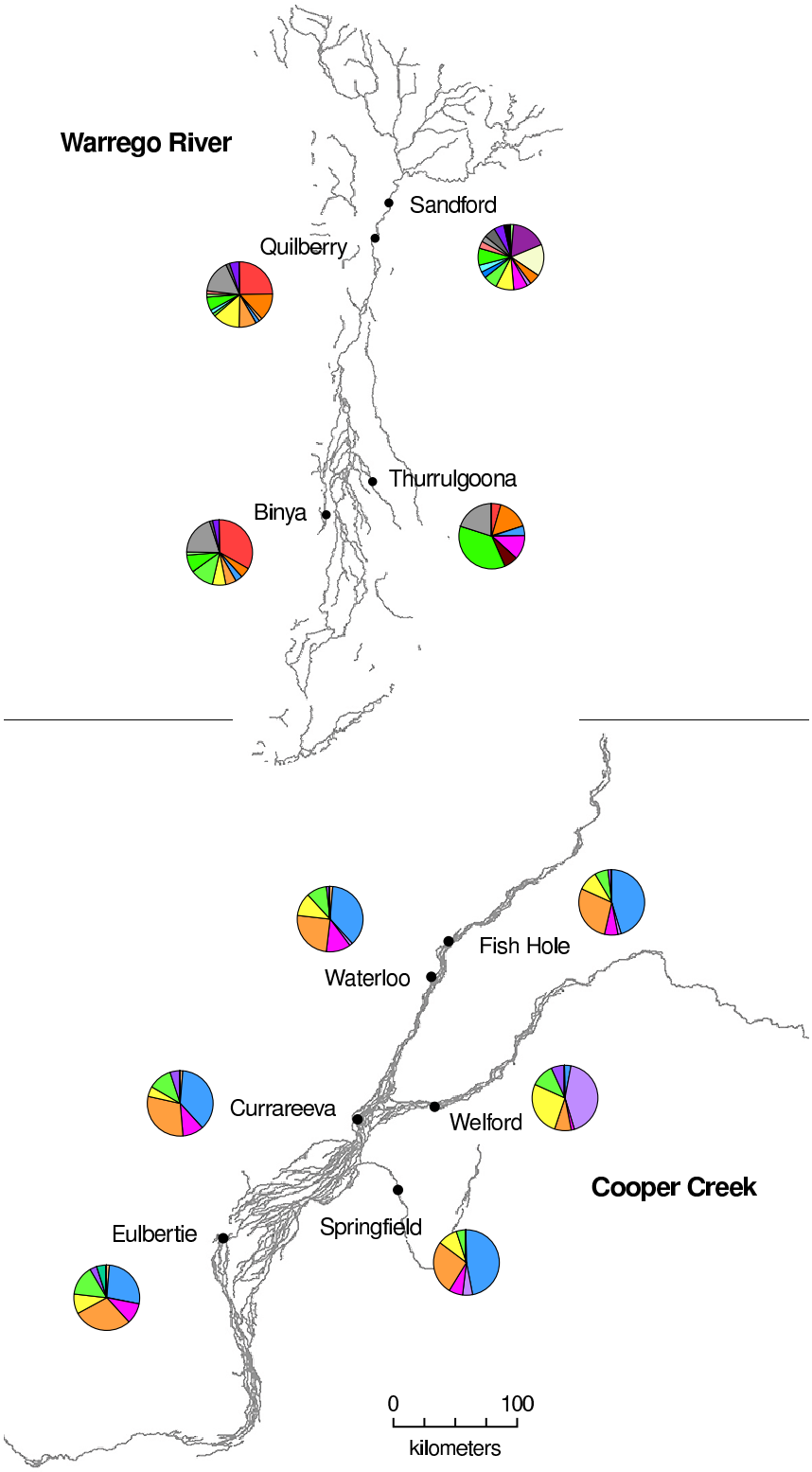

Figure 3.7 Number of alleles and their frequency at locus TLE 13.3 for the Warrego River (above) and Cooper Creek (below). Each allele is shown in a different colour as a portion of the total allelic variation for each site.

3.3 Genetic Divergence of Populations

3.3.1 Estimates of F_{ST}

Estimates of F_{ST} revealed a highly significant genetic divergence between the two catchments, with F_{ST} ranging from 0.296 - 0.340 (Table 3.2). This divergence was also clearly observed when F_{ST} estimates were spatially scaled within a two dimensional area (Figure 3.8). Furthermore, F_{ST} estimates showed significant subdivision between populations within the Cooper and Warrego catchments, where F_{ST} ranged from 0.003 - 0.144 in the Cooper and 0.032 - 0.089 in the Warrego (Table 3.2). In all pairwise tests, only Fish Hole and Waterloo populations in Cooper Creek did not deviate significantly from zero (F_{ST} = 0.003), indicating little evidence of divergence.

3.3.2 Estimates of R_{ST}

Estimates of R_{ST} between Cooper and Warrego populations were on average higher than the associated F_{ST} values (average R_{ST} = 0.375, average F_{ST} = 0.313) (Table 3.2 and 3.3). Similar to F_{ST} values, R_{ST} estimates indicated highly significant genetic subdivision between the Cooper and Warrego catchments, with R_{ST} ranging from 0.290 - 0.548 (Table 3.3). In contrast to F_{ST}, estimates of R_{ST} did not significantly deviate from zero between the populations Welford and Waterloo (R_{ST} = 0.031), or between the populations Eulbertie and Fish Hole (R_{ST} = 0.025), Eulbertie and Waterloo (R_{ST} = 0.027) and Eulbertie and Welford (R_{ST} = 0.044). However, similarly to F_{ST} estimates, R_{ST} did not significantly deviate from zero between Fish Hole and Waterloo populations (R_{ST} = 0.016). Overall, R_{ST} estimates ranged from 0.016 - 0.171 in the Cooper and 0.052 - 0.130 in the Warrego (Table 3.3).

Table 3.2 F_{ST} values for waterhole populations and levels of significance indicated (*p < 0.001, after correction for multiple comparisons). Populations abbreviated as follows: in Cooper Creek, Fish Hole (FH), Waterloo (WO), Welford (WE), Currareeva (CU), Springfield (SP), Eulbertie (EB); in the Warrego River, Sandford (SF), Quilberry (QB), Thurrulgoona (TH) and Binya (BY). Grey areas indicate values between catchments, white areas indicate values within a single catchment.

	FH	WO	WE	CU	SP	EB	SF	QB	TH
WO	0.003								
WE	0.124*	0.100*							
CU	0.058*	0.051*	0.100*						
SP	0.061*	0.078*	0.144*	0.028*					
EB	0.011*	0.026*	0.116*	0.050*	0.067*				
SF	0.321*	0.300*	0.304*	0.324*	0.336*	0.310*			
QB	0.314*	0.293*	0.303*	0.315*	0.320*	0.299*	0.051*		
TH	0.340*	0.321*	0.327*	0.323*	0.331*	0.326*	0.089*	0.074*	
BY	0.312*	0.296*	0.287*	0.300*	0.311*	0.299*	0.047*	0.032*	0.067*

Table 3.3 R_{ST} values for each water hole population and levels of significance indicated (*p < 0.001, after correction for multiple comparisons). Abbreviations and grey areas marked as in Table 3.2.

	FH	WO	WE	CU	SP	EB	SF	QB	TH
WO	0.016								
WE	0.068*	0.031							
CU	0.139*	0.119*	0.080*						
SP	0.128*	0.156*	0.171*	0.094*					
EB	0.025	0.027	0.044	0.089*	0.120*				
SF	0.290*	0.316*	0.309*	0.367*	0.310*	0.295*			
QB	0.338*	0.358*	0.339*	0.422*	0.376*	0.347*	0.061*		
TH	0.537*	0.548*	0.504*	0.450*	0.433*	0.496*	0.130*	0.125*	
BY	0.337*	0.378*	0.364*	0.318*	0.256*	0.312*	0.058*	0.052*	0.076*

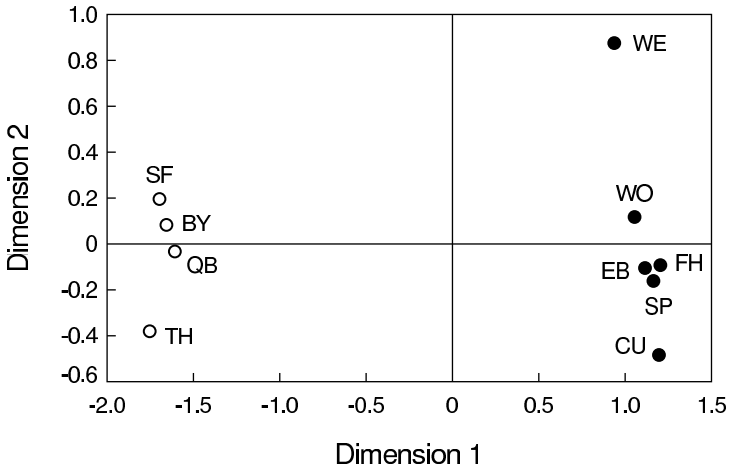

Figure 3.8 Two dimensional scaling of F_{ST} estimates for all populations. Closed circles indicate Cooper Creek populations, open circles indicate Warrego River populations. Site names are abbreviated as follows: in Cooper Creek, Fish Hole (FH), Waterloo (WO), Welford (WE), Currareeva (CU), Springfield (SP), Eulbertie (EB); in the Warrego River, Sandford (SF), Quilberry (QB), Thurrulgoona (TH) and Binya (BY).

Most surprisingly, F_{ST} and R_{ST} estimates were found to be especially inconsistent at one Cooper site, Welford (Table 3.2 and Table 3.3). Comparisons between the Welford population have among the lowest R_{ST} values, and the among highest F_{ST} values in the Cooper catchment. While there were observed differences in estimates of F_{ST} and R_{ST} among populations, none show such contrasting values as those between Welford and the remaining Cooper populations.

Although some sites displayed differences between F_{ST} and R_{ST} values, the two estimates remained correlated (Figure 3.9). Populations within catchments showed lower F_{ST} and R_{ST} values and had less variation, resulting in a gentle correlation slope. In contrast, populations between Cooper and Warrego catchments showed high F_{ST} and R_{ST} values with higher variation that resulted in a steeper correlation.

3.4 Migration of Individuals Among Populations

3.4.1 Assignment Test for the Identification of Migrants

Individuals that were assigned to a single site were given the value 1 and individuals assigned to multiple sites were given the value of 1 divided evenly by the number of sites assigned. However, individuals that were assigned to multiple sites often contained a high allocation to the original population. This has exaggerated the amount of assignment to external populations and has underestimated the proportion of individuals that were actually self-assigned. However, the overall patterns of gene flow and dispersal among populations in the Cooper and Warrego catchments remain clearly detectable.

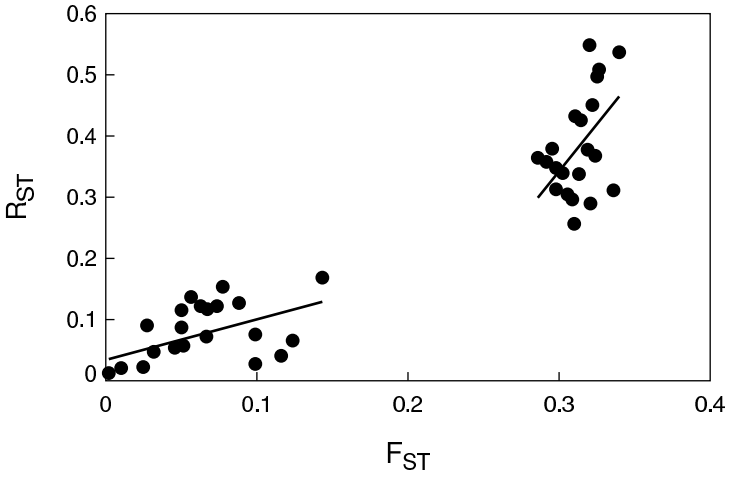

Figure 3.9 Correlation of F_{ST} and R_{ST} estimations of all populations in the Cooper and Warrego catchments. Populations within a single catchment exhibit lower values and show a gentler slope of correlation (left) than populations between catchments (right).

Assignment tests generally confirmed the trends found in F_{ST} and R_{ST} estimations of genetic divergence. There was no migration found between the Cooper and Warrego catchments. There was, however, evidence of migration between populations within Cooper and Warrego catchments (Figures 3.10 and 3.11). In general, dispersal was found to be lower in Warrego River compared to Cooper Creek, as demonstrated by the higher number of individuals that were self-assigned (Cooper average = 49.1, Warrego average = 31.3) (Table 3.4). Both catchments contained a small number of individuals that found no assignment in any populations (Cooper average = 0.55, Warrego average = 3.4) (Table 3.4).

3.4.2 Migration Patterns in Cooper Creek

In agreement with the low levels of genetic separation between Fish Hole and Waterloo that were indicated by F_{ST} and R_{ST} values, these waterholes showed a comparatively high level of dispersal between them (Figure 3.10). Other Cooper sites indicated that Fish Hole and Waterloo provided a relatively high proportion of emigrant individuals to other populations within the Cooper drainage (Figure 3.10 and Table 3.4). Again, Welford waterhole was an unusual population in having the strongest self-assignment in comparison to other sites (Figure 3.10 and Table 3.4). This suggests a low amount of immigration into Welford relative to other populations in Cooper Creek.

3.4.3 Migration Patterns in Warrego River

In general, populations within Warrego River showed stronger self-assignment than in populations from Cooper Creek (Table 3.4). Few individuals were shown to have

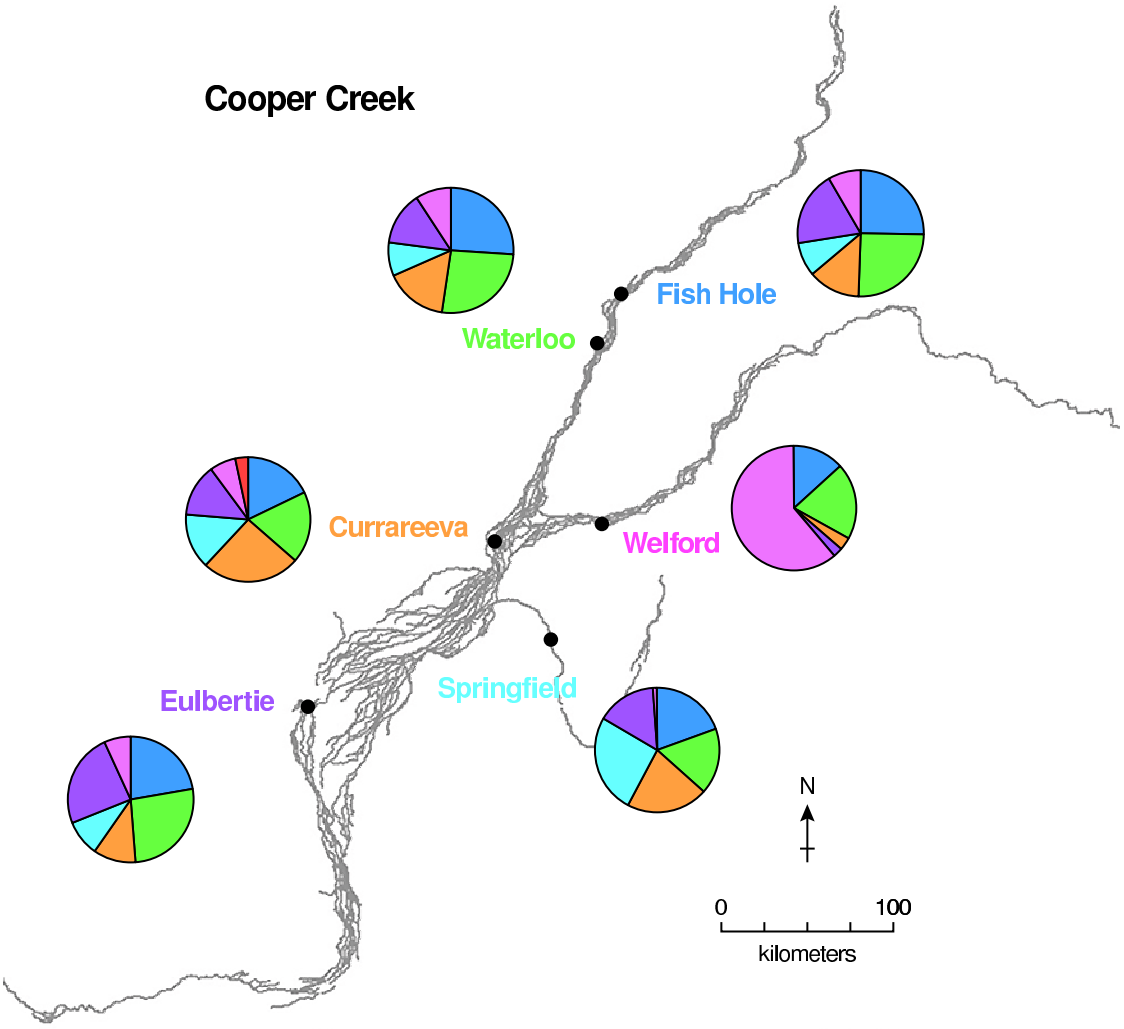

Figure 3.10 Assignment of individuals from each population in the Cooper catchment. Each site is colour coded and identical coloured pie slices indicate the proportion of individuals that were assigned to those corresponding populations. Red pie slices indicate unassigned individuals.

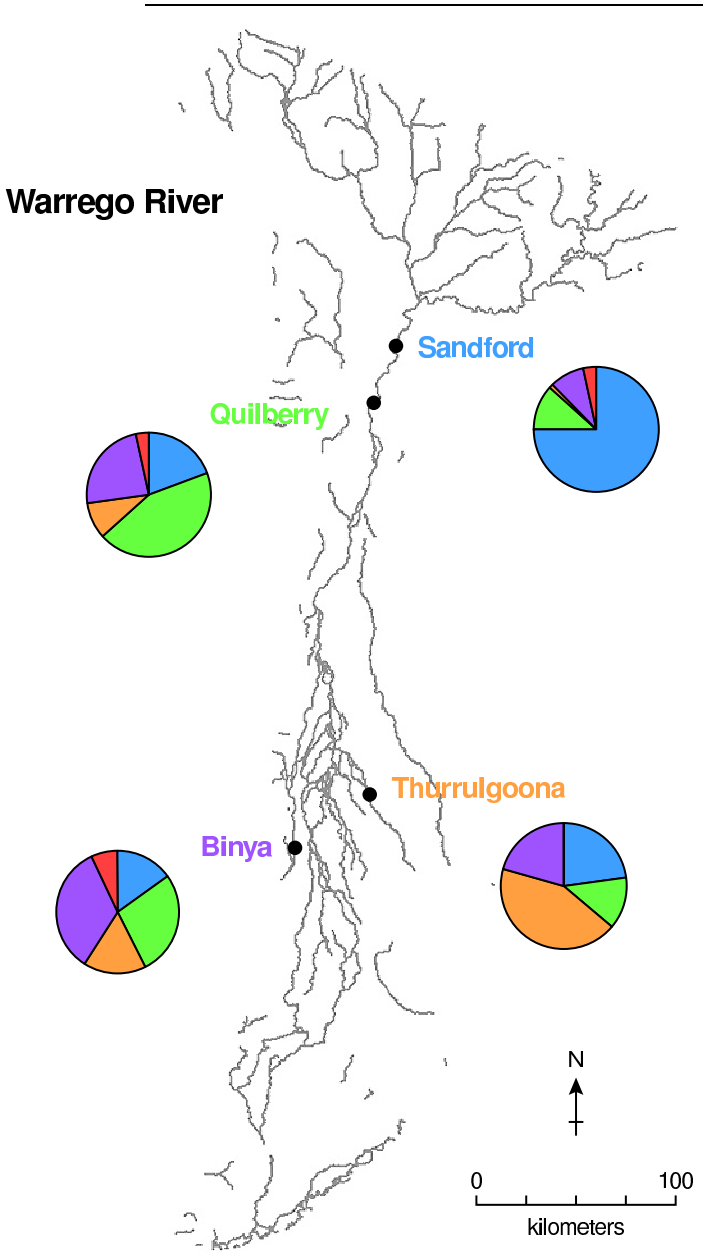

Figure 3.11 Assignment of individuals from each population in the Warrego catchment. Each site is colour coded and identical coloured pie slices indicate the proportion of individuals that were assigned to those corresponding populations. Red pie slices indicate unassigned individuals.

Table 3.4 Percentage of individuals in each population that were shown to be self-assigned, unassigned, immigrant and emigrant. Data obtained from a simulated assignment test with a probalistic threshold (0.01) and then converted into percentages.

	Cooper catchment						Warrego catchment			
	FH	WO	WE	CU	SP	EB	SF	QB	TH	BY
% Self-assignment	25.2	26.1	61.2	25.4	25.8	24.4	75.0	43.9	43.3	34.2
% Unassigned	0.0	0.0	0.0	0.0	3.3	0.0	3.3	3.3	0.0	6.9
% Immigrants	74.7	73.9	38.8	71.2	74.1	75.6	21.7	52.8	56.7	58.9
% Emigrants	16.5	17.9	5.4	10.7	6.8	10.7	14.4	12.9	6.7	13.4

emigrated from Thurrulgoona (Table 3.4) and comparatively high F_{ST} and R_{ST} values suggest a high level of divergence from other sites (Table 3.2 and 3.3). Emigrant individuals from Sandford were relatively common in the Warrego catchment, although conversely the number of immigrants to this population was the lowest observed in this study (Figure 3.11 and Table 3.4). Considering the closeness of Quilberry and Sandford populations (approximately 25 km), little migration was detected between them, in contrast to other neighbouring waterholes such as Fish Hole and Waterloo in the Cooper catchment. Interestingly, individuals from Thurrulgoona were not detected in the Sandford population but were present at the Quilberry site nearby (Figure 3.11).

3.4.4 Sex-biased Migration

Assignment values were used to determine whether migrating individuals were sex-biased (Favre et al., 1997; Waser and Strobeck 1998; Mossman and Waser, 1999). There was no sex-bias found for either Cooper Creek ($p = 0.154$) or Warrego River ($p = 0.145$) using a two-sided statistical test. This relationship was confirmed to be not significant using the corrected assignment index values (AI_c) (Figure 3.12).

3.5 Genetic and Geomorphological Characteristics

3.5.1 Isolation by Distance

Channel distances obtained from satellite imagery were found to have no significant correlation with genetic distance in either Cooper Creek ($p = 0.464$) or in Warrego River ($p = 0.824$). This lack of relationship is apparent when produced graphically (Figure 3.13).

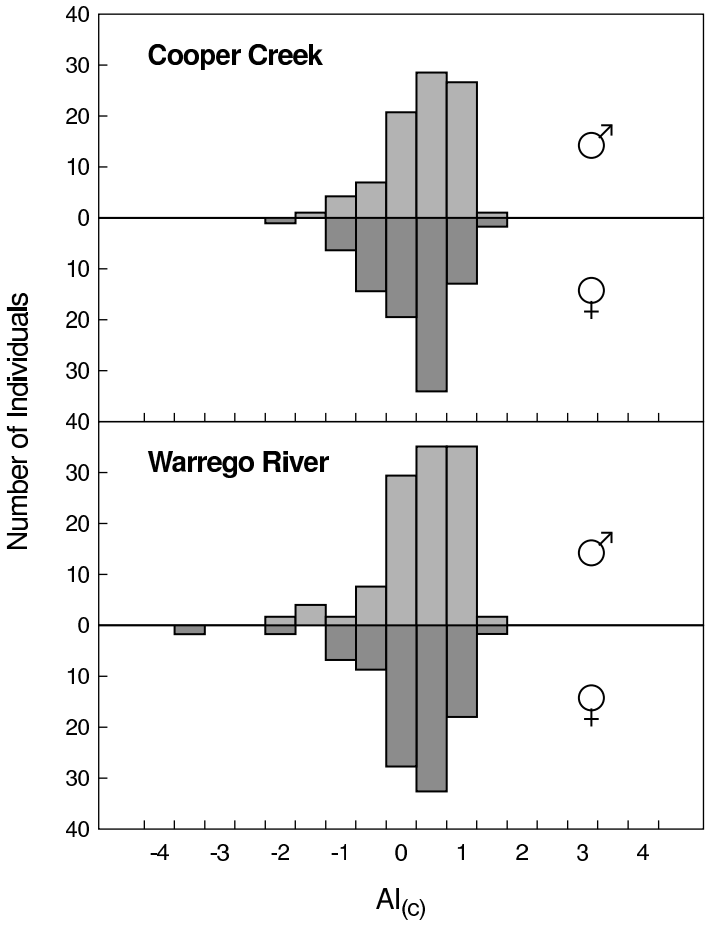

Figure 3.12 Sex-biased migration using corrected assignment index values (AI_c). Separation of male migrants (above) and female migrants (below) are shown for the Cooper and Warrego catchments. Positive integers on the X axis indicate individuals that are more likely to have been born locally, while negative integers indicate possible migrants.

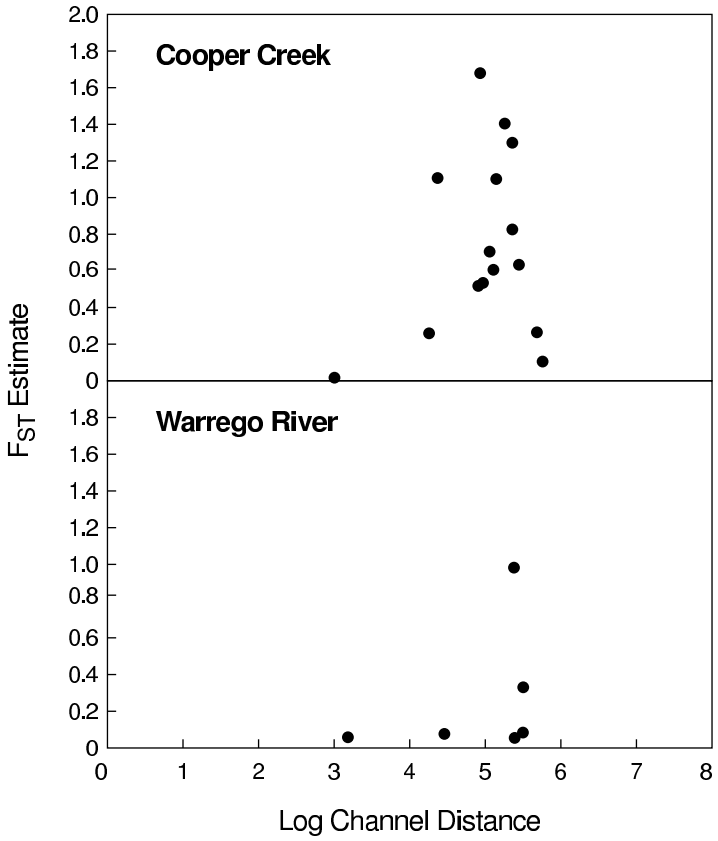

Figure 3.13 Genetic isolation (F_{ST} estimates) and log channel distance show no correlation in Cooper Creek (above) or Warrego River (below).

4.0 Discussion

4.1 Summary of the Principle Findings

4.1.1 Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium was consistent for all loci, except TLE 2.1 where two out of ten populations showed a heterozygote deficiency. Deviation from the expected Hardy-Weinberg equilibrium at this locus may have resulted from the presence of a null allele. This occurs when mutation in the flanking sequence where primers anneal causes heterozygotes to appear as homozygotes, thus raising the overall observed homozygosity. However, null homozygotes would not be expected to amplify at all and as the failure rate of amplified samples was not great, the occurrence of a null allele at locus TLE 2.1 is unlikely.

In any case, the combined data did not significantly deviate from the expected ratios of heterozygosity, suggesting that the few populations that were out of Hardy-Weinberg equilibrium did not significantly influence the results of this study. Conformation to expected ratios of heterozygosity indicate these populations did not violate the underlying assumptions of Hardy-Weinberg equilibrium. The result implies that these freshwater turtles do not show signs of inbreeding within populations and that gene flow is not restricted by fragmentation within waterhole populations in either Cooper Creek or Warrego River (Frankham *et al.*, 2002).

4.1.2 Estimates of Genetic Divergence

F_{ST} and R_{ST} estimates both indicated that the Cooper and Warrego catchments were highly differential, and this separation could be further observed in the variation of allele diversity and frequency recorded between catchments. Although genetic diversity between the Cooper and Warrego catchments was expected, the F_{ST}, and particularly the higher R_{ST} estimates suggested a much greater level of genetic divergence than expected. Moreover, in contrast to predictions, F_{ST} and R_{ST} estimates also detected a moderate level of genetic divergence between populations within the same catchment.

4.1.3 A Comparison Between F_{ST} and R_{ST} Estimates

Although a remarkable pattern of genetic structuring has emerged from these results, there was also a notable variation in the estimates of divergence between populations produced by F_{ST} and R_{ST}. For example, R_{ST} produced much higher estimations of divergence between the Cooper and Warrego catchments, compared to that of F_{ST} (F_{ST} average = 0.313; R_{ST} average = 0.375). In most cases, R_{ST} values were also higher for population comparisons within the same catchment, although more R_{ST} estimates were found to be not significantly different from zero (F_{ST} average for the Cooper = 0.068 and for the Warrego = 0.06; R_{ST} average for the Cooper = 0.087 and for the Warrego = 0.084). Variation between F_{ST} and R_{ST} estimates in genetic studies are often reported and can only be accounted for by contrasts in the assumptions used by these models (Balloux and Lugon-Moulin, 2002). Because the SMM is a more realistic model of microsatellite mutation, it has been suggested that R_{ST} is a better indicator of

interspecific divergence, however as the impact of migration becomes more important than that of mutation, R_{ST} is thought to be less accurate than that of F_{ST} at detecting intraspecific divergence (Balloux and Lugon-Moulin, 2002; Forbes *et al.*, 1995).

This argument would support the higher R_{ST} values found between the Warrego and Cooper catchments as being closer to the true estimates of population divergence. It would also suggest that the consistently significant F_{ST} values reported within each catchment are a better reflection of the actual population subdivision within Cooper Creek and the Warrego River.

4.1.4 Detection of Migration Among Populations

In general, the patterns of dispersal and gene flow detected by the assignment test support the high levels of genetic divergence detected by F_{ST} and R_{ST} estimates. In agreement with the high genetic divergence observed between catchments reported by R_{ST}, no migration was detected between the Cooper and Warrego catchments. Within each catchment, migration of turtles between populations remained relatively consistent, although dispersal between Fish Hole and Waterloo was high, in agreement with F_{ST} and R_{ST} estimates that were not significant. The exception to these results was that of the Welford population, in Cooper Creek.

4.1.5 A Unique Population in Cooper Creek

The Welford population presented an anomaly in the results, where R_{ST} estimates were found to be much lower than F_{ST} estimates. It has been recognised that R_{ST} is reduced

when mutations involve even a small proportion of random events that erases part of the memory of mutation, rather than the addition or deletion of one repeat unit (Balloux *et al.*, 2000). If this was the case, it could be expected that allele size distributions would cover a broader range of allele sizes with an increased number of gaps between them. However, the allele frequencies do not suggest that Welford has any noticably different pattern to that of other Cooper sites. Nevertheless, deviation in the allele mutation process would only have to be small to deflate R_{ST}, and discerning a distinguishable pattern from allele frequencies may not be possible. In any case, estimations of F_{ST} demonstrate a high divergence between Welford and all other populations in Cooper Creek. The genetic isolation of Welford was clearly observed when F_{ST} estimates were spatially scaled within a two-dimensional area (Figure 3.8).

In addition, the Welford population exhibits a strong level of self-assignment than other sites in the Cooper catchment. Furthermore, very few emigrants from Welford were detected in other populations of Cooper Creek. A high level of isolation could also be distinguished from allele frequencies, where Welford contained a number of unique alleles compared to other sites. It may be possible that combining samples from two separate waterholes at Welford has influenced the data and lead to a bias in results. However, F_{IS} values and Hardy-Weinberg ratios of heterozygosity do not significantly deviate from zero, at all but one locus, and therefore do not indicate any further partitioning within this population.

It is clear from these results that Welford represents an unusual and unique population in Cooper Creek. Welford is located in a separate reach within the Cooper catchment, although the site is relatively close to Currareeva on the main Cooper channel. The unique genetic character of Welford clearly demonstrates a low connectivity between this region with the rest of the Cooper catchment. Further exploration and sampling from Welford and upstream areas is required to ascertain whether populations further north continue to demonstrate this level of genetic separation.

4.2 The Biological Significance of High Genetic Divergence

4.2.1 Comparison to Previous Studies of Freshwater Turtles

In comparison to other genetic studies, these estimations of population subdivision represent the highest values recorded for freshwater turtles in the world. Using mtDNA from slider turtles, studies in South Carolina USA reported F_{ST} values below 0.05 for nearby populations and values between 0.021 and 0.132 for populations from different catchments (Scribner *et al.*, 1986). DNA fingerprinting of populations of western pond turtles from the USA recorded F_{ST} values of 0.244, 0.047 and 0.078 between different catchments in Washington, Oregon and California states, respectively (Gray, 1995). A more recent study used highly variable microsatellite loci from giant Amazon River turtles to compare two river systems in South America, the Río Araguaia and Río Tapajós (Sites *et al.*, 1999). F_{ST} estimates fell below 0.04 and R_{ST} below 0.023 for populations within Río Araguaia, while F_{ST} ranged between 0.084-0.23 and R_{ST} ranged from -0.002-0.224 between populations in Río Araguaia and Río Tapajós (Sites *et al.*, 1999). Estimates of population divergence for freshwater turtles in rivers of temperate and

tropical regions are clearly many times lower than those reported in this study. Notably, previous studies were centred on freshwater turtles that inhabited rivers characterised by constant flow.

4.2.2 The Effect of Connectivity on Migration and Genetic Divergence

Although some measure of genetic divergence between the Cooper and Warrego catchments was anticipated, the high estimates recorded for F_{ST} and R_{ST} were well beyond expectations. Moreover, in contrast to predictions, a strong level of genetic subdivision was also detected between populations within separate catchments. The level of population structure of *E. macquarii* is many times greater than those reported for any other freshwater turtle in the world. It is clear that the highly variable environment of Australia's dryland rivers has a strongly segregating influence on the genetic structure of these long-lived turtles.

It is highly likely that the unusual flooding patterns and variation in connectivity among waterholes in dryland rivers have had a significant effect on the population structure of *E. macquarii*. Results from this study indicate a strong genetic separation between populations and is evidence that gene flow is limited in these systems, particularly between catchments but also to some extent within catchments. This suggests that these turtles have a high fidelity to their natal waterholes and exhibit diminished dispersal ability compared to other freshwater turtles in more temperate climates. This behaviour is reflective of the highly variable arid environment in which *E. macquarii* must survive. Certainly, an environment where waterholes may be greatly reduced or could

completely dry out for long periods must place severe constraints upon the migrational behaviour of these freshwater turtles. Once adult turtles have reached a deep, long-term permanent waterhole it would seem a good strategy to remain and reap the rewards of living in highly productive refugia that is somewhat sheltered from effects of drought and flood.

The chances of reproductive success and survival would be high in areas of refugia (Morton *et al.*, 1995; Lancaster and Belyea, 1997). Although competition between individuals may be intense, the selective pressure for an individual to remain in a permanent waterhole would be strong. Interestingly, the restricted level of migration exhibited by *E. macquarii* would also encourage the greatest genetic divergence at a population level. If gene flow is high, subdivided populations behave as a single panmictic population, while if gene flow is too low, small separated populations are prone to genetic deterioration through random genetic drift and inbreeding (Amos and Harwood, 1998). An intermediate level of migration would have the maximum benefit to a metapopulation in terms of heterozygosity and genetic variation.

4.3 The Patterns of Gene Flow Between Populations

4.3.1 Migration in the Cooper and Warrego Catchments

Although gene flow was low enough to produce a substantial genetic subdivision between populations, it is clear that some migration did occur. The assignment test provided evidence for both the amount of dispersal and the pattern of gene flow that exists among these populations of freshwater turtles. No migration at all was detected

between Cooper Creek and the Warrego River, suggesting that dispersal between these catchments must be a very rare event that seems to have little effect on their overall genetic variation. Migration of turtles was present among waterhole populations within each catchment, however, the representation of assignment data used in this study may have inflated the number of migrants compared to those that were self-assigned. In any case, it can be concluded that migration occurred relatively consistently between populations within each catchment, although the level of gene flow was not high enough to maintain a continuous population structure of panmictic nature. It is important to note that these results support the type of population structure indicated by F_{ST} and R_{ST} estimates, even though the assignment test is based on individual genotypes, rather than the population-wide descriptors that F_{ST} and R_{ST} rely on (Waser and Strobeck, 1998). Clearly, the assignment test detected the patterns of migration among populations, and estimates of F_{ST} and R_{ST} further determined that the level of this migration was low enough to produce moderate genetic divergence.

4.3.2 A Comparison Between Cooper and Warrego Populations

The Warrego catchment was observed to have several unassigned individuals in the Sandford, Quilberry and Binya waterholes. In addition to this, Warrego populations contained a higher number of self-assigned individuals compared to that of Cooper Creek. Explanation of these phenomenon may be derived from the presence of high allele numbers, another unique characteristic of Warrego populations. Increases in the number of unassigned individuals in the Warrego may have occurred through a type of sampling bias. As allele diversity was high, it is unlikely that all alleles were detected

and therefore could result in an escalated number of unassigned individuals. Furthermore, the increased number of unique alleles within Warrego populations could have resulted in a higher proportion of self-assigning individuals without increasing the genetic divergence detected by F_{ST} and R_{ST} estimates. A higher allele diversity may have caused Warrego populations to become more genetically distinct, without affecting their overall genetic divergence.

4.3.3 Historical Separation of Cooper and Warrego Populations

Perhaps the most biologically significant conclusion of these patterns is the variation of allele diversity between Cooper Creek and Warrego River. Clearly, the two catchments show a marked difference in the number of alleles present. It is likely that the origin of the distinct genetic characters of the Cooper and Warrego catchments have resulted from separate historical events. The possibility exists that freshwater turtles from Cooper Creek and the Warrego River were originally founded from discrete populations throughout the local region. Broadening this study to include other catchments in the area may shed some light on the relationship of turtles from the Cooper and Warrego catchments in comparison to those in neighbouring rivers.

4.4 Metapopulation Structure of Freshwater Turtles

4.4.1 Dispersal and Channel Distance

Distances between populations did not seem to affect dispersal ability in any way. From the assignment test for example, migration between Fish Hole and Waterloo was relatively high in the Cooper catchment, but the similarly distanced populations of Sandford

and Quilberry or Binya and Thurrulgoona in the Warrego had no such connection. Moreover, the results from an independent statistical test of genetic isolation over channel distance were found to be not significant. However, these findings may not necessarily exclude the possibility of a type of isolation by distance population model for turtles in the Cooper and Warrego catchments. Ecological factors, such as the direction of flow, channel depth, channel complexity or other habitat features may be a confounding influence and could be considered as co-factors with channel distance in the future.

4.4.2 A Metapopulation Model for *E. macquarii*

The relationship between F_{ST} and channel distance was found to be not significant and this suggests that the isolation by distance metapopulation model does not fit the population structure observed in *E. macquarii*. In addition, is seems unlikely that the stepping-stone model, which is similar in construction to that of the isolation by distance model, would compare closely to turtle populations in Cooper Creek and the Warrego River (Table 1.1).

Recent reviews have suggested that the majority of natural populations compares well with the mainland-island population model (Harrison, 1991; Gaggiotti, 1996). A population that fits this metapopulation model would display low immigration into a source population, but high emigration away from the source into isolated sink populations (Table 1.1). Of the waterholes studied there were none that demonstrated particularly strong emigration and weak immigration of individuals within either the Cooper or Warrego catchments, except perhaps for that of Sandford. Nevertheless, recent research

found permanent waterholes contain high numbers of mature males and females, whereas more juveniles were resident in semi-permanent waterholes (White, 2002). This type of demographic structure suggests that permanent waterholes contain a mature source population, while semi-permanent waterholes that have an age structure in disequilibrium accommodate sink populations.

Surprisingly, the permanent waterholes included in this study are most representative of the traditional island metapopulation model (Table 1.1) that does not occur commonly in natural populations (Harrison, 1991). However, including individuals from semi-permanent waterholes in future research would ascertain whether a broader source-sink structure exists between waterholes. It is certainly possible that the metapopulation structure of these freshwater turtles would change with the scale of study. Although permanent waterholes display an island metapopulation structure, higher resolution gained by adding individuals from semi-permanent waterholes, may allow a mainland-island model to emerge.

4.5 Conservation and Management Goals

4.5.1 Maintaining the Genetic Diversity of Populations

Conservation genetics plays an important role in the protection and management of species because it provides evidence for the variability and divergence of populations. When taken with aspects of demography, ecology and landscape, the genetic character of a species can provide an indication of both its immediate viability and its longer-term evolutionary potential (Haig, 1998). This information is critical when

developing a strategy for the conservation and management of any species. To this end, the concepts of Evolutionary Significant Units (ESUs) and Management Units (MUs) have been defined, at one level, to recognise and protect evolutionary heritage and, at a second level, to monitor population structure (Moritz, 1994). For long-term management objectives ESUs recognise populations that have been historically separated and are likely to have a distinct evolutionary potential (Crandall *et al.*, 2000). Over the short-term, MUs address population structure and variability over a geographic gradient (Moritz, 1994).

How does the freshwater turtle *E. macquarii* fall into the categories of ESU and MU? Genetic divergence of these turtles is highly significant between the Cooper and Warrego catchments, but is the separation strong enough to be classified as an ESU? A genetically defined ESU should include reciprocally monophyletic mtDNA haplotypes and show significant divergence of allele frequencies at nuclear loci (Moritz, 1994). Simulation studies indicate that approximately 4Ne generations of separation need to occur for there to be a high probability of this level of divergence (Neigel and Avise, 1986). While this study has demonstrated that divergence of microsatellite regions of nuclear DNA is strong, it is impossible to predict whether this would remain true for other regions of nuclear DNA or for mtDNA.

Further research is required to determine with certainty whether populations of *E. macquarii* exhibit enough divergence to be classified as single ESUs. However, from this study R_{ST} estimates clearly show extremely strong separation and the assignment test

found negligible migration between the Cooper and Warrego catchments, indicating that each catchment may deserve an ESU classification. Furthermore, populations within the Cooper and Warrego catchments can be distinguished as separate MUs, warranting individual management, particularly for that of Welford.

4.5.2 Protection of Species and Environment

The ultimate goal of studies such as this is to better understand the biological and ecological processes of a species to manage its long-term conservation. Of course species do not act as separate entities, they are ecologically linked to the environment in which they inhabit. Protection of a particular species also equates to the conservation of the ecological environment in which it lives. This study clearly demonstrates the influence of a highly variable river environment on the population structure of long-lived freshwater turtles. Episodic flooding events for which dryland rivers are distinctly characterised in Australia, are responsible for the variable aquatic connectivity that ultimately leads to a significantly high level of genetic divergence between turtle populations. The question that remains is how best to manage the agricultural demands of water from dryland rivers in such an arid and variable landscape.

The most environmentally sustainable use of water would involve harvesting excess water during large floods, allowing small flood waters to pass and halting demand during drought (Walker *et al.*, 1997). Connectivity between waterholes would remain open during moderate to large floods and refugia would not diminish during drought. However, even with a strategic management plan, the need for a consistent water

supply to irrigation and agriculture would inevitably provoke demands for access to smaller discharges (Walker *et al*., 1997). Reducing the natural flow during flooding events would decrease dispersal opportunities and further divide genetic populations that would eventually suffer the deleterious effects of random genetic drift and inbreeding (Roach *et al.*, 2001; Ciofi and Bruford, 1999). On the other hand, increasing the number of moderate flows would allow excessive migration to occur and act as a homogenising force on genetic diversity (Roach *et al.*, 2001; Gilpin, 1991). Maintaining the natural variability of flow in dryland rivers is an essential requirement to conserve the genetic divergence and patterns of gene flow between populations of freshwater turtles.

4.6 Conclusion

The freshwater turtle, *E. macquarii*, inhabits highly variable dryland rivers in arid Australia. These environments are characterised by episodic flooding and drying events that result in variable aquatic connectivity between waterholes. The environmental unpredictability and the variation in connectivity between waterhole habitat has greatly influenced genetic subdivision of populations and the gene flow between these populations of freshwater turtles.

Estimates for F_{ST} and R_{ST} showed that turtles had moderate genetic differentiation among populations in each catchment. In addition to this, a very high level of divergence was detected among populations between the Cooper and Warrego catchments. The amount

of genetic differentiation observed between these populations is the highest reported for any freshwater turtle in the world.

Patterns of migration were analysed through the assignment of individuals to populations using their multilocus genotypes. No migration was detected between Cooper Creek and Warrego River and, when added with the high F_{ST} and R_{ST} estimates, suggest that populations of *E. macquarii* have experienced a long historical isolation between these catchments. Migration was detected among all populations within the Cooper and Warrego catchments. The level of gene flow that occurred within each catchment was relatively consistent, although the Welford population demonstrated some genetic isolation.

The population structure of these turtles represents a traditional island model, where individuals are exchanged randomly between isolated populations. However, there is some evidence that this may change with the spatial scale of the study. With the addition of populations from semi-permanent waterholes the metapopulation structure of these freshwater turtles may more closely resemble a mainland-island model.

The strong genetic separation between turtles from Cooper Creek and the Warrego River and the genetic differentiation among populations within those catchments warrants careful management. Clearly, conservation of these freshwater turtles require adaptive strategies that will respond appropriately to this apparent genetic separation. Above all, populations of *E. macquarii* require natural river flow in order to maintain their

complex genetic structure. Long-term protection of the variability and intensity of flow in Australia's dryland rivers will protect this and many other inland species that are vital for the quality of our own existence.

4.7 Future Research

4.7.1 Project Review and Future Funding

This study, along with other investigations that were part of the CRC Dryland Refugia Project, went through an external research review in October of this year. Overall, the CRC Refugia Project was well received and this project in particular was highlighted as an important and essential component. Funding for the CRC Refugia Project and for this study, is expected to continue in the future.

4.7.2 Further Research Objectives

There are a number of areas into which, I believe, this study could be expanded. Firstly, increasing the number of microsatellite loci from six to ten should strengthen the results already obtained. While the divergence detected in this analysis was significant, including more loci will provide an even higher resolution to the level of genetic separation between populations of freshwater turtles.

The addition of new locations should also be included among the major goals for future research. Further sampling from sites north of Welford should provide clarification on the genetic isolation and uniqueness in this region of the Cooper catchment. Another important expansion could be to include sites from neighbouring catchments to deter-

mine if the level of separation detected from this study is common throughout the range of this species. These data would also provide an evolutionary context on the historical expansion of *E. macquarii* into western Queensland.

Because population divergence has been detected between permanent waterholes, samples from semi-permanent waterholes can be used to further explore the type of metapopulation model that these turtles conform to. With the addition of samples from semi-permanent waterholes, it is possible that a mainland-island metapopulation structure may be detected between turtle populations.

Analysis of a broader sample selection would bring clarification of the structure of populations within catchments over time. Due to time constraints and to reduce bias only adult turtles were included in analysis, but analysis of juvenile samples may reveal genetic shifts that have occurred across generations in recent history.

Finally, clarification of the possible ESU or MU status using mtDNA analyses would support a more considered management of this species. Management actions to conserve important populations of *E. macquarii* are likely to aid in the protection of dryland river environments and of many other organisms that depend on this landscape.

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