Call differentiation in the *Limnodynastes tasmaniensis* complex (Anura:Leptodactylidae).

by

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Contents

Summary

This thesis analyses call differentiation in the Leptodactylid frog, *Limnodynastes tasmaniensis*. This analysis assumes that males call to attract females as mates and that calls can therefore also function as premating isolating mechansims. This was investigated experimentally. Males, females and juveniles could all discriminate between their own and grossly different calls. However, the traditional call discrimination set-up, two loudspeakers with a frog released mid-way, was shown to be inadequate to justify inferences about call function.

Acoustic analysis revealed three call races, western, northern and southern, within the morpho-species, *L.tasmaniensis*. The races differ most in notes per call, note repetition rate and dominant frequency. The distribution of the three races was mapped. They are largely allopatric but three contact areas exist. The western and northern call races meet in a series of narrow hybrid zones on the Murray plains in South Australia, the northern and southern in a broad intergrade in central Victoria and the southern and western overlap along the Coorong, South Australia.

Evolution of the three races was discussed in terms of three hypotheses. Evidence allegedly supporting these hypotheses was critically discussed in the introduction.

1. Reinforcement: changes in an isolating mechanism result from selection favouring devices which reduce the frequency of interbreeding between two genetically distinct groups whose hybrids are inviable.

Calls and other possible premating isolating mechanisms were analysed in contact areas, and although in all overlaps there was evidence of hybridisation, there was no indication of reinforcement. Hybridisation studies using combinations of all call races, gave no indication of hybrid inviability suggesting the selective agent required for reinforcement (i.e. gamete wastage) was non-existent. There were no obvious environmental constraints on the expansion of any of the three contact zones. For the Coorong overlap this was confirmed by the survival of southern tadpoles transplanted to sites outside the normal range of this call race. The possible future of all contacts was discussed.

2. Incidental origin: changes in an isolating mechanism are a correlated response to selection in some other context.

Sampling of call structures in populations from a wide range of environments gave no evidence of incidental origin of call differences between populations within call races.

3. Selective origin: changes in a potential isolating mechanism reflect selection to improve their efficiency as mate attractants in the particular local environmant they must function in.

In the northern and western call races, the variance of pulse repetition rate was least in populations with either the greatest number of sympatric anuran species or, where there were synchronic species with call dominant frequencies close to that of L.tasmaniensis. This suggests that acoustic interference may be an important influence on call structure.

The evolution of the three call races was discussed given the above results.

Declaration

This thesis contains no material accepted for the award of any other degree or diploma in this or any other university.

To the best of my knowledge this thesis contains no material previously published or written by another person, except when due reference is made in the text.

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1. The evolution of premating isolating mechanisms: some theories and facts.

1.1 Introduction.

In recent years several authors (Ehrlich 1961; Ehrlich and Raven 1969; Sokal and Crovello 1970; Sokal 1973) have challenged the usefulness, applicability or necessity of the widely accepted biological species concept, defined by Mayr (1969) as, "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups". Critics of this concept have emphasised local populations as evolutionary units (cf. Mayr who considers collections of populations) and have emphasised the role of selection rather than gene flow in maintaining the similarity of such populations.

However, whatever one considers the important grouping to be, recognition of the number of distinct evolutionary units at a particular locality at some point in time will most profitably be based on criteria of effective reproductive isolation (Bigelow 1965). It seems likely that the mechanisms involved in the evolution of this isolation will be similar whether one is looking at species (*sensu* Mayr 1969) or some more limited grouping. In the remainder of this chapter I intend to examine the major theories, and their supportive evidence, relating to the evolution of premating isolating mechanisms. The remainder of this thesis will deal with the evolution of call differences, a potential premating isolating mechanism, within the *Limnodynastes tasmaniensis* complex.

Various categorisations of isolating mechanisms have appeared in the literature (Mayr 1963; Littlejohn 1969; Dobzhansky 1970) but the most significant division is into pre and postmating isolating mechanisms where the former prevent gamete release but the latter do not. Three theories have emerged to explain the evolution of traits that may act as premating isolating mechanisms. The theories are not mutually exclusive and all or any in combination may explain a given situation. Only one, reinforcement, (section 1.3) deals specifically with selection for the isolating function of the character being considered. The other two discuss the evolution of variations in behaviour patterns, ecological attributes, etc., which have the potential to act as premating isolating mechanisms if an appropriate situation arises; e.g. if an area of overlap develops between two previously allopatric taxa (Thielcke 1973).

I will deal with each of the theories in turn, and will present and criticise evidence which is alleged to support them.

1.2 Incidental origin.

1) The theory.

A characteristic of a species, e.g. a behavioural sequence, the time or place of breeding, which has the potential to act as a premating isolating mechanism changes not because of selection directly upon it but as a correlated response to selection on some functionally unrelated trait.

The notion that potential premating isolating mechanisms might have incidental origins stems from Muller (1940, 1942).

To decide that changes in a character with the potential to act as an isolating mechanism have an incidental origin certain criteria must be met.

1. The character (X) should exhibit significant variation through time or over some geographic gradient.
2. A functionally unrelated character (Y) should vary over the same gradient or through the same time span.
3. It must be clearly shown that the variation in Y causes the

variation in X.

2) The evidence supporting the incidental origin hypothesis.

The appearance of a degree of premating isolation has been reported between strains of *Drosophila melanogaster* held in laboratory culture for considerable periods (Koref-Santibañez and Waddington 1958). Similarly, Ayala (1965) and Ayala, Tracy, Barr and Ehrenfeld (1974) reported isolation between strains of various other *Drosophila* species more recently derived from the field. The mechanism producing isolation was not elucidated in any of these cases and therefore there can be no proof that any premating isolating mechanisms present had an incidental origin. Indeed it is conceivable that something altogether different was happening. Ayala *et al.* (1974) suggested that odours specific to the culture medium led to the positive assortative mating by strains that they observed.

Nevo (1969) reported significant geographic variation in the call of the frog, *Acris crepitans*. There was correlated variation in body size and it has been shown elsewhere (Nevo 1973) that these changes probably reflect important adaptations to aridity.

Nevo found variation in spectral and temporal call components. Other authors (e.g. Zweifel 1968; A.A. Martin 1972) have reported negative correlations of body size and call dominant frequency in frogs and it is possible that changes in body size directly cause changes in call structure (cf. W.F. Martin 1971). However, Nevo did not present any proof that the body length variations he studied caused the call structure variance.

Variation in form or function of potential premating isolating mechanisms has been reported in other studies (e.g. Gerhardt 1974; Littlejohn 1964) but it is critical that other causes are eliminated before incidental origin is invoked as an explanation.

1.3 Reinforcement.

1) The theory.

Two taxa with similar mate attracting systems hybridise, but because they are otherwise genetically different hybrids are relatively inviable. In such a situation selection would favour individuals with traits increasing the frequency of interbreeding with their own kind as hybridisation would reduce the chances of leaving progeny.

Mayr (1974) suggested the term, "reinforcement of isolating mechanisms", to describe such phenomena. V. Grant (1963, 1966) attributes the reinforcement concept to A.R. Wallace (1889) and suggested it be called the Wallace effect. However, this apparently stems from confusion of two terms used by Wallace; disinclination to mate and infertility. Wallace's arguments are clearly concerned with increasing the degree of the latter between two interacting forms, not to increasing the former. This is contrary to the meaning of reinforcement where premating isolation (disinclination to mate) is selected for but postmating isolation (infertility) is not. To my knowledge the theory of reinforcement was first unequivocally outlined by Fisher (1930) and was later elaborated by Dobzhansky (1940).

To be certain that reinforcement explains any particular case of variation in a potential premating isolating mechanism I think the following requirements should be met.

1. There should be two distinct taxa which are at least partially sympatric.
2. The form of premating isolating mechanisms should be determined for both taxa in the area of sympatry. The nature of the same characters in pure populations of each taxon outside the area of sympatry should also be studied. Reinforcement may be suspected if characters acting as premating isolating mechanisms in sympatric

populations of either taxon diverge from the form found in appropriate allopatric samples (Figure 1.1).

3. In populations of both taxa in and out of sympatry there must be sufficient documentation of inter and intra-population variation of characters that act as premating isolating mechanisms to eliminate spurious evidence of reinforcement; e.g. from chance effects in small samples or more systematic effects like parallel overlapping clines (cf. P.R. Grant 1972).

4. There should be unequivocal evidence that hybrids occur and are inviable. Sympatric divergence of mate attracting systems may be caused by processes not involving hybridisation (see section 1.4) and hybrids are not necessarily inferior (Littlejohn and Watson 1973; Bull 1973).

5. Changes in the form of premating isolating mechanisms with time in either or both of two interacting taxa may also produce evidence of reinforcement. As in 3. and 4. above, there would have to be adequate controls on the occurrence and nature of hybrids and sufficient studies on variability of the characters concerned. Such studies could, however, be conducted at a single site.

2) The evidence that reinforcement might produce premating isolating mechanisms.

a) Simulation and experimental studies.

Bossert (1963) conducted a computer simulation of reinforcement in sympatric hybridising organisms. I have not seen Bossert's thesis and his work is unpublished. However, Wilson (1965) discussed some of Bossert's results. According to Wilson, Bossert found reinforcement produced complete premating isolation if postmating isolation was high and interspecific gene flow consequently low. At the other extreme, when gene flow between the two hypothetical species was high, fusion occurred. In the large grey area in between Wilson states (page 19), "displacement will compete with gene flow, and the outcome will depend on their relative rates". The time required to achieve efficient premating isolation was not given even for the most extreme case. Wilson's only relevant comment is on page 20 where he states, "Displacement is likely to be both clear-cut and rapid in many ordinary situations".

Crosby (1970) also did a computer simulation of reinforcement using hybridising "plants" and found that efficient isolation was attained after 140 to 290 generations. This was dependent on there being an initial difference in flowering time (the method chosen for giving isolation); and if such a difference was absent no progress towards reinforcement was made until one arose by chance. Generally, relying on chance to produce the initial difference in flowering time considerably lengthened the time needed to produce complete isolation.

There is good evidence from laboratory studies with various species and strains of *Drosophila* that selection against hybrids will increase the relative frequency of intraspecific or intra-strain mating (Ehrman 1971, 1973; B. Wallace 1954; Koopman 1950; Kessler 1966; Knight, Robertson and Waddington 1956; Dobzhansky and Pavlovsky 1971-which should be read in conjunction with Dobzhansky 1972, for data on some vital control experiments).

Comparable experiments using other sorts of animals have not been reported but one has been done with two maize strains (Paterniani 1969). Paterniani found flowering times of the two sorts of maize diverged; but as the experiment was only run for three generations the long term stability of the observed changes may be questioned.

Reproductive isolation which might have resulted from reinforcement has been observed in disruptive selection experiments with *Drosophila* (Thoday and Gibson 1962), and with the house fly, *Musca* domestica (Soans, Pimentel and Soans 1974). In the latter study the selected characters, positive and negative geotaxis, may well have contributed to the observed isolation. And, as Thoday and Gibson point out, their results may reflect a lack of hybrid survivors rather than premating isolation though later work (Gibson and Thoday 1963, 1964) suggests hybrids are not produced.

b) Reinforcement in natural populations.

Despite the abundance of experimental demonstrations of reinforcement there is little adequate data from natural populations though many have interpreted their data in terms of this hypothesis (Fouquette 1975; Loftus-Hills 1975).

i) Isolation in related allopatric and sympatric species.

It has often been noted that sympatric species exhibit efficient premating isolation but that related allopatric or allochronic species do not (W.F. Blair 1958a; Alexander 1962; Grun and Radlow 1961; Rubinoff and Rubinoff 1971). The studies on allopatric species involved either mate choice experiments or investigations of the form of traits that promote premating isolation between sympatric species.

However interesting these studies, they tell us nothing about the role of reinforcement in producing premating isolation. They tell only about the requirements for living sympatrically, no more.

ii) Comparisons of two hybridising species at different points in time.

Although there have been several studies on the temporal stability of hybrid zones (Johnsgard 1967; Yang and Selander 1968; Hunt and Selander 1973), only one person (Jones 1973) has attempted an analysis of temporal variation in the level of premating isolation between two hybridising species.

Jones compared characteristics of a mixed population of Bufo americanus and Bufo woodhousii fowleri with data from the same population collected thirty years before and reported by A.P. Blair (1941). Blair's data is from a period when the species were allegedly hybridising. Jones concluded that at present, "no hybridisation is occurring", and further, "The two species are now separated by several ethological barriers". However, such conclusions are not supported by Jones' data.

Blair made three statements about hybrids in the mixed B.americanus, B.woodhousii fowleri population at Bloomington, Indiana. Using external morphology he distinguished the two species and categorised some toads as intermediate. With the same morphological characters he scored the number of matings involving a male of one species and a female of the other. Third, he noted that the two species' calls differed but that toads with intermediate calls did occur.

Jones conducted an extensive morphological analysis using Blair's criteria and some of his own. He found that toads of intermediate morphology still occur. Jones did not study mating pairs but he did analyse calls from a large number of toads. As Blair had suggested, Jones found the two species' calls did differ but he did not record any intermediate calls. This may mean hybridisation has ceased or, that no hybrids were calling when Jones was in the field or, that hybrids, although produced, have not survived to an age when they can call (Loftus-Hills 1975). The absence of toads with intermediate calls is no proof that these two species do not still interbreed. Nor is it proof that there is now effective premating isolation, e.g. mediated by the call differences, which has evolved by reinforcement since Blair's study in 1941.

Jones made one other observation on calls which he thought might contribute to maintaining premating isolation between B.americanus and B.woodhousii fowleri. The calling seasons of these two species overlap and in that period the variance of one call component, pulse repetition rate, was reduced in both species. Pulse repetition rate best distinguished the calls of these two toad species. It is not unreasonable to assume that this call component might be important in call discrimination if calls function at all in preventing interspecific matings (Chapter 3; Loftus-Hills and Littlejohn 1971; Straughan 1975). The reduced pulse repetition rate variance might have resulted from reinforcement but is more likely an artefact.

Both Jones and Zweifel (1968) found that the value of this particular call component was signficantly affected by environmental temperature. In his calculations, Jones made no allowance for the temperature influence and therefore, changes in pulse repetition rate variance through time may well reflect the variance in environmental temperature rather than anything else (Loftus-Hills 1975).

Although Jones' study may have given some superficial evidence of reinforcement (see W.F. Blair's (1974) unqualified acceptance of this example) his analyses and interpretations are inadequate.

iii) Comparisons of premating isolating mechanisms in sympatric and allopatric populations of two species with overlapping distributions.

The most commonly used method for seeking evidence of reinforcement is by comparing form or function of putative premating isolating mechanisms in sympatric and allopatric populations of two species with at least partly overlapping geographic ranges. Studies of this sort have been done with a variety of organisms.

1. Frogs.

Littlejohn (1965) showed that the calls of Litoria ewingi and L.verrauxi (Litoria equals Hyla; Tyler 1971) were virtually identical except in areas where the two species are sympatric. There, the calls have diverged with particularly obvious differences in pulse repetition rate. Subsequent work (Littlejohn and Loftus-Hills 1968; Loftus-Hills and Littlejohn 1971) has shown that the diverged calls may act as efficient premating isolating mechanisms but that present allopatric calls are very unlikely to prevent interspecific hybridisation. Despite this, hetero-specific pairing still occurs in the area of sympatry (3/66 pairs, Littlejohn and Loftus-Hills 1968) although no adult hybrids have been reported. These facts, coupled with Watson and Martin's (1968) data showing that hybrid embryoes are relatively inviable, all support the conclusion that the sympatric call divergence is a result of reinforcement.

Fouquette (1975) reported similar results from a study of call structure in two other hylid frogs, Pseudacris nigrita nigrita and P.triseriata feriarum. Fouquette found a great divergence in pulse repetition rates in areas where the two species are sympatric but other call components were not so severely affected.

Crenshaw and Blair (1959) reported a low frequency (4% of specimens examined) of natural hybrids between these two species. Mecham (1965) found no evidence of hybrid inviability in laboratory studies but this does not necessarily reflect performance in nature.

No tests of the function of the diverged calls have been done but it is known that in one of these species calls may attract females (Michaud 1962).

Reinforcement may explain Fouquette's data but, until there is clearer evidence of hybrid inviability and some more detailed tests of call function have been done, other possibilities cannot be excluded. The most likely alternative is the theory discussed in section 1.4: adaptive differentiation in differing reproductive environments.

W.F. Blair (1974) referred to two studies of his own which he alleges are examples of reinforcement. In one of these (W.F. Blair 1958) he gave almost no data. Nevo (1969) analysed this same example much more intensively and concluded that, although both _Acris gryllus_ and _A.crepitans_ exhibited differences in call between allopatric and sympatric samples, this resulted from overlapping clines (cf. P.R. Grant 1972) not reinforcement. According to Nevo, the call structure variations represent incidental effects of adaptations to increasing aridity.

Blair's other study (W.F. Blair 1958) is not convincing either. Call duration in _Microhyla carolinensis_ was the same in sympatric and allopatric samples. However, in _M.olivacea_, call duration was more different from that of _M.carolinensis_ in a sample from west of the zone of sympatry than it was within the zone of sympatry. An analagous situation existed in the midpoint frequency data for _M.carolinensis_. Here, mean values in the samples from the overlap and east of sympatry were virtually identical but the mean from a more distant allopatric sample was closer to values in _M.olivacea_.

Until these variations in call parameters are sensibly explained, and there is some assessment of the role of various call components in interspecific discrimination, Blair's data cannot be accepted as evidence of reinforcement.

Ball and Jameson (1966) reported a reduced dominant frequency variance in calls of _Hyla regilla_ sympatric with _Hyla californiae_ and suggested this may have been caused by reinforcement. Alternatively, it may be because the samples analysed from sympatric sites were from two to six times larger than those from elsewhere. Littlejohn (1969, 1971) has given additional criticisms of this study.

2. Crickets and other soniferous arthropods.

The results of at least three studies of soniferous arthropods have been proposed as supporting the reinforcement hypothesis. In two, (Walker 1963; Alexander 1957) insufficient data is available to make any assessment. Walker's (1962) data from the tree crickets, *Oecanthus fultoni* and *O.rileyi* are more interesting, though there is only one allopatric sample, and the significance of chirp rate (the diverged character) as a premating isolating mechanism is unknown.

Walker (1974) critically reviewed his earlier work and referred to a number of other cricket species pairs which might, after more detailed study, provide evidence of call divergence in areas of sympatry. Walker did not specifically discuss the forces responsible for producing call divergence and it may be that these examples should be considered in section 1.4 where mutual interference of signals leading to inefficient mate attraction, rather than gamete wastage, is the selective agent.

3. Fish.

Hubbs and Delco (1962) suggested reinforcement of courtship preferences had occurred in populations of *Gambusia affinis* sympatric with *G.geiseri*. They conducted two series of experiments and the results are contradictory. Allopatric *G.affinis* males from Houston were poorer, and those from Brownsville were better, at interspecific discrimination than sympatric males. In addition, results from sympatric *G.affinis* males differed considerably between trials. In one experiment they directed 23.3% of courtship activity to *G.geiseri* females while in the other, 33.6% of activity was so aimed. I am not convinced that the behavioural traits used to assess isolation were reliable.

4. Lizards.

Webster and Burns (1973) reported significant geographic variation of dewlap colour in a lizard, *Anolis brevirostris*. Dewlap colour may be important in mate attraction and or interspecific discrimination and Webster and Burns speculated that at least some of the variation they observed might have resulted from reinforcement. However, their data were too limited to be certain.

5. Birds.

The data from avian studies claiming to show evidence of reinforcement or its converse, character release (Marler 1960), was carefully examined by Thielcke (1969, 1973). Thielcke stated in his later paper, "the 'best substantiated evidence for reinforcement of isolating mechanisms' is nil". His criticisms seem very apt and I do not intend to comment further on data from bird studies.

6. Mammals.

Two studies on mammalian mating preferences are alleged to support the reinforcement theory. Smith (1965) placed male _Peromyscus eremicus_ in a five chambered apparatus. The chambers were in a row and the males could move freely between the centre three. Males could not enter the end chambers and in one of these Smith placed a conspecific female and in the other a female _P.californicus_. The time that males spent in any particular compartment was recorded electronically. There were no differences between _P.californicus_ males from populations sympatric with and allopatric to _P.eremicus_. Allopatric _P.eremicus_ males spent less time in the compartment next to a conspecific female than males from sympatry implying sympatric males are better at discriminating appropriate mates.

However, these results probably reflect the distribution of male nests rather than anything else. Smith states that once a male had nested it rarely left the chamber the nest was in. Therefore, the distribution of nests might be a more meaningful parameter to look at. Eight of eight sympatric and five of eight allopatric

P.eremicus males nested next to a conspecific female. Of the remaining allopatric males, two nested in the neutral chamber and the third next to a *P.californicus* female.

If neutral males are excluded and the distribution of nests in allopatric and sympatric males compared using a Fisher exact probability test (Siegel 1956, page 96) the chance is greater than .05 that the distributions do not differ. If neutral males are treated as having selected heterospecific mates and the test repeated, the probability of no difference is again greater than .05. Thus the apparent evidence of reinforcement disappears.

McCarley (1964) did a study similar to Smith's but it was methodologically weaker. There was no neutral chamber so non-reactive individuals could not be scored. Some subjects were retested but all the data were lumped thus obscuring any bias that individual, behavioural quirks might have produced. McCarley tested animals of both sexes and found that allopatric, female *Peromyscus leucopus* preferred *P.gossypinus* to conspecific males. Either there was some bias (see above) or this experimental design is not really telling anything about *Peromyscus* mating preferences.

7. *Drosophila*.

Although there ~~is~~ are extensive data from experimental populations of *Drosophila* demonstrating the reality of reinforcement, data from field studies or field collected strains are severely lacking.

Ehrman (1965) reported on isolation within and between various strains of the *Drosophila paulistorum* complex. She summarised her results as comparisons of allopatric and sympatric isolation indices between particular pairs of races. The allopatric indices were generally much lower, indicating much greater interracial crossing in these tests than in trials with sympatric strains. Interracial hybrids in the *D.paulistorum* complex are generally sterile and it may follow that the difference in degree of isolation between races is a result of reinforcement.

However, a close examination of the nature of Ehrman's "allopatric" crosses reveals an inconsistency with the generally used meaning of this word.

Consider two hypothetical taxa, X and Y, distributed as in Figure 1.2(a). These taxa are sympatric in region 2 which contains localities A, B and C. X is allopatric to Y in area 1 containing sites D, E and F and similarly Y is allopatric to X in area 3 with sites G, H and I. This is my understanding of the terms sympatric and allopatric.

If I were was to search for evidence of reinforcement in this situation I would take X and Y from area 2 and determine the level of premating isolation. Then, I would take X from area 1 and Y from area 3 and again determine the level of premating isolation. If the isolation was greater in the first test, involving X and Y from area 2, this would be evidence for reinforcement. By analogy with Ehrman's terminology I would call the first a sympatric and the second an allopatric determination of isolation.

But, this is not what Ehrman always did. In many cases, allopatric tests of isolation between pairs of races in the *D.paulistorum* complex involved the equivalent of, a stock of race X from site A and a Y stock from C in area 2 (Figure 1.2(a)). If allopatric is used in this sense the reinforcement prediction of greater isolation in sympatric tests is not necessarily true.

Assume that Figure 1.2(b) represents the form of a character, P, acting as a premating isolating mechanism in two taxa, X and Y, which are undergoing reinforcement. Assume further that the degree of isolation between X and Y is a function of the difference in magnitude of P. A sympatric comparison of X and Y from B (Figure 1.2(b)) would

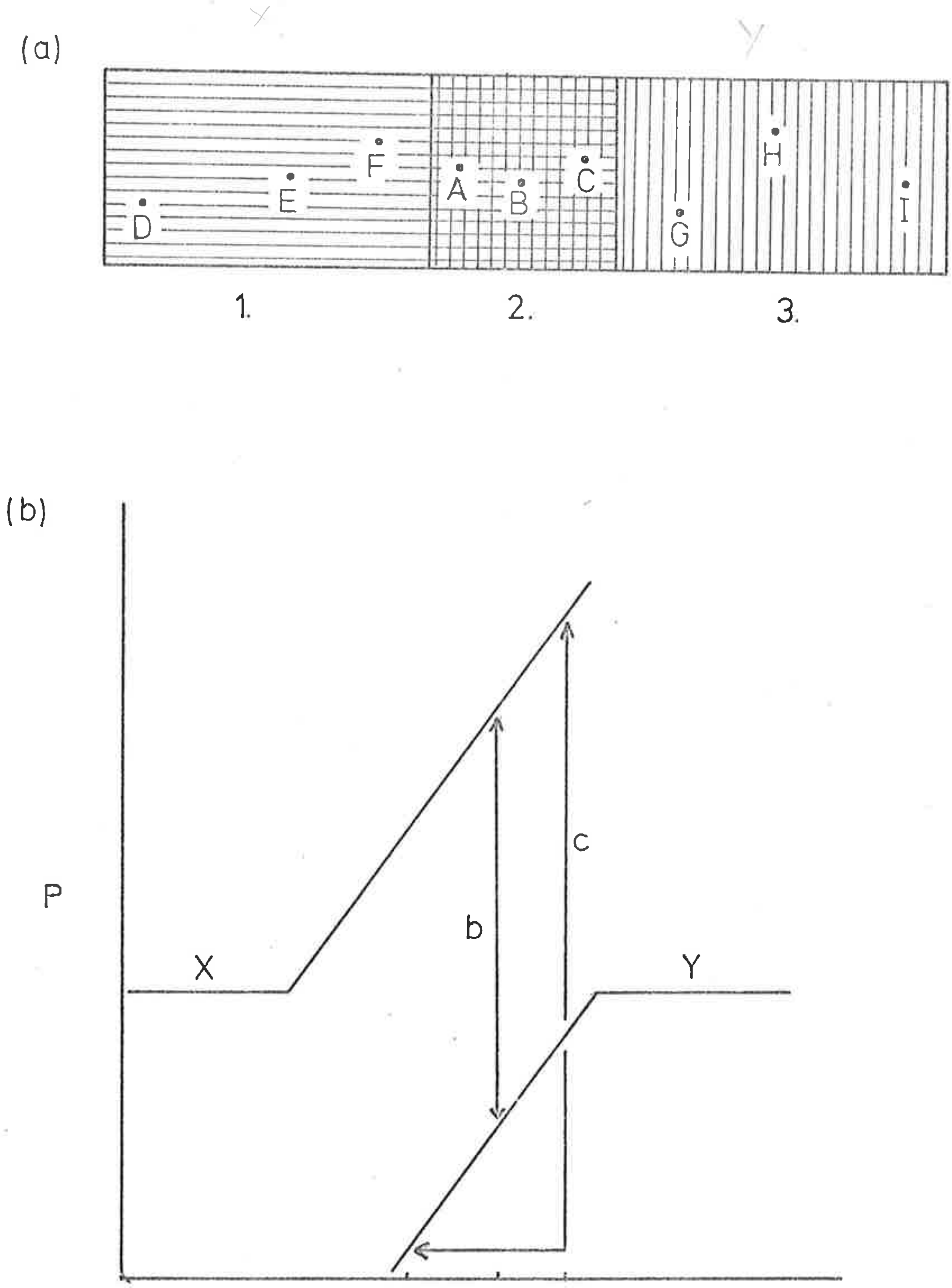


Figure 1.2 (a) The ranges of two hypothetical taxa, X (horizontal hatching) and Y (vertical hatching). X only is found in area 1, Y only in area 3 and both X and Y in area 2. A to I represent the position of sites in the ranges of each taxon.
(b) Representation of geographic variation in a character P in two taxa X and Y. Axes as in Figure 1.1. A, B and C are sites where X and Y are sympatric.

give an isolation index, b. An allopatric test, sensu Ehrman, with X from C and Y from A would give an isolation index c, where c indicates more effective isolation than b (Figure 1.2(b)). If allopatric and sympatric are interpreted Ehrman's way, the reinforcement prediction of greater isolation in the allopatric test (see above) would have to be reversed.

If reinforcement has occurred between pairs of races in the D.paulistorum complex and the characters giving isolation vary as in Figure 1.2(b) or as in Figure 1.1(3) then at least four of Ehrman's tests should have given greater isolation in the allopatric comparisons (tests 4, 5 and 6 in the Amazonian, Andean-Brazilian series, test 5 in the Orinocan, Andean-Brazilian series; see Ehrman 1965, section headed Material; Spassky et al. 1971 for distribution data). I will note that in two of the more likely examples of reinforcement (Littlejohn 1965; Fouquette 1975) the character probably critical in interspecific discrimination (pulse repetition rate) does vary roughly as P does in Figure 1.2(b). There may also be additional complications to the simple conditions for producing evidence of reinforcement as at many sites three or four, not two, races of the Drosophila paulistorum complex are found (Ehrman 1965; Spassky et al. 1971).

8. Plants.

V. Grant (1966) discussed the evolution of incompatability barriers (apparently a postmating isolating mechanism, Littlejohn 1969) in the plant genus Gilia. Grant found the greatest incompatability between sympatric species but relatively little between allopatric forms and presumed that reinforcement had produced this situation. Mayr (1963), Mecham (1961), Littlejohn (1969) and Dobzhansky (1970) all discussed the difficulties with the hypothesis that natural selection can increase the degree of postmating isolation between sympatric taxa (but see Coyne 1974). Grant's use of the term reinforcement to describe his results seems inappropriate.

Levin and Kerster (1967) reported that *Phlox pilosa pilosa* is polymorphic for flower colour being red-pink or white. Generally the frequency of white flowered plants is low except in populations sympatric with *P.glaberrima*. There, white flowered plants are common. Pollen of the two species is distinct and Levin and Kerster found that *P.glaberrima* pollen often occurred on red-pink *P.pilosa pilosa* flowers but rarely on white. This was attributed to the major pollinators preference for red flowers. Clearly the occurrence of white flowers reduces the incidence of interspecific pollen flow but it has not been shown that the frequency of white flowers has increased as a result of some interaction with *P.glaberrima*. The presence of white flowered plants in allopatric populations of *P.pilosa pilosa* presumably indicates superiority under some conditions; such conditions may be particularly favourable for sympatry with *P.glaberrima*.

McNeilly and Antanovics (1968) discussed the evolution of significant flowering time differences between adjacent populations in two species of grass, *Agrostis tenuis* and *Anthoxanthum odoratum*. In both cases there is good evidence of interpopulation gene flow, and that hybrids would be at a considerable disadvantage. In both grasses one of the populations was specifically adapted to growing on ground contaminated with heavy metals. There was no apparent ecological factor, e.g. soil type, which would have effected flowering times and reinforcement seems a reasonable explanation of these authors' data.

Antanovics (1968) also studied plants growing on old mine spoil. He argued that reinforcement had produced the self-fertility observed in plants growing on metal polluted soil. Antanovics' data are not clear cut and LeFèbvre (1973) has offered a plausible alternative explanation. Although the plant species Antanovics studied usually outcross some individuals can and do self-fertilise. Self-fertilisation is a common attribute of colonising species and LeFèbvre argued that Antanovics' findings are a remnant of the colonisation process, not the result of reinforcement.

iv) Hybrid zones and reinforcement.

It is a vexed question whether premating isolation will evolve, by reinforcement, in and around narrow hybrid zones. Remington (1968) and Wilson (1965) feel that hybrid zones are generally ephemeral and that either fusion or the attainment of reproductive isolation are the inevitable results.

Moore (1957) and Littlejohn (1969) present contrary ideas. Moore suggested that although a "reinforced" phenotype may become established within the area of sympatry it will not spread, as elsewhere it has no selective advantage. Littlejohn argued that if reinforcement occurred within a hybrid zone its effects would be nullified by migrants from adjacent populations never exposed to hybridisation and that the hybrid zone might thus stabilise.

Crosby (1970) conducted a computer simulation of this situation and his results contradict both Moore's and Littlejohn's suggestions. Reinforcement not only occurred in a narrow hybrid zone but it also spread through the adjacent essentially allopatric areas. This happened whether or not there was an ecological barrier to the achievement of sympatry. Crosby fully realised the limitations of his work and emphasised the relatively small size of his interacting populations and the importance of random events in determining or contributing to some of his results.

Crosby did not attempt to resolve the conflict between his results and others' predictions though it is perhaps not difficult to do so. For example, in answer to Moore (1957), at the edge of the area of sympatry the nature of the reinforcement may be such that "reinforced" individuals may appear as little more than extreme types from the normal population and as such are reasonably likely to obtain mates if they move out of the zone. This may also apply to more extreme "reinforced" individuals moving into populations exhibiting a moderate degree of reinforcement. "Non-reinforced" phenotypes will probably do poorly in the zone because of the risk of hybridisation and it is therefore possible that the nett effective gene flow is away from the area of sympatry. The more widespread establishment of reinforced phenotypes away from the zone could be analagous to the spread of a neutral gene with the numbers of immigrant animals, particularly, playing a significant role.

Littlejohn's suggestions imply that conditions are such in the hybrid zone that potential swampees can survive and reproduce there. Appropriate knowledge of the population dynamics and other ecological parameters of hybrid zones, is minimal (Levin 1973). It is true, however, that, at least in other situations, the unifying effects of gene flow have been considerably overestimated (Ehrlich and Raven 1969; Endler 1973; Soans, Pimentel and Soans 1974; Thoday 1972). Littlejohn's prediction that immigrants will swamp any tendency to reinforcement is unsubstantiated and may be an unlikely event.

As mentioned earlier, one of the drawbacks of Crosby's (1970) simulation was the relatively small size of the hybridising populations. Hall and Selander (1973) and Littlejohn and Watson (1973) suggest that a reinforced premating isolating mechanism is more likely to become established in such situations as swamping effects will be less and because a greater proportion of the population may have been exposed to the hybridisation test. Neither authors' results support their contention but the idea receives some support from McNeilly and Antanovics' (1968) data on flowering time in an analagous, though not directly comparable situation in two plant species.

1.4 Differing reproductive environments.

1) The theory.

Characters that may act as premating isolating mechanisms undergo adaptive change in situations where their normal function is interfered with by features of either the physical or biotic environment.

Perdeck (1958) first suggested that isolating mechanisms might change this way when he attempted to explain song differentiation in grasshoppers. The idea was elaborated by Littlejohn (1969a) and Thielcke (1973).

To be certain that variation in a potential premating isolating mechanism is the result of adaptive change to a differing reproductive environment certain criteria should be met.

1. Significant differences in a possible premating isolating mechanism should exist between two populations (spatially or temporally separated) of a single species or between two species which are known to have shared an immediate common ancestor.
2. Some feature of the environment used for reproduction should differ in the two populations. That is, the environment of one population (say A) should contain a factor (F) not found in the environment of the other (B).
3. It must be shown that if F occurred in B (refers to 2. above) then reproduction would be impaired.

2) The evidence.

To my knowledge there is no unequivocal evidence that a character that might act as a premating isolating mechanism has adaptively changed in response to a source of interference in the reproductive environment. However, there are some suggestive examples.

Littlejohn and Martin (1969) demonstrated interference between calls of the frogs *Geocrinia victoriana* (*Geocrinia* equals *Crinia* in part; Blake 1972) and *Pseudophryne semimarmorata*. In the absence of *G.victoriana*, *P.semimarmorata* males averaged 5.5 calls per minute. When *G.victoriana* starts calling *P.semimarmorata* males stop and only resume when the former species ceases vocalising. In periods after experimental inhibition of calling (by playback of *G.victoriana* calls) *P.semimarmorata* males averaged 19.0 calls per minute.

It has not been established whether this phenomenon of call inhibition and subsequent increased calling rate is confined to populations of *P.semimarmorata* sympatric with *G.victoriana* (or populations derived from presently or formerly sympatric stocks) or whether it is a general property of the communication system of *P.semimarmorata*.

Webster and Burns (1973) claim that adaptive differentiation may in part explain the variation of dewlap colour found in the lizard, *Anolis brevirostris*. They suggest there may have been confusion with the sympatric *A.distichus*. Dewlap colour may be important in attracting mates (references in Webster and Burns 1973).

G.W. Ferguson (1973) has also used this theory to explain variation in push-up displays in the lizard genus *Sceloporus*. These displays are probably of some importance in either attracting or keeping mates (G.W. Ferguson 1973).

Ferguson analysed displays of sympatric *Sceloporus undulatus elongatus* and *S.graciosus graciosus*. He found divergence of the of the *S.u.elongatus* display pattern from that in a single allopatric sample of *S.u.erythrocheilus*. The *S.g.graciosus* display was similar to that in *S.u.erythrocheilus*. There was no allopatric sample for *S.g.graciosus* or *S.u.elongatus*.

Two criticisms can be levelled at this study. The relationship of *S.u.elongatus* and *S.u.erythrocheilus* was not elaborated and it is therefore unclear whether the latter is an appropriate allopatric comparison. Additionally, if one assumes it is reasonable to compare these two subspecies, there is only one allopatric sample which can give no idea of the range of variation expected in Sceloporus undulatus populations. Ferguson did not distinguish between reinforcement and adaptive differentiation and I have included his data here rather than in my discussion of reinforcement as he makes no mention of hybridisation between S.graciosus and S.undulatus.

Apart from these few examples in which the two interacting species are known, adaptive differentiation in differing reproductive environments had been advanced as a possible cause of geographic variation in frog calls (Littlejohn 1959, 1964, 1965) and as a reason why push-up displays of lizards might vary (G.W. Ferguson 1971). However, in none of these cases was there any real evidence in favour of this hypothesis.

Despite this dearth of examples, interference, either from other species' signals or by features of the environment, e.g. vegetation, may constitute a potent selective force and thus be important in changing signals that may act as premating isolating mechanisms. For example, in soniferous animals a variety of adaptations have been described which may improve the efficiency of intra- or interspecific communication (Loftus-Hills 1971, 1974; Walker 1969; Cody and Brown 1969; Ficken, Ficken and Hailman 1974; Morton 1975; Nottebohm 1975).

1.5 Discussion.

1) The theories and the evidence.

This review of the evolution of premating isolating mechanisms has concentrated on reinforcement. I have dealt particularly with field studies that have gained wide acceptance as illustrators of the reinforcement process (e.g. in the reviews of V. Grant 1963, 1966;

Littlejohn 1969; Dobzhansky 1970; Levin 1970; Murray 1972; Thoday 1972) and have I hope shown that the majority are inadequate in some way. Incidental change and adaptative differentiation in varying reproductive environments are almost as well documented as ways of changing premating isolating mechanisms, particularly if only investigations of natural populations are considered.

However, the emphasis on reinforcement is not wholly my own. It is my distinct impression that reinforcement has inspired many more studies than either of the other theories. This is clearly true of experimental and simulation investigations as here, other ideas have virtually been ignored.

I find it hard to understand this bias. Perhaps it stems from the fact that reinforcement is an end point in one of the more commonly accepted speciation models.

Speciation is often said to occur as follows.

1. A species range is split into geographically separate isolates.
2. The isolates adapt to their particular local environments and thus diverge genetically.
3. The range of either or both isolates expands and they contact.
4. Hybridisation occurs and reinforcement leads to the evolution of effective premating isolating mechanisms.

An alternative to step 4. is: in isolation, the populations incidentally acquire characteristics which act as premating isolating mechanisms and prevent interbreeding on contact. With these alternative endings, this is essentially the speciation process as outlined by Mayr (1963) and it is one, especially with the reinforcement option, that has gained wide acceptance (e.g. MacArthur 1972; Ayala, Tracey, Hedgecock and Richmond 1974).

The popularity of this model to the exclusion of others (cf. White 1968, stasipatric speciation; Thoday 1972, sympatric speciation) may account for the interest shown in reinforcement. Further, experimental studies on reinforcement are relatively easy to design and conduct (e.g. Ehrman's 1971; 1973 automatic hybrid lethal system) and given the number of precedents a positive result seems almost assured (though see Ehrman 1971, where reinforcement did not occur in all lines).

In a discussion of genetic differentiation and speciation Sokal (1973) emphasised the importance of not being restricted by popular theories or interpretations. Persons investigating the evolution of premating isolating mechanisms should heed Sokal's advice.

2) The *Limnodynastes tasmaniensis* complex.

The described species, *Limnodynastes tasmaniensis* contains a heterogeneous assemblage of at least three distinct call races (Chapter 2). This thesis contains my analysis of call structure differentiation in this complex. I am interested in the evolution of premating isolating mechanisms and this study was designed around the three theories discussed in this introduction. The effects reinforcement might have had on *L.tasmaniensis* calls is dealt with in chapter 4 while incidental origin and adaptative differentiation in varying reproductive environments are considered in chapter 5.

This study was initiated after making one major assumption. That was, that male *L.tasmaniensis* call to attract mates and that call may therefore act as a premating isolating mechanism. The assumption was tested experimentally and the results are given in chapter 3.

2. Call structures in the Limnodynastes tasmaniensis complex: objective delineation of the call races.

2.1 Introduction.

Parker (1940) first commented on L.tasmaniensis calls. He described two, "... a lower 'cook-kuk-kuk-cook' and a higher (? female) 'Kuk-ku-kuk'". Moore (1961) claimed the call was, "a very rapid 'uk-uk-uk-uk...,' reminding one of a small boy imitating a machine gun". Tyler (1966) repeated Moore's analogy and suggested the call was, "A rapid and staccato burst rather like an imitation of machine-gun fire". None of these authors gave any data on where the calls described were heard or on their geographic distribution.

Littlejohn (1963), discussing frogs from the immediate environs of Melbourne, described the call of L.tasmaniensis as, "A single short sharp 'click' similar to the sound produced by striking two stones together". This call is distinctive in that it consists of a single sound motif whereas alternative representations refer to a repeated motif or motifs.

Littlejohn (1966) made the first positive statement about heterogeneity in the call of L.tasmaniensis when he discussed two call races, northern and southern. The northern call was described as, "... consisting of 2 - 5 short pulses which are rapidly repeated so that the sound has a staccato quality". The southern call was as in Littlejohn (1963) and had been the subject of objective acoustic analysis by Littlejohn and Martin (1965a).

Littlejohn (1966) roughly described the ranges of the two call types and (1967) mapped the distribution in more detail. In the latter paper the northern call race was figured as extending into northern South Australia via the Murray Valley and the southern call race into the south eastern region of that state.

Loftus-Hills (1973) referred to a third call race; the western. He reported two localities where this race occurred, 29.6 km south of Meningie, S.A. and 11.2 km south of Myponga, S.A. He gave the data presented in Table 2.1 on call dominant frequencies for the three call races.

The data presented so far indicate there is temporal and spectral heterogeneity in the call of L.tasmaniensis. I have quantified this by analysing calls from six localities. At two sites, Moyston and Mount Hope Drain (Table 2.2), calls were clearly from the southern call race. At two others, Blanchetown and Big Bend (Table 2.2), calls appeared to have the known characteristics of the northern call race and at the remaining two, Gawler and Murray Bridge (Table 2.2), calls seemed to have the lower dominant frequencies ascribed to the western call race.

2.2 Methods.

1) Call recording and analysis.

Calls were recorded in the field using a Kudelski Paudex Vaud, Nagra III NP, portable tape recorder and a Beyer M 100 omnidirectional microphone. The connecting lead was either 4.5 or 4.5 plus 27 m long. Data supplied by M.J. Littlejohn were recorded on a Nagra III BH recorder with either a Beyer M 69, Beyer M 88, Electro-Voice EV644 or Sennhaiser MKH 805 microphone with various lengths of connecting lead. Tape speed was 19 cm per second on both recorders.

During recording the microphone was clamped to a rigid steel stake planted firmly in the ground. I attempted to position the microphone as close as possible to the calling frog (within 20 cm if possible) but I was not always successful as the frogs, particularly those of the putative northern and western call races, generally

Call Race	Sample Size	Call Dominant Frequency (Hz)	Locality
Southern	5	1900 (1650 - 2000)	3.2 km N.E. of Whittlesea, Victoria.
Northern	7	1779 (1700 - 1900)	5.6 km S. of Berrigan, New South Wales.
Western	5	1330 (1300 - 1400)	29.6 km S. of Meningie, South Australia.

Table 2.1 Call dominant frequencies for the three call races of *Limnodynastes tasmaniensis* from Loftus-Hills (1973). Data are given as the mean with the range in brackets.

Locality	Map and Grid Reference	Recording Date	Recorder	Number of Recordings
Western				
1. Murray Bridge, South Australia	SI 54-13 229662	27.viii.70	M.J.L.	5
		1.ix.71	J.D.R.	6
		10.ix.71	J.D.R.	6
2. 7.5 km north west of Gawler, South Australia	SI 54-9 167729	27.iii.73	J.D.R.	5
		29.iii.73	J.D.R.	11
		2.iv.73	J.D.R.	4
Northern				
1. Blanchetown and 4.5 km south of Blanchetown, South Australia	SI 54-10 261756	22.ix.71	J.D.R.	5
	SI 54-10 260752	23.ix.71	J.D.R.	9
2. Big Bend, 8.7 km south south east of Swan Reach, South Australia	SI 54-10 261720	29.ix.71	J.D.R.	18
Southern				
1. 10.0 km south west of Moyston, Victoria	SJ 54-8 562391	10.x.73	J.D.R.	9
			M.J.L.	9
2. Mount Hope Drain, 17.0 km west north west of Millicent, South Australia.	SJ 54-6 319370	31.viii.73	J.D.R.	10
		9.x.73	J.D.R.	7

Table 2.2 Recording localities and other recording details. Grid references are for Series R 502, Edition 1 maps (scale 1:250,000 Department of National Development, Canberra). M.J.L. is M.J.Littlejohn, Zoology Department, University of Melbourne; J.D.R. is myself. Number of recordings is the number of frogs recorded, not the number of calls.

called from concealed sites. Recording levels were set below -4 dB to minimize overload distortion which could arise from level meter inertia with signals of short duration.

Water temperatures were measured directly below the microphone when recording of each individual was completed. If the animal was captured I attempted to take cloacal temperatures. However, water temperature is probably a sufficient measure of cloacal temperature (Appendix 1). All temperatures were taken with a Schultheis (Wesco) reptilian thermometer with interpolation generally to the nearest one tenth of a degree.

If a chorus of L.tasmaniensis is repeatedly disturbed and all frogs stop calling it is my impression that the same one or two individuals are always the first to start calling each time the disturbance ends (Whitney and Krebs 1975). Such frogs seemed easier to record and to reduce possible bias from only recording chorus leaders I always attempted to record all vocalising frogs in a particular area. On repeated visits to the same locality I worked in different sections of the dam, swamp or whatever to reduce the possibility of individuals being re-recorded.

All call data were taken from oscillograms made on Ilford, NS6 recording paper using either a Grass C4 camera plus Tektronix 502 oscilloscope or Philips PM320 oscilloscope plus Philips PP1014 camera. Playback was from either of the Nagra records used for field recording at either the recorded, or half the recorded speed. A time marker (100 pulses per second, derived from the 50 Hz mains frequency) was displayed on the second trace of the oscilloscope. The time marker was at least as accurate as the time bases of the Tektronix oscilloscope.

An Allison 2AB passive filter was inserted during the production of some oscillograms to reduce background interference; e.g. from wind or the calls of other species of frogs. The filter had no important effect on any of the call components measured (Appendix 2).

2) Call components and their measurement.

Oscillograms were made of the last interference-free recorded call of each frog and five call components derived. However, before I can discuss the derived call components I must define some basic call components.

a) Basic call components (see Figure 2.1).

1. A call is a temporally isolated sound motif or group of sound motifs.

2. A note is the major repeated sound motif in L.tasmaniensis calls with a repetitive structure. In calls with a single major sound motif, note and call are equivalent terms.

3. Pulses result from amplitude modulation within notes and are an oscillation or train of oscillations separated by a space, aberrant oscillation or abrupt change in amplitude from any other such oscillation or oscillation train.

b) Derived call components.

1. Note repetition rate (Notes per second): the reciprocal of the time from the start of the first note to the start of the second. I could have averaged note repetition rate over the whole call but I wanted to maintain a direct comparability between calls with two and higher note numbers.

2. Pulse repetition rate (pulses per second): the reciprocal of the time from the peak of the first oscillation of the pulse of maximum amplitude in the second note to the peak of the analagous oscillation in the immediately subsequent pulse.

3. Dominant frequency (Hertz): the number of oscillations in the pulse measured for pulse repetition rate over the time for their

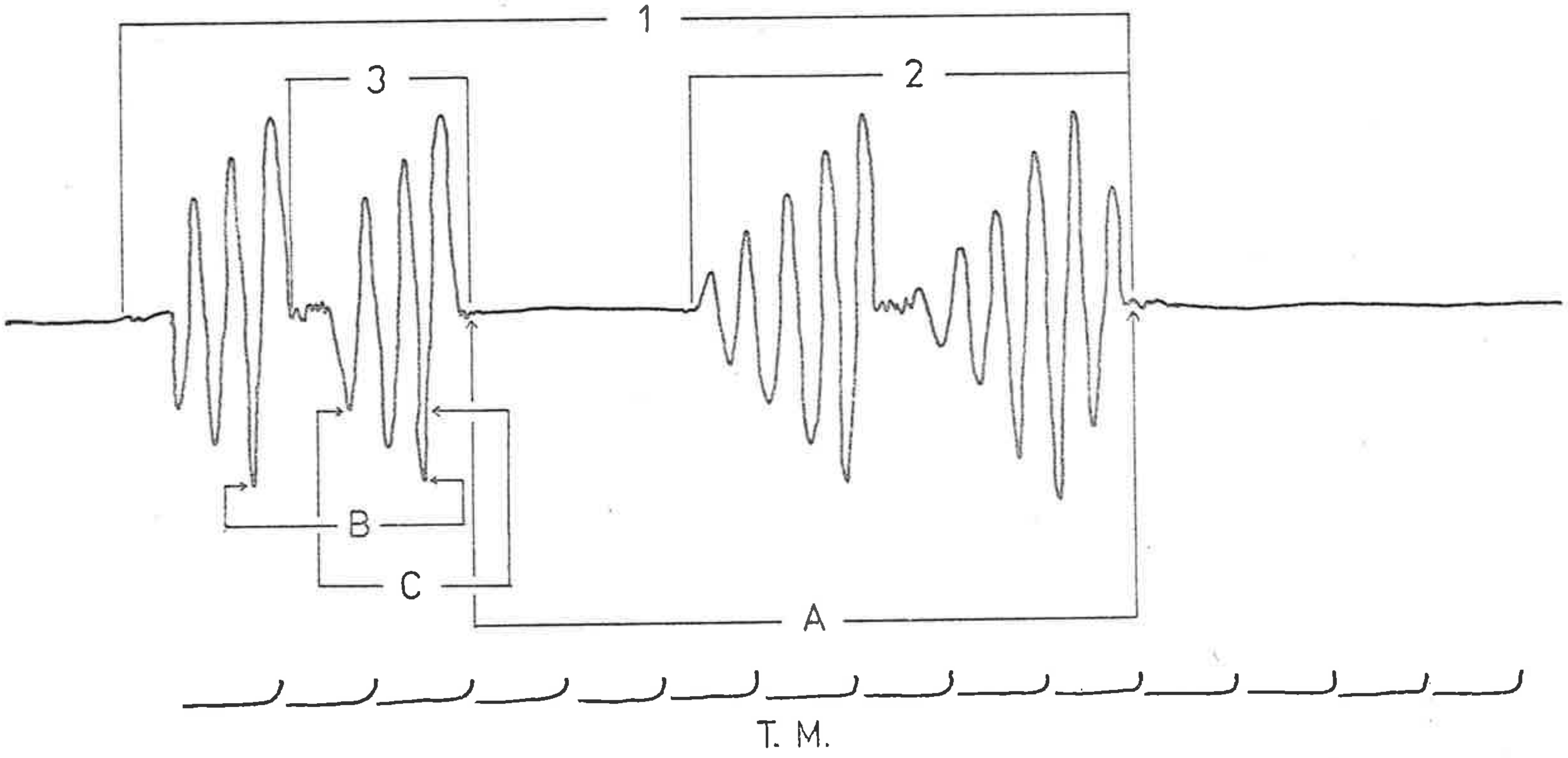


Figure 2.1 A schematic oscillogram. 1, Call 2, Note 3, Pulse A, distance measured for note repetition rate B, distance for pulse repetition rate C, distance for dominant frequency, this distance contains two complete oscillations.

production. The time was measured from the peak of the first to the peak of the last measurable oscillation and the number of oscillations counted accordingly.

4. Pulses per second note: counted in the second note by visual inspection of the oscillogram.

5. Note duration (millisecond): measured from the point where the second note first became distinct from the background to the point where it became indistinct again.

6. Notes per call: counted in the last five calls by listening to tapes played back at reduced speed. Difficult calls or calls with high note repetition rates were simultaneously monitored on an oscilloscope.

Five calls were counted for notes per call, as for any particular frog this character may vary considerably between calls. This contrasts with the stereotyped nature of notes within and between calls of individual frogs.

The measurements made to give the basic data for production of the derived call components are illustrated in Figure 2.1.

c) Measurement.

All measurements made on the oscillograms were corrected for film speed variation using two correction coefficients based on the time marker. The oscillogram film runs through the camera at a rated speed. Thus, distance measurements should represent exact elapsed times. However, the actual film speed varies considerably and was generally slightly less than the given speed. Assuming the time marker is accurate the oscillogram distance actually representing the passage of some interval, say 100 m s, can be measured and used to correct other measurements. For example, if the rated film speed is 1000 mm per second but the actual distance for the elapse of 100 m s is 9.27 mm, against an expectation of 10.0 mm, then the correction coefficient would be 0.927. All measurements taken from that particular oscillogram would be multiplied by 0.927 and would thus be slightly reduced.

For each measured call I calculated two correction coefficients.

1. Raw data giving note duration and note repetition rate were measured with vernier calipers accurate to .05 mm. For these data a distance representing the elapse of 100 m s was used as the correction coefficient.

2. Data giving dominant frequencies and pulse repetition rates were measured with a dial gauge accurate to .01 mm. The oscillogram was fixed to a movable stage and shifted relative to a set of crosshairs in one eyepiece of a binocular microscope. The stage movement activated a dial caliper. For these data the distance representing the elapse of 10 m s was used to correct other measures.

All measurements were done twice and if the difference between the derived parameters was greater than 1% of the initial value the data were checked and if necessary re-measured. Otherwise the second value was used.

In single note calls, components 2, 3, 4 and 5 were measured in the call's only note. Components 1 and 6 are obviously irrelevant in such calls.

Call repetition rate was not investigated as this may be markedly affected by the calls of neighbouring frogs (Loftus-Hills 1971, 1974).

d) Temperature correction methods.

Temperature dependent variation of call components has been widely reported in the literature on anuran calls (e.g. Schneider 1974; A.A. Martin 1972; Zweifel 1968; Littlejohn 1964). I will therefore preface discussion of the analyses used on my own data with a survey of temperature effects and methods of dealing with them.

Many authors (e.g. A.A. Martin 1972; Littlejohn 1964, 1965; Gerhardt 1974a) have attempted to remove this sort of variability by "correcting" their data to some selected temperature. They used the slope of a regression line fitted to population data in an equation as below.

$$C = b(C - 0) + P$$

Where C is the temperature the datum is corrected to, b the slope of a fitted regression line, 0 the temperature the original datum was collected at and P is the datum value at 0.

However, there are some difficulties with this method. First, if the slopes of regression lines fitted to data from various populations differ then selection of the correction temperature will effect the magnitude of differences between samples (see Figure 2.2(a)). Second,plots of how an individual's call varies with temperature may not coincide with the same plot for a population sample. For example, one might be linear and the other curvilinear which would invalidate use of the equation above. This is in fact the case in at least one frog, Bombina bombina. Lörcher (1969) gave a plot of call duration against temperature for a population sample of B.bombina. Schneider and Eichelberg (1974), working with the same species, gave plots of call duration against temperature for single frogs. Even allowing for the fact that these two sets of authors plotted their data with different scales there was no agreement between the individual and population data.

In analysing temperature dependent data I see no reason why one should not look at exact population characteristics, the slope and elevation of the fitted regression line, rather than some parameter of dubious validity, e.g. a mean and variance based on temperature corrected data. Slopes and elevations of regression lines can be compared with relatively simple statistical procedures (Snedecor and Cochran 1967). This is the method I have used when analysing temperature effected data.

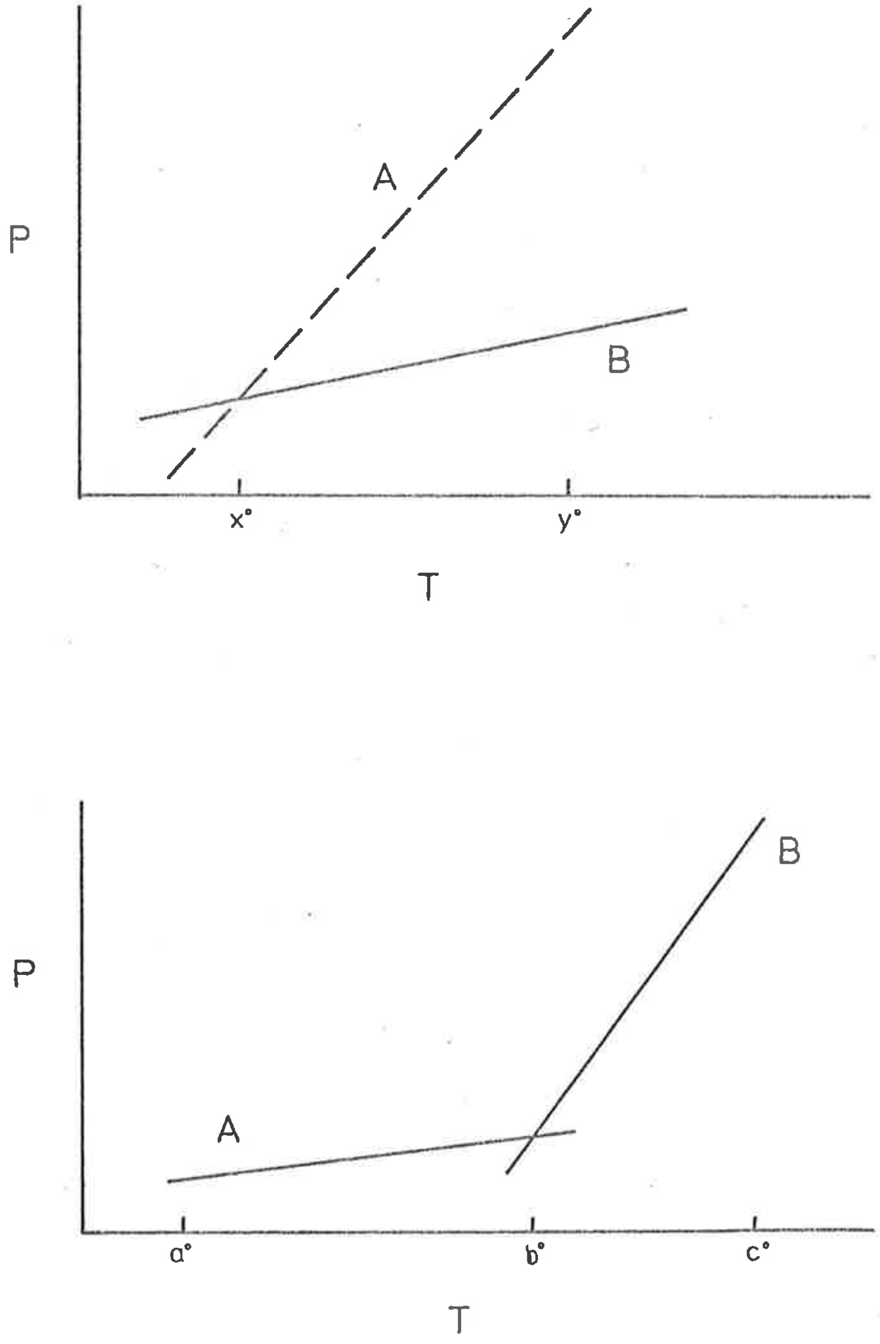


Figure 2.2 (a) Lines A and B represent regression lines fitted to plots of call component P against environment temperature in two populations A and B. If data are corrected to a common temperature x^{o}, A and B would be considered identical. If corrected to y^{o} P would have a greater value in A.

(b) as for (a) except that A and B are two samples from a single population. Sampling the temperature range a^{o} to b^{o} gives a very different result from the range b^{o} to c^{o}. The actual relationship between T and P is non-linear.

There is one final point about temperature effects. To eliminate the chance of representing curvilinear temperature relationships as linear the full range of calling temperatures must be covered (if practicable). If linear regressions are fitted to data covering different temperature ranges for a variable which truly has a non-linear temperature response, considerable heterogeneity between samples may result however the data are analysed (Figure 2.2(b)).

2.3 Results

As stated earlier *L.tasmaniensis* calls were recorded at six localities representing two sites for each of the call races. Exact geographic locations, recording dates and the number of frogs recorded are all given in Table 2.2. The position of recording sites in south eastern Australia is shown on Figure 5.1.

In analysing my own data all call components were initially regressed on water temperature. The results of these regressions are summarised in Table 2.3. There are two call components, note duration and note repetition rate which show significant temperature effects in at least two of the samples. The effects on note duration are suggestive of a curvilinear relation with temperature (at least for the Murray Bridge/Gawler data; Figure 2.3) and because of inadequate temperature ranges for most data this component was not analysed in detail.

Note repetition rate dat~~e~~a were analysed by comparing slopes and elevations of fitted regression lines (Snedecor and Cochran 1967) whilst other variables were compared by anova with *a posteriori* comparisons amongst means by the Student-Newman-Keuls test (Sokal and Rholf 1969). If the assumptions of anova did not seem to be met non-parametric analyses of variance were also done (Siegel 1956).

Summaries of call component values for each locality are given in

Locality	n	Dominant Frequency	Pulses per Second	Note Duration	Notes per Call	Notes per Second	Water Temperature
Murray Bridge	17	11.63 n.s.	24.02 *	- 2.31 **	0.023 n.s.	0.7222 **	9.92 (6.0 - 12.5)
Gawler	20	- 14.65 n.s.	16.09 n.s.	- 0.99 **	- 0.1 n.s.	0.9708 **	18.03 (15.8 - 22.3)
Blanchetown	14	13.30 n.s.	- 1.27 n.s.	0.54 n.s.	- 0.043 n.s.	0.7128 **	13.74 (11.0 - 14.9)
Big Bend	18	- 22.07 n.s.	- 2.21 n.s.	- 0.68 n.s.	- 0.003 n.s.	0.8632 **	18.47 (16.4 - 20.0)
Moyston	18	- 17.27 n.s.	----	- 0.213 n.s.	----	----	15.5 (14.1 - 17.0)
Mount Hope Drain	17	20.68 n.s.	----	- 0.258 n.s.	----	----	13.05 (11.0 - 16.0)

Table 2.3 Summary of temperature effects on call components in *L.tasmaniensis*. Data given are, n, sample size; regression coefficients for plots of call component on water temperature plus the significance level for comparison of the coefficient with zero (n.s., not significant; * p less than .05; ** p less than .01). Temperature data are means with ranges in brackets.

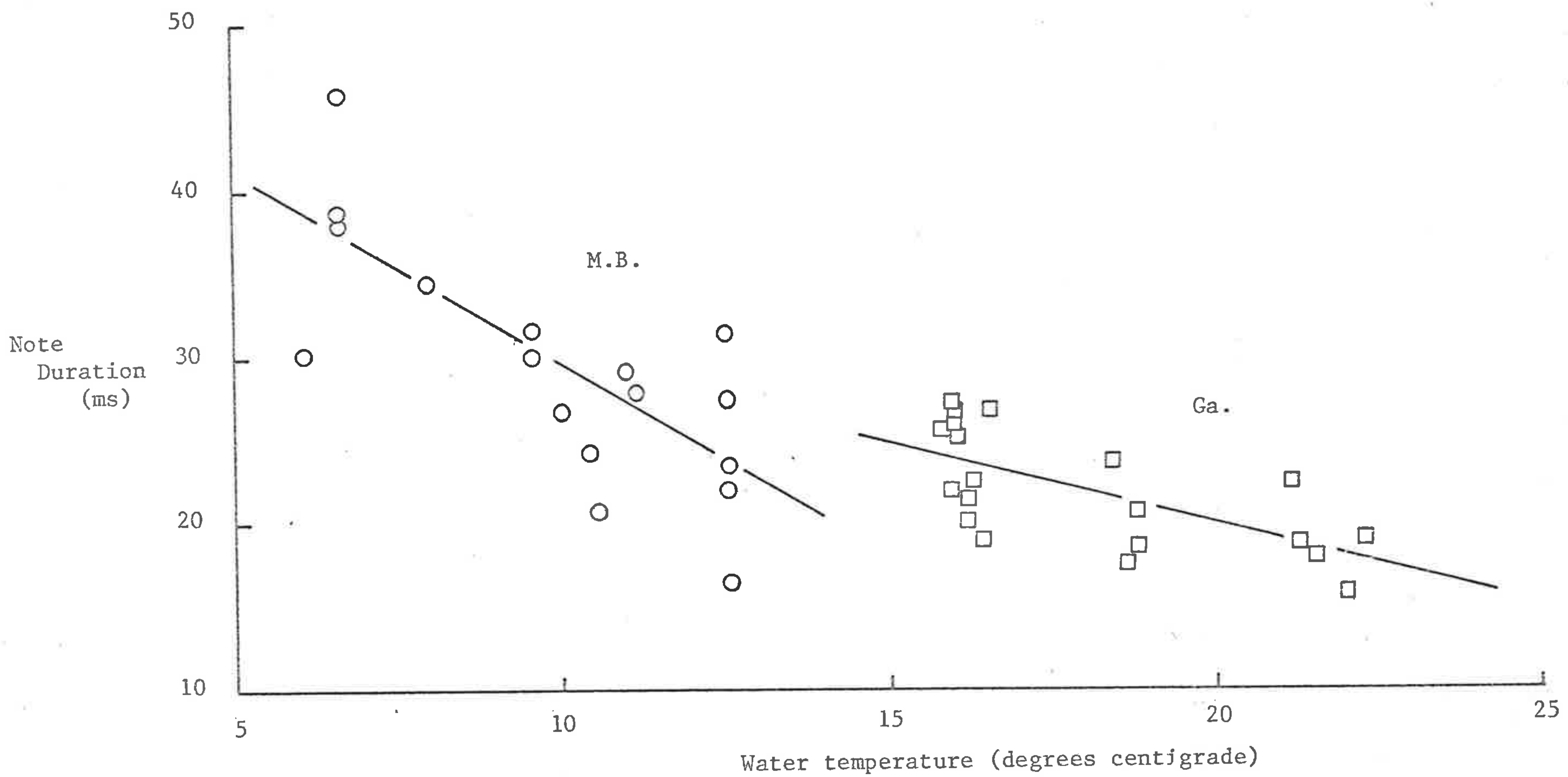


Figure 2.3 Plot of note duration against water temperature for Murray Bridge (M.B.) and Gawler (Ga.). Lines are fitted regression lines whose slopes differ significantly ($F_{1,33} = 7.94$, p less than .01).

Table 2.4. Equations of regression lines fitted to the note repetition rate, water temperature data are given in Table 2.5.

Calls from the two most southern localities, Moyston and Mount Hope Drain, consist of a single note as expected for the southern call race (section 2.1). Calls from the other four sites all had at least two notes. This difference obviates the need for detailed statistical comparison of northern data with that from Moyston and Mount Hope Drain.

Calls of what I will henceforth refer to as the southern call race also differ in other respects. Pulse repetition rate could not be measured meaningfully as intra-note amplitude modulation did not occur consistently and if present did not take any regular form. Mean note durations in southern calls are roughly half those in the other four call samples (Table 2.4).

Note durations at Moyston and Mt. Hope Drain differed significantly (Table 2.4). The causes of this difference will be discussed elsewhere (section 5.2 2)).

For the remaining four localities the results of anova on dominant frequency, pulse repetition rate and notes per call data are given in Table 2.6. The results of comparisons amongst means are appended to Table 2.4. The analysis of note repetition rate data is presented in Table 2.7. Means, ranges, standard deviations and 95% confidence limits on the means are illustrated in Figure 2.4 for temperature independent variables. Exact values of note repetition rate are plotted in Figure 2.5.

All anova gave significant F ratios indicating differences in sample means. For two call components, pulse repetition rate and notes per call, sample mean squares were not homogeneous (Table 2.6). This may indicate anova is an inappropriate technique to use on these data (but see reservations concerning the use of Bartlett's test,

	Locality	n	Dominant Frequency (Hz)	Pulses per Second	Note Duration (ms)	Notes per Call
W	Murray Bridge	17	1302 (1171 - 1474)]	242.51 (118.02 - 527.12)]	29.37 (16.53 - 45.74)	4.92 (3 - 8)]
	Gawler	20	1422 (1237 - 1654)]	317.86 (148.96 - 801.62)]	21.74 (15.57 - 27.02)	4.19 (3 - 7)]
N	Blanchetown	14	1771 (1552 - 2054)]	213.19 (171.19 - 438.83)]	25.34 (19.03 - 35.59)	3.13 (3 - 4)]
	Big Bend	18	1841 (1616 - 2039)]	213.45 (180.39 - 395.98)]	20.16 (16.13 - 25.46)	2.9 (2 - 4)]
S	Moyston	18	1996 (1832 - 2262)]	------	10.64 (8.0 - 12.90)]	1
	Mount Hope Drain	17	1940 (1845 - 2160)]	------	12.79 (9.61 - 15.48)]	1

Table 2.4 Call parameter values in various _L.tasmaniensis_ populations. Data are means with ranges in brackets. n is the sample size. Square brackets contain means that were not significantly different at the 5% level. _A posteriori_ comparisons of means from the top four localities were done with the Student-Newman-Keuls test. Moyston and Mount Hope Drain were treated separately and compared with a t test.

	Locality	Locality data			Pooled data	
		Slope	S.E.	Intercept	Slope	Intercept
W	Murray Bridge	0.7222	0.0654	3.03	0.8419	1.853
	Gawler	0.9708	0.0778	- 0.46		
N	Blanchetown	0.7128	0.1250	- 0.08	0.8419	- 1.924
	Big Bend	0.8632	0.1971	- 2.37		

Table 2.5 Parameters defining the regression lines fitted to the water temperature, note repetition rate data. S.E. is the standard error of the individual locality slope estimate. The pooled data column contains a slope estimate based on all data, and intercept values from western call race (Murray Bridge and Gawler) and northern call race (Big Bend and Blanchetown) sites.

(a) Dominant Frequency

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	3	3541969.09	1180656.36	91.82 **
Within localities	65	835752.65	12857.73	

Variance homogeneity, Chi^2 = 5.09, n.s.

(b) Pulses per Second

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	3	136019.52	45339.84	4.47 **
Within localities	65	658934.65	10137.46	

Variance homogeneity, Chi^2 = 16.71 *** , Kruksal-Wallis H = 12.07 **

(c) Notes per Call

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	3	45.15	15.05	41.54
Within localities	65	23.55	0.36	

Variance homogeneity, Chi^2 = 20.26 ***, Kruksal-Wallis H = 50.31 ***

Table 2.6 Inter-locality anova for temperature independent components of calls from putative western and northern call race populations. n.s. not significant, ** p less than .01, *** p less than .001.

(a) Comparison of slopes

Variation	d.f.	Sum of Squares	Mean Square	F
Among regression coefficients	3	3.5878	1.1959	1.8934 n.s.
Deviations from regression	61	38.5253	0.6316	

Deviation from regression mean square homogeneity, Chi^2 = 2.36 n.s.

(b) Comparison of elevations

Variation	d.f.	Sum of Squares	Mean Square	F
Between intercepts	3	227.4843	75.8281	115.24 ***
Deviations from pooled estimate of regression	64	42.1131	0.6580	

Table 2.7 Analysis of covariance among regression coefficents and intercepts for notes per second, water temperature data. n.s. etc. as in Table 2.6

page 375 of Sokal and Rohlf 1969). However, Kruksal-Wallis non-parametric analyses of variance also indicated significant differences between sites (Table 2.6).

Mean dominant frequencies at Murray Bridge and Gawler, although significantly different, are both low relative to means at Big Bend and Blanchetown (Table 2.4). It is obvious (Figure 2.4) that the major cause of the significant F ratio in the anova on this variable is the split between putative western and northern sites. Average notes per call means vary analagously in that means at Murray Bridge and Gawler are much closer to each other than either is to Big Bend or Blanchetown (Table 2.4; Figure 2.4). Pulses per second shows a different pattern of variation with the Gawler mean much higher than any of the others (Table 2.4; Figure 2.4). Slopes of regression lines fitted to note repetition rate, water temperature data were homogeneous but there were significant differences among elevations (Table 2.7). Again the Blanchetown and Big Bend samples are quite distinct from those from Gawler and Murray Bridge (Table 2.5; Figure 2.5).

2.4 Discussion.

1) The call races.

The call of the southern call race is, as Littlejohn (1966) has already stated, quite distinct from other *L.tasmaniensis* calls. Southern calls can be readily distinguished by two features; their short duration (8.0 - 15.5 m s) and the fact that they have only one note.

It may be less clear, however, that two call races need be delineated amongst *L.tasmaniensis* making multiple note calls.

If a single *L.tasmaniensis* were was recorded at one of Murray Bridge, Gawler, Blanchetown or Big Bend and one was given information on the call's dominant frequency, number of notes, note repetition rate and

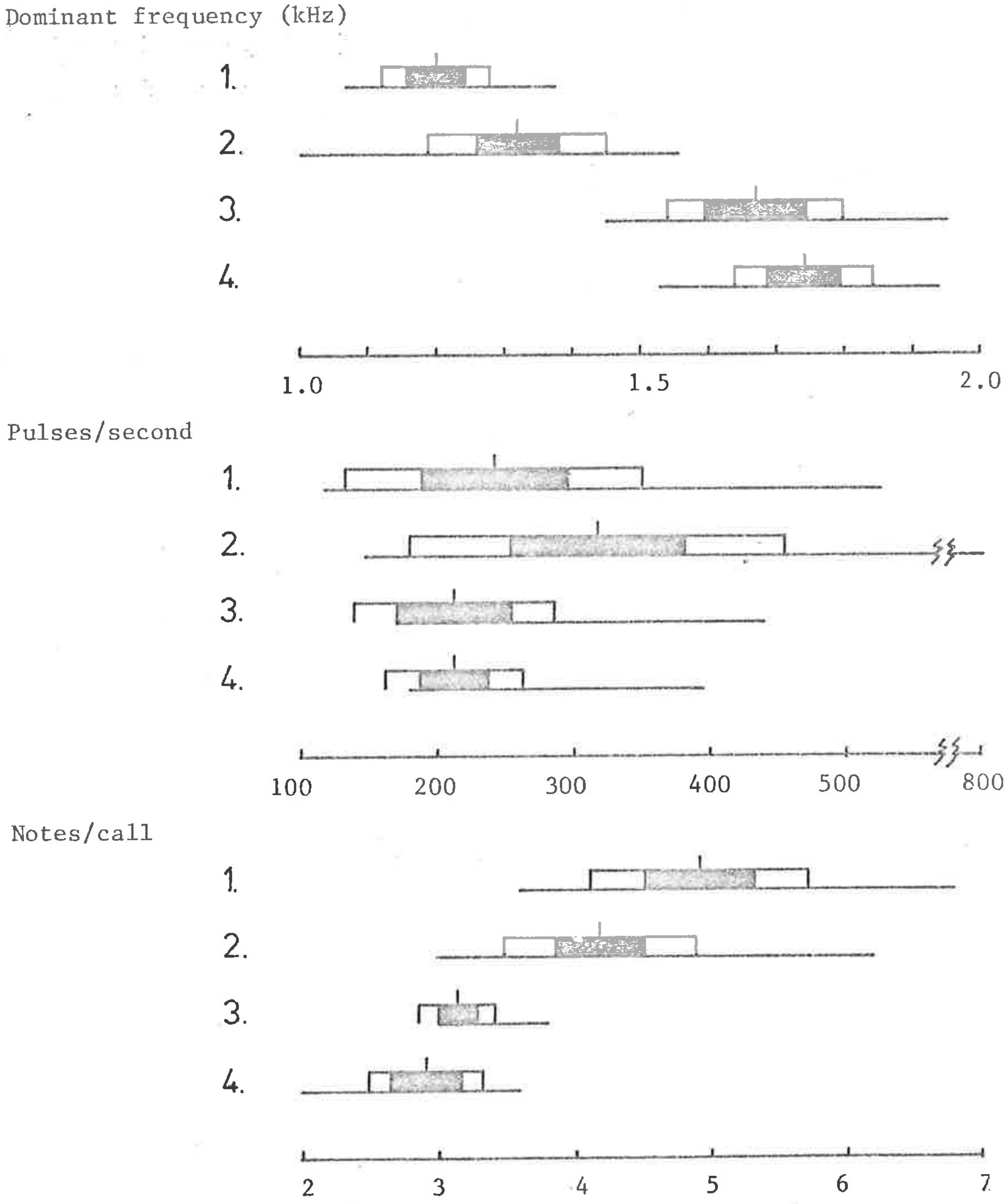


Figure 2.4 Summary of call component values in the western and northern call races. Vertical lines mark the mean, lower horizontal lines the range, open box symmetric about the mean contains two standard deviations and the blackened box spans the 95% confidence limits on the mean. 1., Murray Bridge; 2., Gawler; 3., Big Bend; 4., Blanchetown.

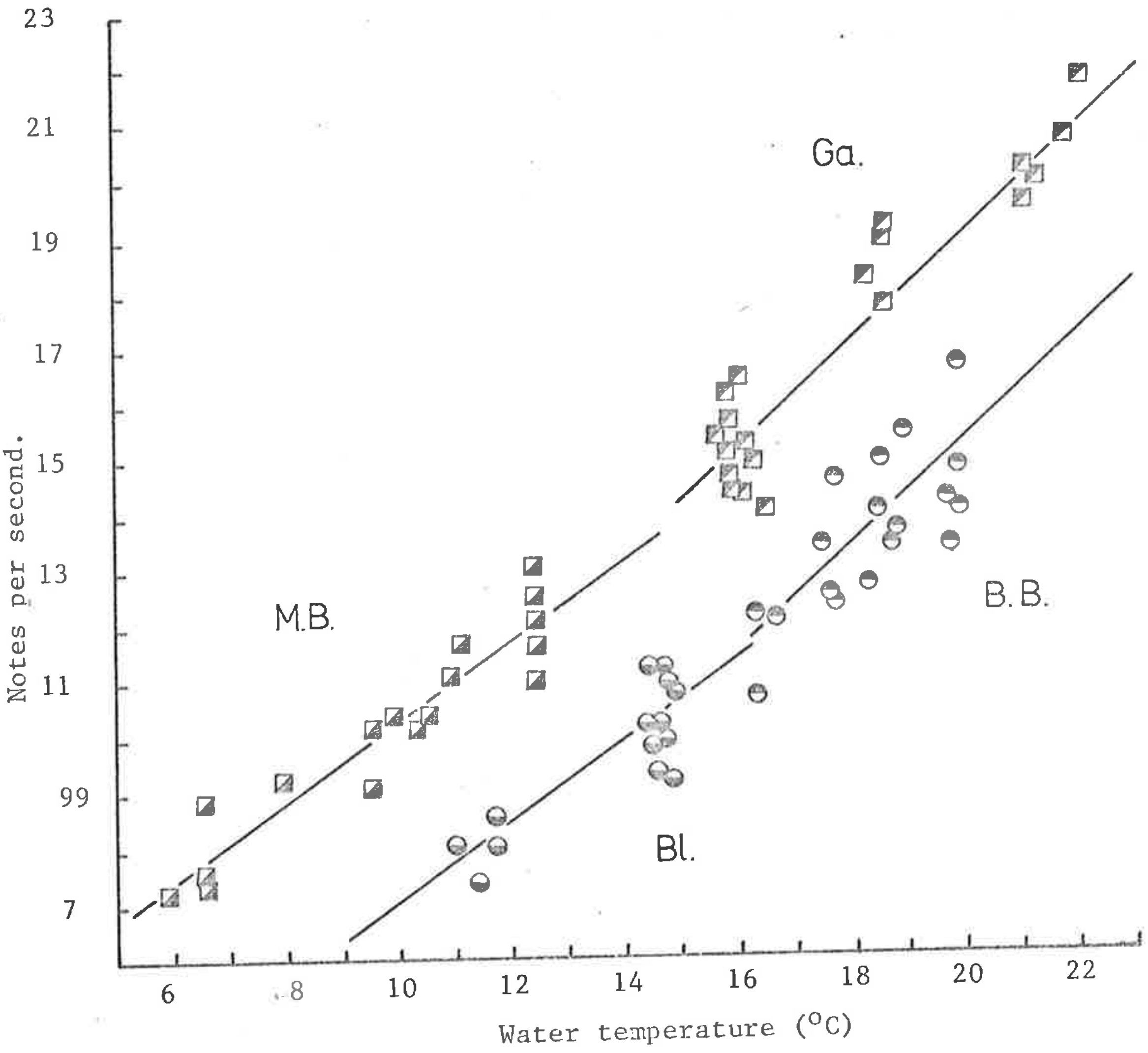


Figure 2.5 Plots of note repetition rate against water temperature for various *L.tasmaniensis* populations. Lines are fitted to individual locality data (see Table 2.5 for details). M.B., Murray Bridge; Ga., Gawler; Bl., Blanchetown; B.B., Big Bend.

water temperature, the call's actual origin could not be assigned with complete certainty. However, I am confident one could say whether the call was from one of two pairs of localities: Big Bend, Blanchetown or, Murray Bridge, Gawler.

L.tasmaniensis calls at Murray Bridge and Gawler are characterised by low dominant frequencies (1100 - 1650 Hz), high note repetition rates (Figure 2.5) and a call with usually four or five notes. Calls of this type I will refer to as the western call race following Loftus-Hills (1973). The differences between the Gawler and Murray Bridge samples will be discussed elsewhere (section 5.4).

Although calls at Murray Bridge and Gawler differed, to my ears they were virtually indistinguishable. This contrasts with the audible differences between western calls and those recorded at Big Bend and Blanchetown. At the latter sites dominant frequences are high (1550 - 2100 Hz), note repetition rate low (Figure 2.5) and there are most commonly two or three notes per call. I will refer calls of this type to the northern call race.

2) Distribution of the call types.

The call types, particularly the southern, can be distinguished readily and I think reliably without recourse to detailed acoustic analysis. The most distinct component is notes per call. Southern calls with a single note cannot be confused with those of either of the other races. The production of two note calls, either throughout or at the beginning of calling sequences, by northern males makes distinction of this race from the western relatively simple. The differencesin dominant frequency also simplify this latter discrimination.

Geographic distributions of the call races were determined by noting calls heard on road traverses throughout South Australia and Victoria. R.J. Swaby provided an extensive series of chorus recordings from various parts of Australia which gave additional data. Information was also provided by M.J. Littlejohn and taken from Littlejohn (1967).

All these data were combined to give the generalised distribution map in Figure 2.6. Detailed data for contact areas will be given in chapter 4. The known distribution of L.tasmaniensis is much wider than the known range of call types given in Figure 2.6. I have specimens of this species from Dalhousie Springs, 114 km north of Oodnadatta, South Australia and from two sites on Cooper's Creek; one in north east South Australia and the other in south west Queensland. Moore (1961) reported L.tasmaniensis from far west New South Wales. Cogger (1975) gave a small distribution map with no specific supporting data and figured L.tasmaniensis as occurring throughout New South Wales and Victoria, in southern and eastern Queensland and in most of the southern and eastern portions of South Australia.

I know nothing of call structures in L.tasmaniensis from these more remote areas of Australia. Neither am I certain of call structures in northern New South Wales and Queensland. Straughan's (1966) data for frogs recorded in these latter areas indicated calls quite different from the typical northern call I have described.

Moore (1961) reported L.tasmaniensis from Port Lincoln, South Australia. I conducted intensive searches in this area in October 1974 but neither heard nor found any evidence of this species. In other areas of South Australia all call races were calling and breeding strongly at this time.

Although there may be some breakdown of the differences between the western and northern call races in more northern parts of the range of L.tasmaniensis (cf. Straughan 1966, discussed above) I am satisfied that the three races I have defined are distinct enough to warrant the investigations that I report in the remainder of this thesis.

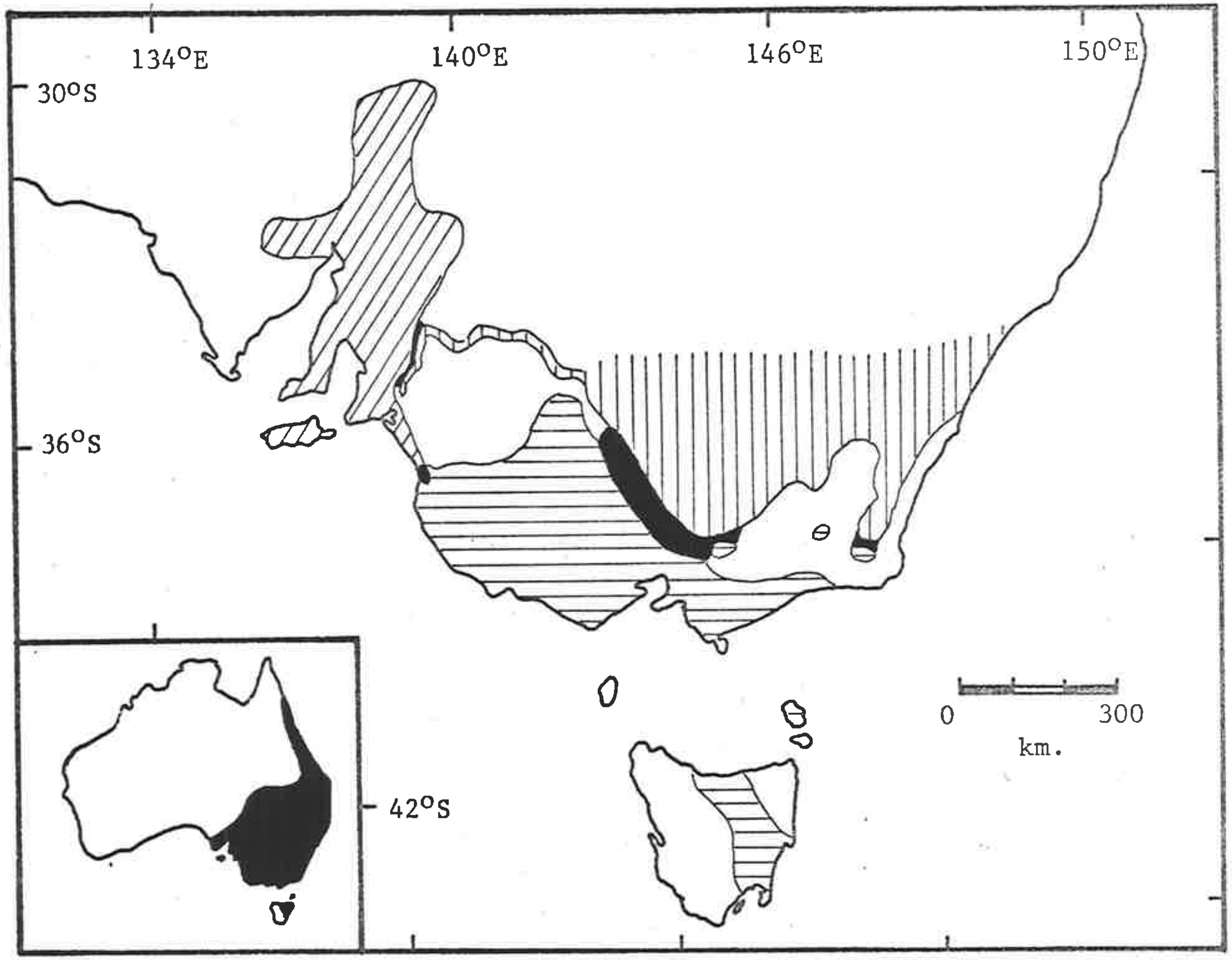


Figure 2.6 Known distribution of the southern (horizontal hatching), northern (vertical hatching) and western (diagonal hatching) call races of L.tasmaniensis in south eastern Australia. The blackened area of the inset covers the total range of this species according to Cogger (1975).

3. Call function in *L.tasmaniensis*: an experimental analysis.

3.1 Introduction.

There is considerable circumstantial evidence to suggest that male call is an important factor in anuran reproduction. Calling is inevitably associated with breeding for any given species although in some species calling occurs over a longer period than spawning (Main 1968; W.F. Blair 1961). There are also several observations of females apparently deliberately approaching calling conspecific males and subsequently being amplexed (e.g. Noble 1923; further references in Bogert 1960; Martof and Thompson 1958).

Martof and Thompson (1958) achieved some control over the sorts of observations that Noble had made in the field, by watching frogs contained in a large tank. They reported that gravid female *Pseudacris nigrita* approached calling males of the same species and were amplexed. When males were not calling no such movement was seen. Furthermore, if the calling frog was totally concealed females still approached. Orientation to calls played back through a small loudspeaker was also noted. Martof and Thompson's data strongly support the idea that anuran calls play an important role in bringing males and females together to mate.

A corollary of this is that frog calls may act as premating isolating mechanisms. Sympatric frog species generally have well differentiated calls (Littlejohn 1969) and if they do not, other attributes effect premating isolation (Gerhardt 1974b; Awbrey 1968). For premating isolation to be attained only requires that females respond selectively to conspecific calls. The aim of virtually all investigations of anuran call function post Martof and Thompson (1958) has been to determine whether females do respond selectively or not (see appendix 3 for references). There have also been some studies of the importance of particular call components in eliciting responses from females (Loftus-Hills and Littlejohn 1971; Gerhardt 1974c and d; Straughan 1975).

All these experiments were conducted in essentially the same manner. A female frog, often collected in amplexus (Table 3.1), was released midway between two loudspeakers; one broadcasting a conspecific call and the other some alternative sound. The movements and positions assumed by the female or females under test were noted. After females responded to a call the signals were generally reversed and the frog either released midway between the speakers again or left to respond from where it was at the time the signals were transposed. Many authors tested individual females repeatedly, usually with consistent results.

Although these experiments have produced an impressive body of data showing that calls of male frogs can attract conspecific females certain features of the experimental design are bad.

1. Use of amplexed females: The hypothesis being tested by call discrimination trials is that male call can attract females. The presumption is that subsequently, if a male were present, she would be amplexed. This latter aspect clearly cannot be tested in experiments as detailed above. However, the inference concerning amplexus will inevitably be made or implied if females do respond to call playbacks. Because there is no guarantee that already amplexed females are equivalent physiologically, behaviourally or whatever to females striving for amplexus, frogs collected in amplexus should not be used in call discrimination trials.

2. Testing of females only: The fact that only females have been tested limits the functions that might be ascribed to anuran calls. Field trials with mole crickets (Scapteriscus species) have shown that mated and virgin females and males are attracted to playbacks of male calls (Ulagaraj and Walker 1973). The expectation in this case would

Amplexed only	Amplexed and other	Other	No information	Total
8	7	4	3	22

Table 3.1 Source of females used in call discrimination trials conducted by authors listed in Appendix 3.

have been to attract only virgin females. Morris (1972) demonstrated phonotaxis by male katydids (Orchelimum species) and stated, "It is commonly assumed that the function of male song in katydids is to attract and guide the female. For these two species the male song also stimulates aggressive behaviour and guides its initial approach phase".

3. Lack of soundless control trials: This is not a serious criticism as most authors have effectively controlled their experiments by transposing the test signals after each trial and by testing responsive females repeatedly. However, soundless control runs may be of more importance when only a small proportion of animals are responding and positive results may be due to chance. Hill (1974) found that 3/40 female crickets (Teleogryllus species) responded in a call discrimination apparatus similar to those used in anuran studies, listed in Appendix 3.

3.2 Methods.

1) Experimental series one:

Two series of experiments were conducted to determine call function in Limnodynastes tasmaniensis. The first involved females of northern and western call races collected during the breeding season at 3.2 km south of Morgan, S.A. and 3.3 km north of Mount Mary, S.A. respectively. These two forms hybridise west of Morgan and east of Mount Mary (section 4.4) but the populations sampled are probably free of genetic contamination by the contrary call race.

The first experiments were run on fairly classic lines with northern and western calls played back through loudspeakers 2.4 m apart (cf. experimental set-ups described in papers listed in Appendix 3). On a line perpendicular to and bisecting that joining the speakers were two dummy speaker boxes the same size and distance apart. The speakers and dummies were set out on a grid marked on cement covered with silver painted bituminous felt (Figure 3.1). The sides of the grid were marked with numbers and letters and the tracks taken by frogs were noted with reference to these coordinates.

The observations were conducted in the open on the roof of the R.A. Fisher building, University of Adelaide. This location is about 130 km from Morgan and 75 km from Dutton; the collecting sites for frogs used in the first and second series of experiments respectively.

All experiments were run at night on still evenings. Frogs were observed by moonlight and or with a red spotlight. Only one observer was present sitting just outside the grid and to one side of the speaker broadcasting the call of the female under tests call race (at A or B, Figure 3.1). If there was no movement within ten minutes a trial was abandoned. Trials also ended if a frog showed a persistent interest in a speaker or dummy, moved off and well away from the grid or moved but did not respond or leave the grid within fifteen minutes.

Frogs were placed under an opaque white container for roughly two minutes before a trial commenced but calls were not played back during this period. The cover was removed by the observer who then returned to his seat and started call playback. The release point was equidistant from the speakers and the dummies.

Calls were played from a programme tape on Uher 4400 Report stereo recorders coupled to Plessey Rola 2C loudspeakers. Tape speed was 9.5 cm s^{-1}. The tape was prepared by repeatedly transcribing a loop of two to five calls of an individual frog at a normal call repetition rate. This sound reproduction system did not produce any obvious distortions (Figure 3.2). Loops were produced from field recordings made in populations of both call races in South Australia. Programme tapes for both northern and western calls were made from recordings of

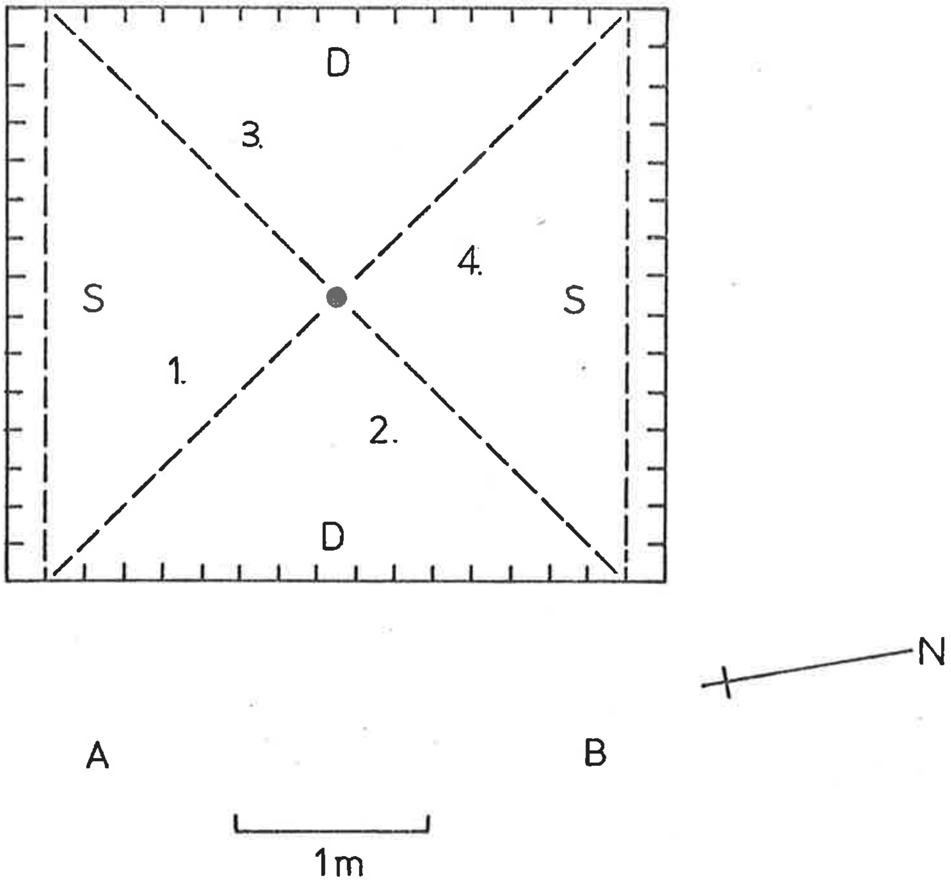


Figure 3.1 Test grid arrangement. S and D indicate speaker and dummy positions respectively. Solid circle was the release point. The observer sat at A or B. The dashed lines delineate the grid quarters referred to in section 3.2 (3). The numbers 1 to 4 are the quarter numbers for an observer seated at A. If the observer was at B, 1 and 4 are transposed.

frogs at water temperatures of about 16° and $20^\circ C$. On any particular night calls initially recorded at the temperature closest to the cloacal temperature of the frogs being tested were used. Particulars of call structure are given in Table 3.2 and oscillograms showing major differences in Figure 3.2.

Gerhardt (1974b) found that intensity differences between two similar calls of different species may influence the behaviour of females in call discrimination trials. Accordingly I tried to make the intensities of the northern and western calls approximately equal at the release point and equivalent to those of natural calls.

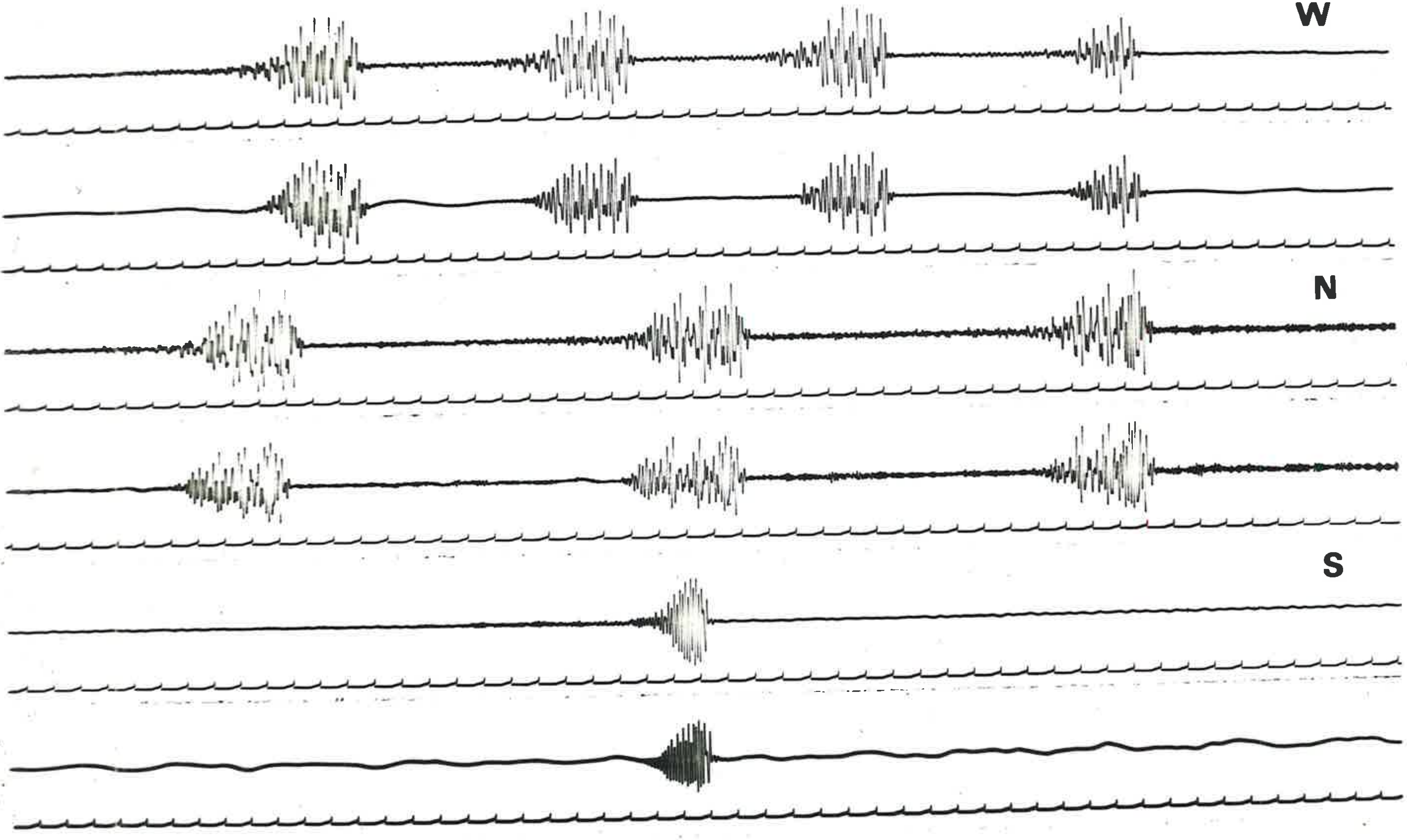
Sound intensity was controlled with the amplifier of the Uher recorder. Natural sound levels were approximated using a Beyer M100 microphone and Nagra III N.P. recorder. The microphone was placed 5 cm in front of the western speaker and the loudness adjusted until the level meter reading was the same as that obtained from a calling male at a similar distance and with a similar microphone input level control setting. The microphone was then put at the release point and the loudness of the northern call adjusted to equal that of the western. Appropriate apparatus was not available for more sophisticated sound level manipulation.

A western then a northen female or vice versa were tested with the western and northern speakers at particular locations on the grid; say, at the southern and northern ends respectively (Figure 3.1). The speakers were then alternated and another western then northern female tested. The dummy speakers were never moved as although it would have been desirable to test all permutations of dummy and speaker positions, two sides of the grid were close to brick walls and I felt that echoes from these may have effected the results (Figure 3.1).

Call type	Notes per second	Note duration (ms)	Dominant frequency (Hz)	Pulses per second	Notes per call
Western 20.8°C	21.07	18.62	1450	362.37	4
" 16.0°C	16.51	23.05	1388	300.66	6
Northern 19.8°C	13.26	20.23	1829	226.85	2 - 3
" 16.0°C	11.41	19.10	2018	206.71	3
Southern 16.0°C	-	13.66	1978	-	1

Table 3.2 Detailed structure of calls used in both series of call discrimination trials.

Figure 3.2 Oscillograms of calls used in call discrimination trials. W, western (20.8°C); N, northern (19.8°C); S, southern (16.0°C). The upper oscillogram in each pair was made from recordings made with a Beyer M 100 microphone and Nagra III N.P. recorder of the sound output of the Uher 4400 plus Rola loudspeaker. The lower oscillogram in each pair was made direct from the programme tape played on the Uher 4400. The time base on all oscillograms effectively represents 5 ms intervals.


W
N
S

2) Experimental series two.

The second series of experiments used only western call race frogs from 3.5 km north of Dutton, South Australia but females, males and juveniles were tested. There were also several other differences. Illumination was provided by a 15 watt red globe suspended 1.5 m above the release point. The opaque cover the frogs were placed under was attached to a long pole and raised from the observer's seat. The frogs were given a choice of western calls (from a frog recorded at an appropriate temperature) and southern call race calls (always from a frog recorded at 16°C). (Details of call structure see Table 3.2 and Figure 3.2.) Playback system and programme tape production, etc. was as before.

Frogs were collected in December 1974 and January 1975. For the first group the observer sat at 'A' and western calls were played through the speaker at the southern end of the grid. For the second group the observer sat at 'B' and calls were reversed (see Figure 3.1). Each frog was run twice. The first trial was a soundless control and in the second frogs were tested as in the first series of experiments except for modifications noted above. Control and experimental runs were always done on different nights. Conditions were generally similar as is indicated by the fact that cloacal temperatures of frogs in control and experimental runs did not differ significantly (t_{58} = 1.44; p greater than 0.1). Additionally, the frogs collected in 1974 were tested a third time 24 hours after they had been injected intraperitoneally with 200 I.U. of H.C.G. (Human Chorionic Gonadotrophin). H.C.G. stimulates öocyte development in anurans (Dumont 1972) and has been used to induce breeding activity in <u>Xenopus laevis</u> (New 1966) and <u>Bombina</u> species (Schneider and Eichelberg 1974). These injections were given in the hope that ovulation or some other change would ensue and thus females, at least, in several physiological states would have been tested. Frogs were killed after this third trial and their reproductive systems examined macroscopically.

In all trials in both experimental series responses to speakers or dummies were scored as multiple or single. For the latter a frog entered the speaker/dummy square once and did not move again or only moved out of the square. Multiple responses occurred when a frog entered the speaker/dummy square and either moved around the speaker or dummy or, repeatedly entered then left the speaker/dummy square (i.e. made a series of movements each equivalent to one single response). Frogs were not re-run, except as indicated above, and thus each frog tested only contributes one result to the combined control and experimental data.

The rationale for the experimental design set out above is fairly simple. The dummy speakers were put in to minimise the possibility that frogs were attracted to objects, or the shadows cast by them, on the test area. The soundless control runs were an additional control to show whether frogs were attracted to the speaker boxes but not to the dummies. The first series was a straight forward test of the discriminatory ability of females. The second could be treated similarly, but equally, southern calls could be described as a control for attraction to sound. Southern and western calls are qualitatively very different.

3) Effects of observer position.

I hoped that if the observer influenced frog movements at all, then this influence would minimise the chance of a frog responding to the call of its own call race. I tested whether this was so by noting the point where each frog left the grid or ended its trial. On control runs, if all frogs exited via the corner diagonally opposite the observer (Figure 3.1), there is a much reduced likelihood that responses seen on experimental runs are due to chance movements.

For the purposes of the above analysis the grid was divided into quarters around the release point (Figure 3.1). The diagonals of the square formed equal area sections numbered 1 to 4 as follows. Quarter 1 contained the speaker broadcasting conspecific calls or was the equivalent quarter on control runs. Quarter 4 contained the other speaker. Quarter 2 contained the dummy speaker closest to the observer and Quarter 3 the other dummy. Quarters three and four thus form the half of the grid furthest from the observer.

As well as detecting observer effects this analysis may also indicate whether frogs are moving in the general direction of a sound source but not directly to it.

3.3 Results.

1) Experimental series one.

Results are summarised in Table 3.3. No females in this series responded to dummies suggesting the responses scored do not represent chance events. It seems that western and northern females cannot distinguish the two types of call, even though there are a number of differences between them (Table 3.2; Chapter 2). However, the data are few and it would be foolish to make much of them. Though, if this result is valid it is not surprising as these two call races hybridise where their ranges meet (section 4.4).

2) Experimental series two.

a) Call discrimination trials.

Call discrimination results (for non H.C.G. injected frogs) are summarised in Table 3.4. No responses to speakers or dummies were observed during control trials and only one response to a dummy during

♀

Frog's call race	Dummy		Western		Northern		No response	No movement	Total frogs tested
	M.	S.	M.	S.	M.	S.			
Western	0	0	0	0	1	0	8	1	10
Northern	0	0	2	1	1	0	7	1	12

Table 3.3 Results of the first series of call discrimination trials. M and S indicate multiple and single responses respectively to either the dummy, northern or western loudspeaker. No movement means the frog never shifted from the release point. No response means the frog moved about the grid but did not make any responses to the loudspeakers.

	Dummy		Western		Southern		No response	No movement	Total frogs tested
	M.	S.	M.	S.	M.	S.			
Juveniles									
Control	0	0	0	0	0	0	4	1	5
Experimental	0	0	0	1	0	0	4	0	5
Males									
Control	0	0	0	0	0	0	12	3	15
Experimental	0	0	1	2	0	0	10	2	15
Females									
Control	0	0	0	0	0	0	11	2	13
Experimental	0	1	4	2	0	0	6	0	13

Table 3.4 Results of the second series of call discrimination trials. M, S etc. as in Table 3.3. All frogs tested were of the western call race (see text).

sound playback. Given this, I interpret the responses noted in experimental trials as representing deliberate choices rather than some chance phenomenon. Some female, male and one juvenile western call race L.tasmaniensis moved to conspecific calls and discriminated against those of the southern call race. Injection with H.C.G. did not produce any marked change in the number of responsive animals. Four of seven injected females responded to western calls and one to southern calls, compared with three responding to western before H.C.G. treatment. However, all females moving to the western calls made multiple responses post H.C.G. compared with two multiple and one single response in previous trials. Only two males and one juvenile were H.C.G. treated. The juvenile never responded and one of the two males did before treatment but not afterwards. Representative tracks taken by frogs on control and experimental runs are illustrated in Figure 3.3.

b) Ovarian state of responsive females.

Only females injected with H.C.G. were investigated. One of the seven had no mature öocytes in its ovaries; this frog did not respond to calls. The ovaries of all other females were similar and contained a fairly high proportion of apparently mature öocytes.

c) Observer effects on frog movement.

The data from this analysis are given in Table 3.5. On the control trials most frogs end in Quarters 3 or 4; i.e. as far away from the observer as possible. This pattern is totally destroyed in the experimental trials with about half of all frogs ending in Quarters 1 or 2. This change is partly caused by responsive animals but can also be attributed to frogs moving towards the speaker broadcasting conspecific calls but not actually responding to it. The observer position did minimise the chance of frogs moving to the loudspeaker

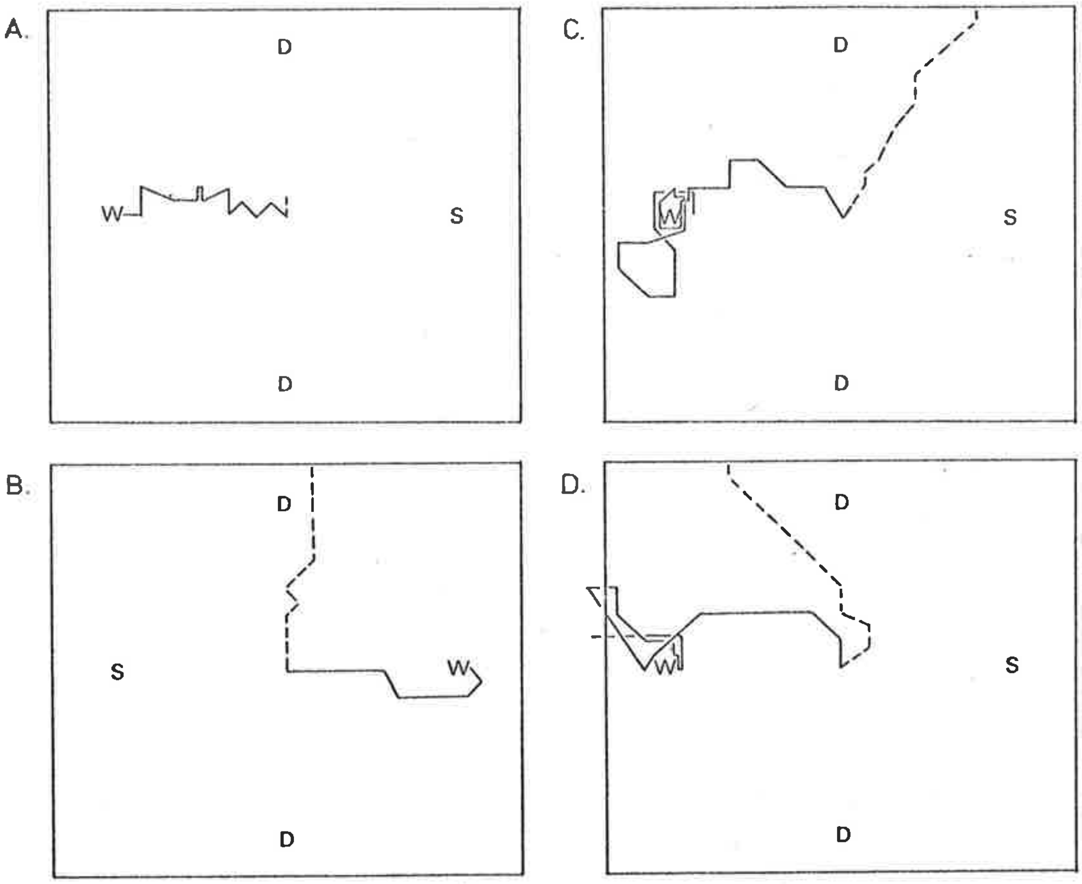


Figure 3.3 Tracks taken by frogs in experimental series two. Solid lines represent tracks taken by responsive frogs. Dashed lines are paths for frogs on control runs. A. and B. are tracks taken by female and male frogs respectively which made single responses. C. and D. are paths taken by female and male frogs making multiple responses. W, western loudspeaker; S, southern loudspeaker; D, dummy.

	Grid Quarter				
	1.	2.	3.	4.	No movement
Control					
Juveniles	0	0	2	2	1
Males	2	0	5	5	3
Females	0	1	6	4	2
Experimental					
Juveniles	3#	1	1	0	0
Males	7##	0	4	2	2
Females	5###	1	7	0	0

Table 3.5 Numbers of frogs ending in indicated quarters of the grid in the second series of call discrimination trials.

includes one responsive frog, ## includes three responsive frogs, ### includes four responsive frogs, two other responsives ended in quarter 3.

broadcasting conspecific calls.

3.4 Discussion.

These experiments demonstrate that female western L.tasmaniensis can discriminate calls of western males from grossly different signals but may have difficulty distinguishing calls which are structurally similar to those of their own males. This result is similar to that obtained in numerous other investigations of Anuran call function (e.g. Littlejohn and Loftus-Hills 1968; Awbrey 1968). However, the results differ in that females were not ovulating at the time of test nor were they obviously ripe or gravid.

Western L.tasmaniensis breed frantically in the spring and early summer, and breeding continues at a low level during later summer and early autumn although in this latter period intense breeding may occur immediately after heavy rain. These observations suggest that some or maybe most, females must be capable of ovulating at virtually any time. If this is so, and if amplexus can occur before ovulation (cf. Gosner and Rossman 1959), then it may not be surprising that a reasonable proportion of females orient to calls. This of course assumes that females orient to calls so that they may be amplexed.

Calling western L.tasmaniensis sometimes fight and/or produce an altered call. These actions usually involve males initially calling very close to each other and may constitute calling site defence mechanisms. It may be that male phonotaxis, either as a direct response (Table 3.4) or as a tendency to move in the direction of the loudspeaker playing conspecific calls (Table 3.5) is a preliminary to some form of territorial defence or challenge (cf. Morrison 1972).

This leaves the juvenile response (Tables 3.4 and 3.5) unexplained. However, juvenile frogs are of one sex or the other and conceivably, responses typical of mature frogs are sometimes given by immatures.

Considerable work has been done on elucidating the cues that frogs use for orientation. Landreth and Ferguson (1966, using *Pseudacris triseriata*) and Ferguson and Landreth (1966, using *Bufo fowleri*) tested the effects on frog movements of playbacks of conspecific chorus recordings. They used both males and females. The tests were conducted some distance from the site where the frogs were initially caught. They found that frogs of both sexes tended to move toward the loudspeaker when it was broadcasting conspecific calls. Otherwise frogs moved randomly or in a direction which would have taken them to a breeding pond at the location where they were caught.

Oldham (1966, 1967 using *Bufo americanus* and *Rana clamitans* respectively) ran experiments similar to Ferguson and Landreth's with similar results. However, Oldham also tested frogs within a few metres of their home ponds. Natural conspecific choruses were silenced but chorus playbacks still did not affect frog movements.

Thus, it seems that in unfamiliar situations frogs move to the only available familiar cue; conspecific calls. The reason for this behaviour is not obvious but possibly, frogs use calls as an indication of a suitable place to survive or reproduce (cf. Ulagaraj and Walker 1973). The movement to calls of its own call race by western *L.tasmaniensis* may represent behaviour similar to that seen by Oldham, Landreth and Ferguson.

Although calls may effect frog movements in experimental situations it seems unlikely this is an important role in nature. Oldham's (1966, 1967) results suggest otherwise. Further, males are not inevitably calling or may not be audible (Heusser 1958; Dole 1969; Tracy and Dole 1969), and it is reasonably well established that anurans can use many other, perhaps more reliable, cues for orientation (Adler 1970; D.E. Ferguson 1971; Fitzgerald and Bider 1974).

What then should I conclude about call function in frogs generally and in *L.tasmaniensis* in particular? First, as suggested for my own work, the result of many call discrimination trials may not relate to reality. Of the papers listed in Appendix 3, only Forrester (1973) conducted trials close to the pond where females tested were caught. This does not mean that male calls do not attract females in nature, nor that calls do not act as premating isolating mechanisms. However, it does mean that much of the evidence amassed to support these views maybe unreliable. Second, I now know that male, female and probably juvenile western *L.tasmaniensis* can distinguish their own calls from those of the southern call race but that northern and western females probably cannot discriminate between northern and western male calls. Third, calls of male *L.tasmaniensis* may attract females and therefore may act as premating isolating mechanisms but calls might also have other functions; e.g. as mediators of interactions between males.

4. Contacts between the call races of *L.tasmaniensis*.

4.1 Introduction

For reinforcement to produce premating isolating mechanisms two genetically distinct taxa must hybridise and there must be some degree of hybrid inviability (section 1.3 1)). In this chapter I report my investigations of characters which might act as pre- or postmating isolating mechanisms in areas where the southern, western and northern call races meet.

My aims were two. First, to search for evidence of reinforcement, particularly of differences in call structure. Second, if there was no evidence of reinforcement I wished to make some prediction about the future of the contacts between the various call races. This prediction was to be based on the information I collected about pre- and postmating isolating mechanisms and on data gathered on factors which might limit the geographic ranges of the three call races.

Criteria for the recognition of reinforcement have been given in section 1.3 1). The sampling of call structures in overlap areas was designed to maximise the chance of detecting reinforcement if it had occurred. Details of the particular sampling routine adopted in each case are given in the text.

At the commencement of this study the position and nature of any contacts between the three call races were poorly known.

Littlejohn (1966) gave a verbal description of calls heard in areas where the northern and southern call races met in south eastern Australia. After differentiating southern and northern calls as single and multiple pulsed respectively (Littlejohn's pulse is equivalent to my note) he stated, "Where the geographic ranges of the two forms contact, zones of intergradation are produced, in which individual frogs may be heard producing both single and multiple pulsed calls within one calling sequence". Littlejohn (1967) made further reference to this contact and presented a generalised distribution map which included four intergrade zones; the largest, is in central Victoria extending from Kerang in the north west to Wandong in the south east.

Contacts between the northern and western call races were unknown before I started work, though Littlejohn (pers. comm.) had heard northern calls at three localities on the upper Murray in South Australia (Kingston, Morgan and Blanchetown), and western calls at Murray Bridge and close to the mouths of two streams flowing east from the Mount Lofty ranges into the river Murray (Reedy creek, 8 km W.S.W. of Mannum and on the Marne river, 4.5 km west of Wongulla). Subsequent investigation revealed several narrow overlaps just west of the Murray river (see Chapter 2).

The southern and western call races were known to be sympatric in fresh water swamps along the eastern edge of the Coorong. Littlejohn (pers. comm.) had recorded both types at Banff (91 km S.E. of Meningie) and Henry creek road corner (42 km N.N.E. of Kingston). However, the complete area of sympatry had not been delineated.

A priori, it was not expected that modern contacts between the races of _L.tasmaniensis_ would produce evidence that reinforcement was responsible for the evolution of the southern, western or northern call races. The differences in call structure are widespread and apparently stable in form (Figure 2.6). Furthermore, the split of the southern and western call races, at least, is ancient. The western call race is found on Kangaroo island and the southern on Flinder's island and in Tasmania. These islands were last joined to the Australian mainland eight to ten thousand years ago (based on water depths on Admiralty charts; Australia, southern portion, 2759b, and information on eustatic sea level changes in Milliman and Emery 1968).

Evidence of reinforcement might still be found, however, if existing call differences between races in the L.tasmaniensis complex were inefficient premating isolating mechanisms. The data in Chapter 3 are few and may not be reliable, but if male call does act as a mate attractant then I would expect the southern and western call races to be isolated but not the northern and western.

I will consider each of the contact areas separately starting with the overlap between the southern and western call races.

4.2 The Coorong contact: the southern and western call races.

This section is divided into four parts. The first deals with a comparison of southern and western call race characters which might act as premating isolating mechanisms. This is to determine whether any of the characters show evidence of reinforcement. The second part deals with postmating isolating mechanisms and an analysis of the viability of southern x western hybrids. The third part discusses geographic ranges of each call race in the area where they are sympatric and the factors that might impose range limits. The final section is a general discussion.

1) Premating isolating mechanisms.

a) Calls.

Calls of the southern and western call races differ in many ways but most obviously in notes per call (Chapter 2). Using this character the calls cannot be confused. Detailed geographic distribution of the call races was determined by noting call types heard on road traverses. The majority of data were collected in the spring of 1971 but additional information was obtained from 1972 to 1975. The distribution data are illustrated in Figure 4.2.

Calls were recorded at five sites (Table 4.1; Figure 4.2). Data from stations 1 and 2 in Table 4.1 were lumped and treated as one

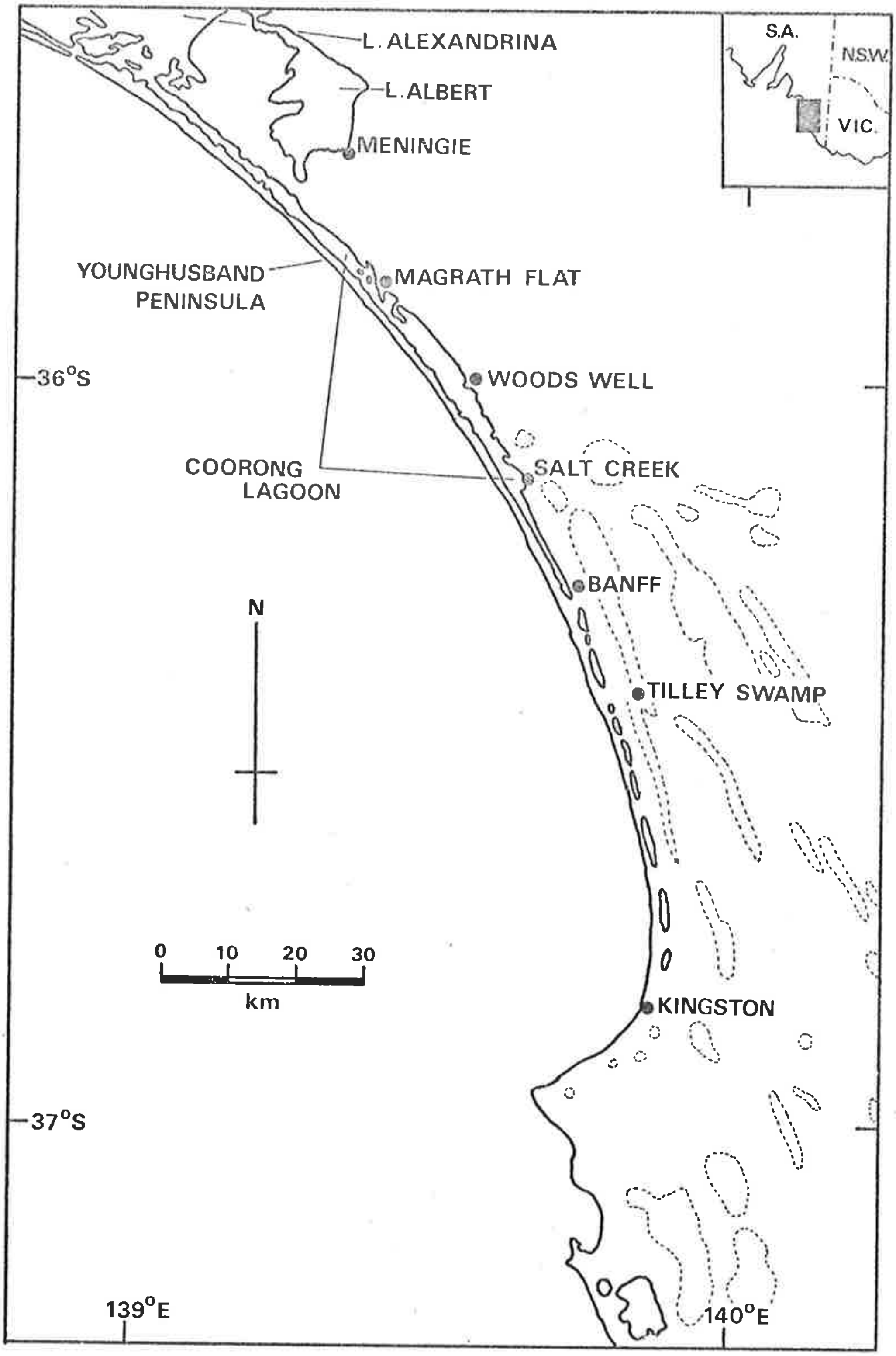
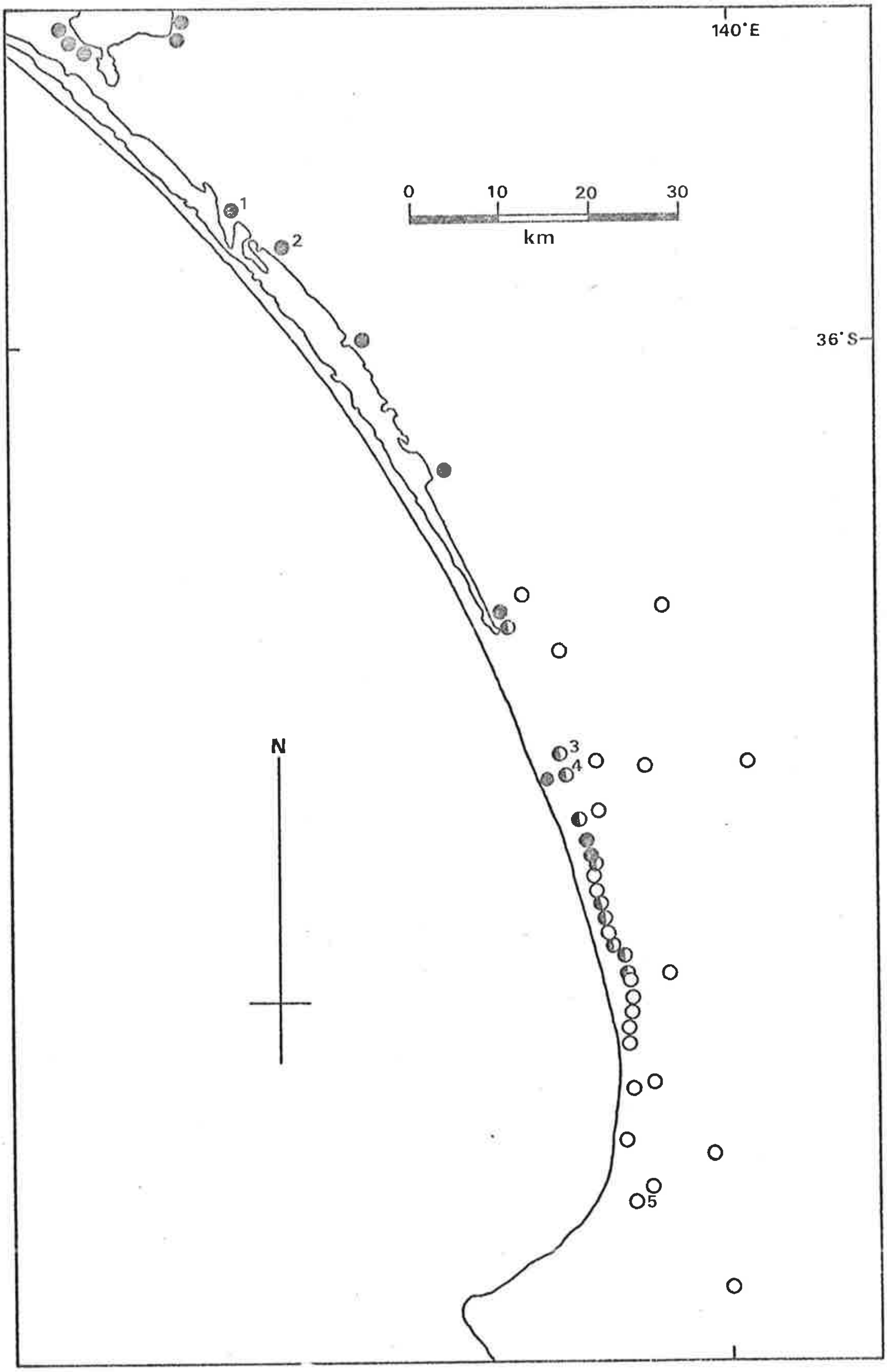


Figure 4.1 Principal localities mentioned in section 4.2. Solid lines indicate coast line, boundaries of permanent fresh water bodies or salt lakes. Dashed lines indicate extent of major freshwater swamplands.

Figure 4.2 Known distribution of L.tasmaniensis on the Coorong. Closed circles are sites where only the western call race has been heard; open circles, southern only; half closed, both races. Numbers to the right of some symbols indicate recording localities and refer to sites listed in Table 4.1.



Locality	Map and Grid reference	Recording date	Recorder	Number of frogs recorded
1. Magrath Flat	SI 54-13 242573	15.ix.1971	J.D.R.	15 W
2. 7.2 km S.E. of Magrath Flat	SI 54-13 248567	14.ix.1968	M.J.L.	5 W
3. 3.5 km W.N.W. of Tilley Swamp	SJ 54-2 281507	14.ix.1971	J.D.R.	2 W, 1 ?H
4. 3.0 km W.S.W. of Tilley Swamp	SJ 54-2 282505	13 & 14.ix.1971	J.D.R.	13 W, 6 S
5. 2.7 km S.E. of Kingston	SJ 54-2	12.ix.1971	J.D.R.	17 S

Table 4.1 Recording localities in the Coorong region , South Australia. Map and grid references are for Edition 1, series R 502 maps (scale 1:250,000, Department of National Development Canberra). W, western call race; S, southern call race: ?H, possible hybrid. The position of major localities referred to in this table is given on Figure 4.1. The position of sites 1 to 5 is given exactly on Figure 4.2.

sample which I will refer to as from Magrath Flat. Data from stations 3 and 4 (Table 4.1) will be referred to as coming from Petherick Road. This sample is the only one from the area where the southern and western call races are sympatric.

Calls were recorded and analysed as in Chapter 2 with the two call races treated separately. Statistical analyses were also as in Chapter 2 except that note duration in the western call race was plotted against water temperature and examined visually.

For the western call race, samples from Petherick Road and Magrath Flat were compared with each other and with a western reference sample formed by combining data from Gawler and Murray Bridge (data summaries etc. for the latter two sites, see Chapter 2). Thus, there is one sample from sympatry (Petherick Road), and two from allopatry. Magrath Flat is relatively close to the overlap zone while the western reference sample is from deep allopatry.

A similar procedure was adopted for the southern call race. I compared samples from sympatry (Petherick Road), close allopatry (Kingston) and deep allopatry (Mt. Hope Drain, see Table 2.2).

Calls were recorded from one frog at locality 3, Table 4.1, which could not be unequivocally scored as either western or southern and which were not from any other anuran species occurring there. These calls are presumably from a hybrid as they show characteristics of both call races. Details of their structure are appended to Tables 4.2 and 4.3.

Summaries of call component values for both call races are given in Tables 4.2 and 4.3 and are illustrated in Figures 4.3, 4.4 and 4.5. Results of anova on individual call components and analysis of co-variance for note repetition rate data from the western call race are given in Table 4.4. Similar information for the southern call race is given in Table 4.5.

Sample	n	Dominant Frequency (Hz)	Pulses per Second	Note Duration (ms)	Notes per Call
Western reference	37	1367 (1105 - 1654)	283.24 (118.0 - 801.0)	25.25 (15.57 - 45.74)	4.52 (3 - 8)
Magrath Flat	20	1292 (1073 - 1521)	255.08 (142.0 - 454.9)	26.76 (22.21 - 33.32)	4.66 (3 - 7)
Petherick Road	15	1455 (1189 - 1646)	248.51 (169.1 - 343.3)	21.11 (17.53 - 24.40)	4.44 (4 - 7)
Mount Hope Drain	17	1940 (1845 - 2160)	-----	12.79 (9.61 - 15.48)	1
Kingston	17	1785 (1005 - 1953)	-----	12.26 (9.17 - 13.81)	1
Petherick Road	6	1777 (1717 - 1841)	-----	12.45 (8.67 - 14.81)	1
Possible Hybrid	1	1501	254.74	19.90	1.80 (1 - 2)

Table 4.2 Sample sizes (n) and means and ranges (in brackets) for analysed call components in southern and western call samples from the Coorong area and in allopatric reference samples. Western reference formed from Gawler and Murray Bridge data (Table 2.2); Magrath Flat, data from sites 2 and 3, Table 4.1; Petherick Road, sites 3 and 4, Table 4.1. Means were compared with the Student- Newman-Keuls test using a 5% significance level. This test detected no differences among means except in western dominant frequencies where all means differed significantly (but see also Table 4.5 where heterogeneity of southern dominant frequencies is indicated).

	Sample	Slope	Standard error of slope	Intercept	Temperature range (°C)
(1)	Western Reference	0.8474	0.0271	1.7748	6.0 - 22.3
	Magrath Flat	0.4075	0.2312	8.1902	12.4 - 17.0
	Petherick Road	0.9160	0.1986	0.3756	15.0 - 18.4
	Mt. Hope Drain	-	-	-	11.0 - 16.0
	Kingston	-	-	-	12.0 - 15.6
	Petherick Road	-	-	-	13.2 - 17.0
(2)	Hybrid	Notes per second, 9.19 at 16.0°C.			

Table 4.3 (1) Parameters defining regression lines fitted to note repetition rate, water temperature data for western call race samples and temperature ranges for all data. (2) Note repetition rate data for the putative hybrid. Expected notes per second values for the Petherick Road and Western Reference lines respectively are 15.03 and 13.56.

(a) Dominant Frequency

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	2	226583.2	113291.60	7.512 **
Within "	69	1040656.5	15081.98	

(b) Pulses per Second

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	2	17628.02	8814.01	0.773 n.s.
Within "	69	786508.22	11398.67	

Kruksal-Wallis H = 0.56 n.s.

(c) Notes per Call

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	2	0.4457	0.2229	0.3942 n.s.
Within "	69	39.0121	0.5654	

(d) Note Repetition Rate on Water Temperature

(i) Comparison of slopes

Variation	d.f.	Sum of Squares	Mean Square	F
Among regression coefficients	2	3.7057	1.8529	2.5185 n.s.
Deviations from regression	66	48.5536	0.7357	

(ii) Comparison of elevations

Variation	d.f.	Sum of Squares	Mean Square	F
Among intercepts	2	0.5706	0.2853	0.3712 n.s.
Deviations from pooled estimate of regression	68	52.2593	0.7685	

Table 4.4 (a) to (c), interlocality anova of various call components for western call race frogs on the Coorong. (d) Comparisons of slopes and elevations of regression lines fitted to note repetition rate, water temperature data for the same frogs. For (b) Kruksal - Wallis H also given as Bartlett's test indicated significant differences in sample variances (Chi^2 10.3, p less than .01). n.s. not significant, ** p less than .01.

(a) Dominant Frequency

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	2	241605.1	120802.55	5.248 *
Within "	37	851688.5	23018.61	

Kruksal-Wallis H = 18.92 ***

(b) Note Duration

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	2	2.374	1.187	0.4026 n.s.
Within "	37	109.097	2.949	

Table 4.5 Interlocality anova of southern call race components for frogs recorded on the Coorong. In (a) Kruksal-Wallis H also given as Bartlett's test indicated significant heterogeneity of sample variances (Chi^2 = 20.7 **). n.s. not significant, * p less than .05, ** p less than .01, *** p less than .001.

In the western call race notes/call, pulses/second and notes/second did not differ significantly between the three samples compared. Note duration data from Magrath Flat and sympatry fit well within the range found in the western reference sample (Figure 4.4). Dominant frequency data differ significantly between the three areas with western call race frogs sympatric with the southern call race having highest values (Table 4.2; Figure 4.3).

The cause of this pattern of variation in western dominant frequencies is unclear though it is clearly irrelevant to the reinforcement hypothesis as in sympatry dominant frequencies of the two races are more similar than anywhere else.

In the southern call race the only variation of significance was in dominant frequency where mean dominant frequencies were heterogeneous (Table 4.5). This was caused by the high mean at Mt. Hope Drain (Table 4.2; Figure 4.3). Again, this cannot be related to reinforcement and the causes will be discussed elsewhere (section 5.2 1)).

b) Breeding and calling seasons.

i) Field data.

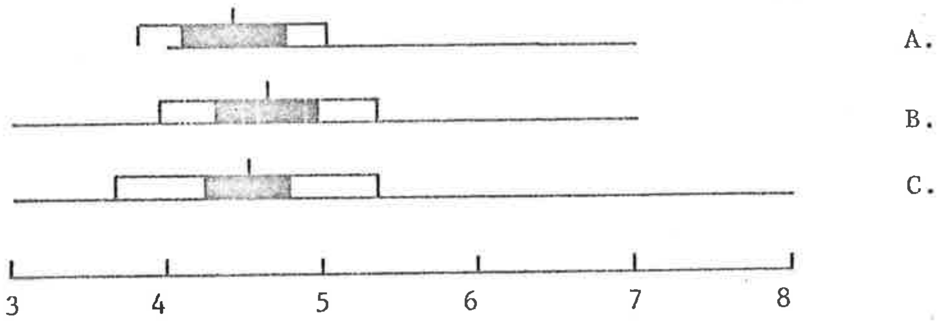
Observations on calling and breeding activity were made in the area of sympatry, in pure western populations north of the overlap and in pure southern populations south and east of the overlap. Observations were made at about monthly intervals during the winter and spring of 1972 and 1973. In summer and autumn when swamps were dry visits were made at longer intervals. On each occasion data were collected as follows.

In a single night a transect was made from Meningie to Kingston and notes made on calling activity at at least five sites: Magrath Flat; Banff (the northern limit of sympatry); Petherick Road; 24.5

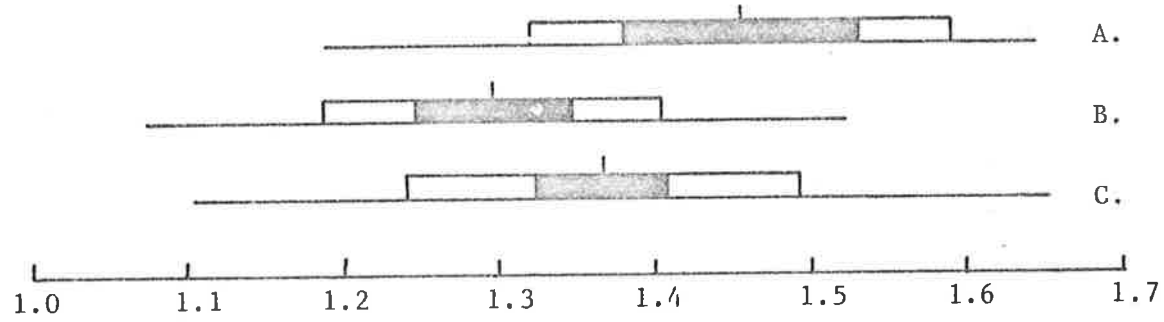
Figure 4.3 Summary (as in Figure 2.4) of various call component values in southern and western call race populations on the Coorong. Sites are: A. Petherick Road; B. Magrath Flat; C. Western reference; D. Kingston; E. Mount Hope Drain. See tables 4.1 and 2.2 and text for sample details.

WESTERN

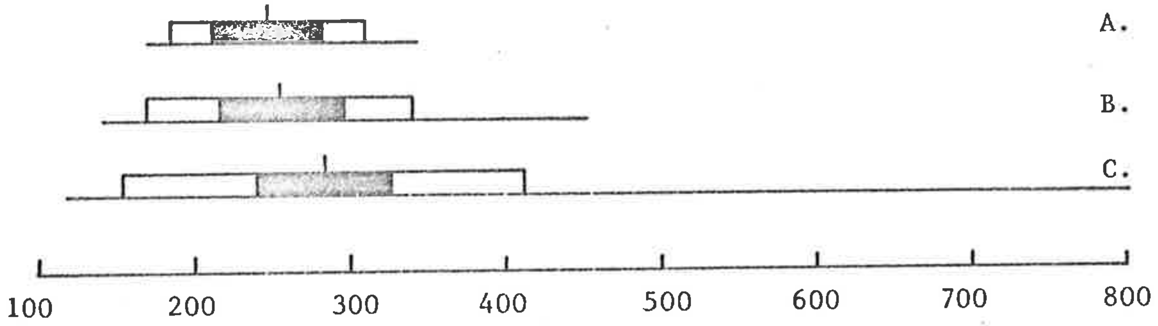
Average Notes/Call



Dominant Frequency (kHz)

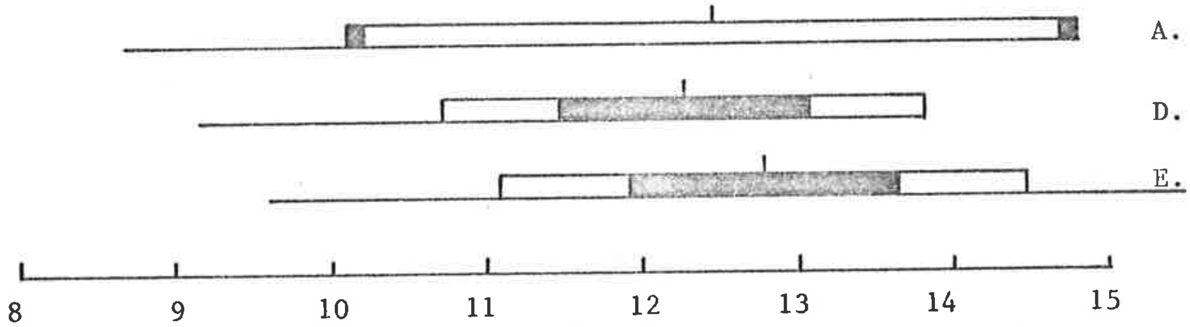


Pulses per Second

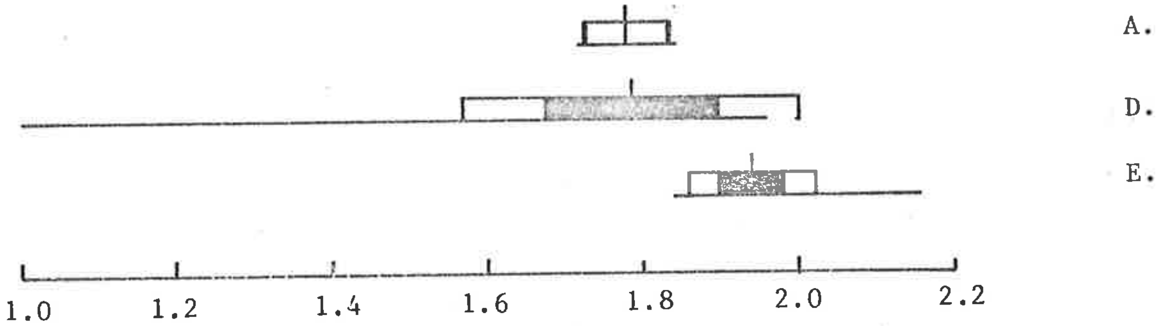


SOUTHERN

Note Duration (ms)



Dominant Frequency (kHz)



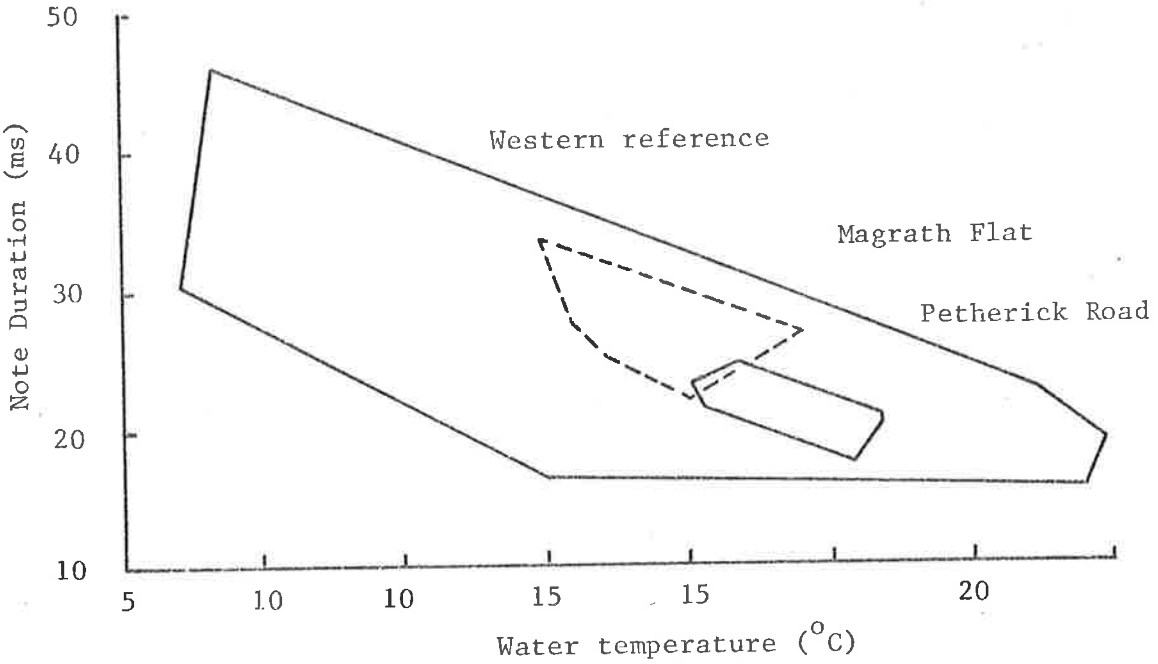


Figure 4.4 Plot of note duration against water temperature for western call race frogs from the Coorong and reference sample. Data are presented as maximum area polygons containing all points for any sample

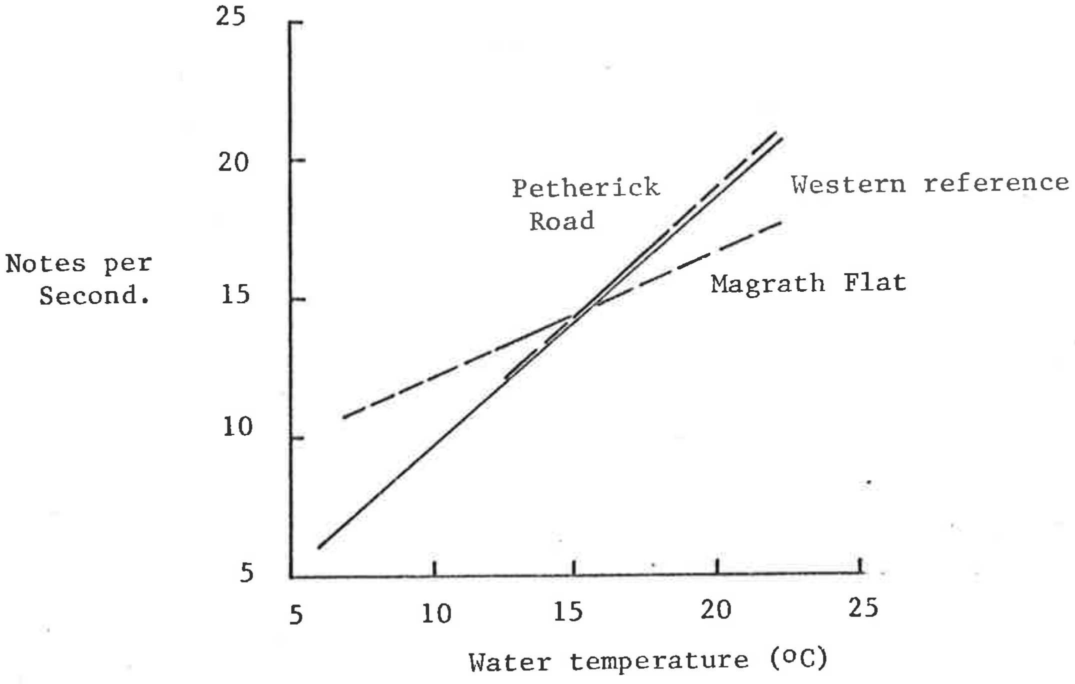


Figure 4.5 Fitted regression lines for note repetition rate verses water temperature plots for western call race frogs from the Coorong and the western reference sample. Solid lines cover the range of water temperatures in the sample and dashed lines are projections.

km north of Kingston (the southern limit of sympatry) and Kingston. As short visits at various stages of the evening may miss rare calls or temporal shifts in calling activity, observation over a twenty-four-hour period was done four times at Petherick Road (i.e. about mid-sympatry).

Calling activity data were placed into one of four categories which very probably reflect the numbers of males calling, though I have no proof of this. The four classes were; 0, no frogs calling; 1, very few frogs calling; 2, many frogs calling; 3, very many frogs calling. Occurrence of breeding was determined by daylight searches for egg masses on the day preceding or following the night transect.

Calling and breeding data for five Coorong sites are given in Figure 4.6. Virtually nothing happened at Magrath Flat, Banff or Petherick Road during 1972 and 1973. At Kingston and the southern end of sympatry calling and breeding may start as early as July, though this is not inevitably so. Western calls have been heard in July at Magrath Flat but I have no data on the commencement of breeding there. In other western populations breeding does not start until mid to late August (see Murray Bridge data appended to Figure 4.6; see also section 4.4 1) g)).

The twenty-four-hour observations at Petherick Road were fruitless, with significant activity occurring only once, in July 1973. On that occasion southern frogs called throughout the night and during the next day. Some observations were also made when field recording at Petherick Road in September 1971. A few western calls were heard in late afternoon but most frogs started calling about sunset. The first southern calls were heard soon after sunset. Both races called continuously for at least six hours.

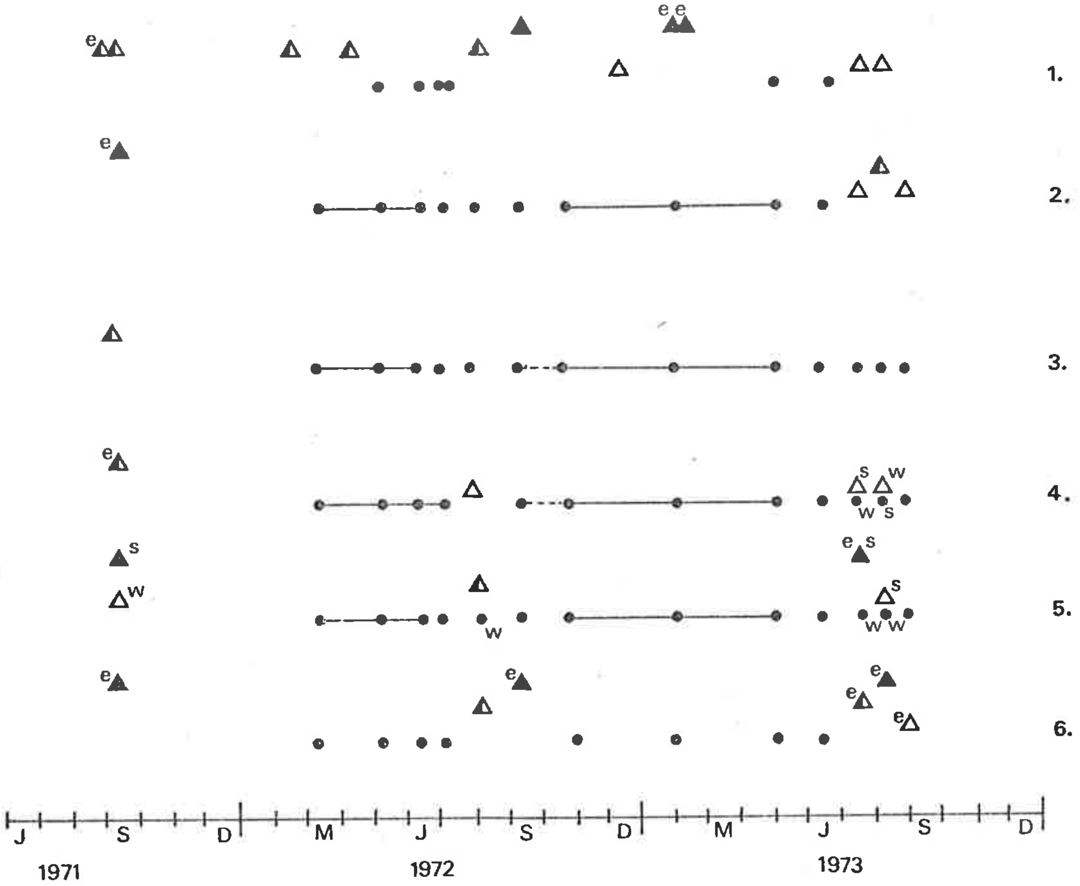


Figure 4.6 Calling activity of L.tasmaniensis on the Coorong. Solid circle, open triangle, half-closed triangle and closed triangle indicate calling levels 0 to 3 respectively (see text). S or W to the right of a symbol means it refers to the southern or western call race only. e to the left of a symbol means at least one egg mass was seen. No observations were made in the period October 1971 to February 1972 as a broken leg inhibited field work. Sites are, 1., Murray Bridge; 2., Magrath Flat; 3., Banff; 4.,Petherick Road; 5.,southern end of sympatry; 6., Kingston (see Figures 4.1, 4.2 for sites). Murray Bridge is not on the Coorong but these data are included to indicate normal activity in western call race populations.

ii) Laboratory data.

Calling activity and breeding data were also collected from groups of frogs of both call races held (call races separate) in cages in Adelaide. The cages were in the open, had galvanised iron walls and were 1.2 m square and 0.9 m deep. The floor was covered with sand 10 to 15 cm deep. Water was provided in a plastic tray 55 by 30 by 10 cm deep and by an overhead (relative to the frogs) spraying system. The sprayers were only used during summer to ensure the frogs did not die of desiccation. Cages were checked daily for the presence of eggs and observations on calling activity were made on two or three nights per week. Laboratory data were collected from May 1972 to February 1973.

No calls were heard, or eggs produced, in May or June. Southern calls were first heard on 17 July 1972, and an egg mass appeared was present two days later. Initial western calls were heard about one month later on 14 August, 1972 and the first egg mass produced on 4 September, 1972. Last egg masses were found on 30.x.72 and 12.xii.72 for southern and western frogs respectively and last calls heard 7.xii.72 and 8.xii.72 again for southern and western frogs respectively.

Southern frogs used in these laboratory cages came from Kingston and western from the Mount Lofty ranges or the Adelaide plains. In the southern call race there is a close correlation between field and laboratory data.

Differences in breeding season might act as potent premating isolating mechanisms. However, although the southern call race may start breeding slightly earlier than the western, there is still a very broad overlap of the periods when the two races reproduce. In the absence of good data for western frogs in sympatry I cannot say whether reinforcement has effected calling or breeding seasons in either call race.

c) Calling sites used by calling males.

It is obvious that if males of the southern and western call races use very different calling sites, e.g. one on land and the other in open water, and if females will only mate with males calling from appropriate locations, then male call site can act as a premating isolating mechanism.

All frogs recorded on the Coorong were calling in water, or if they were not seen could be reasonably inferred to be in water. Males of both call races call in a similar position with the head and inflated vocal sac out of the water but with the rest of the body submerged (cf. Tyler 1971b). However, the specific sites used by the two races were generally quite different.

Calling southern males were usually in the open clear of any cover provided by emergent or overhanging vegetation. At Kingston all recorded males were in an open area of water where the stems of flooded grass had bent or broken and were lying flat on the water surface. The pond margins were flooded unbroken grass which provided dense cover for the other anuran species calling in the area. At Kingston, L.tasmaniensis did not call in such sites. At Magrath Flat, where only the western call race occurs, frogs recorded were hidden deep in grass at the pond edge or in other flooded vegetation. Other frog species calling there were using similar sites.

At Petherick Road corner a similar situation ensued with western males calling from dense cover and southern males much further from the pond margin in exposed sites. These differences are reflected in the number of frogs sighted at some stage of the recording process; either during placement of the microphone or during its removal (Table 4.6).

Southern frogs in other areas used similar call sites. Fifteen of seventeen males recorded at Mt. Hope Drain and eight of nine at Moyston were seen. Littlejohn and Martin (1974) reported that in

	Seen	Not Seen	Total
Magrath Flat	0	15	15*
Petherick Road (Western)	2	13	15
Petherick Road (Southern)	2	4	6
Kingston	7	10	17

* No data for five frogs recorded by M.J.Littlejohn

Table 4.6 Numbers of frogs seen during some stage of the recording process.

W male, W female	S male, W female	W male, S female	S male S female
4	3	0	0

Table 4.7 Numbers of fertilised egg masses found in cages containing frogs as indicated. Data were collected from September 1973 to March 1974.

Tasmania, L.tasmaniensis eggs are found in open water, suggesting males use similar calling sites there. Western males at Murray Bridge were well concealed and none that I recorded were was seen. However, at Gawler, where little cover was available, only four of twenty frogs recorded were not seen.

Thus, this character may contribute to the maintenance of premating isolation between the southern and western call races. The differences in call site are not confined to the area of sympatry and indeed seem characteristic of both races throughout their respective ranges. Therefore, there is no justification for saying reinforcement has effected this character.

d) Amplexus position and behaviour in amplexus.

Differences between call races in amplexus position, e.g. axillary verses inguinal, or behaviour once in amplexus, may result in breakup of an amplexed pair. This might be voluntary because one or both partners received inadequate or inappropriate stimuli from the other or mechanical if one call race had a complicated and the other a very simple behaviour in amplexus (e.g. compare egg deposition in Pseudacris triseriata and Hymenochirus boettgeri described in Rabb 1973). If pair dissolution occurred before gamete release, then the differences in amplexus position or behaviour could act as premating isolating mechanisms.

No field observations of amplexus were made for either call race in the area of sympatry.

In laboratory cages both had an inguinal amplexus as has been reported for eastern Australian L.tasmaniensis (Fletcher 1889). Southern call race males can successfully mate with western females, though whether the converse applies is not known (Table 4.7). Southern females did not oviposit in the control cage, and accordingly I attach no significance to the absence of eggs in the western male, southern female combination. No unfertilised egg masses were found in any of the cages.

Frogs used in the experiments reported in Table 4.7 were not from sites where the southern and western call races are sympatric. I had extreme difficulty in catching frogs of either call race in the overlap area. I cannot say anything about amplexus and associated behaviour in sympatric populations of southern and western L.tasmaniensis.

2) Postmating isolating mechanisms.

Reinforcement, as I conceive it, results when both interacting taxa waste gametes in hybrid matings. The gamete wastage may be because such matings do not produce zygotes, e.g. a mechanical difference may mean eggs are not fertilised, or because hybrids are relatively inviable, e.g. infertile or unable to attract mates.

Artificial hybridisation has been widely used in anuran studies to determine degrees of genetic divergence and relationships between species (e.g. W.F. Blair 1972). In investigations of naturally hybridising taxa, degrees or forms of inviability determined in the laboratory generally agree with those observed in the field (Volpe 1960; Watson 1972).

I investigated several avenues which might have shown whether hybrid inviability existed between the southern and western call races of L.tasmaniensis. I scored the frequency of morphologically abnormal embryoes in, hybrid egg masses and fertilised eggs from artificial hybridisations. I also looked at success and time to metamorphosis of hybrid larvae. One cross of a known hybrid male was also conducted.

As noted in section 4.2 1) d) I did not make field observations of amplexus or oviposition in either call race. However, the two races do exhibit differences in morphology and egg mass form which might bear on whether hybrid matings will work. This will be discussed after I have dealt with my hybridisation studies.

a) Hybridisation and hybrid viability.

i) Artificial hybridisations.

Artificial hybridisations were done by the methods outlined in Hamburger (1960). Males were killed and their testes macerated in a small amount of 10% Holtfreter's solution. Eggs were taken from an ovulating female, either by stripping or by killing the frog and taking the eggs direct from the oviduct, and placed in the sperm suspension. Ovulating females were taken from amplexed pairs found in laboratory cages (amplectant pairs were rarely encountered in the field), or ovulation was induced by injection of macerated whole pituitaries from conspecific or Bufo marinus females.

Eggs were raised in full strength Holtfreter's solution in 9 cm plastic petri dishes (circa 1 egg ml^{-1}), or individual eggs were put in the cells of plastic haemagglutination blocks (volume of a single cell is about 1 ml).

Embryoes were checked regularly for the appearance of abnormalities until just after hatching. It was impossible to determine whether eggs had rotated or not and consequently only eggs which went through initial cleavage were studied. In most crosses some eggs did not obviously cleave but underwent other gross morphological changes. These changes could occur in eggs not exposed to sperm and such eggs were therefore excluded from my analyses.

In any cross some eggs from a single female were fertilised by sperm from a male of the same call type, generally from the same locality, and some by sperm from one or more males of the alternate call type.

The single cross attempted with a known hybrid was conducted as above.

Frequencies of abnormal embryoes, etc. resulting from artificial hybridisations between the southern and western call races are given in Table 4.8(a)(i). Southern females were never stimulated to ovulate and so no crosses of southern females to western males were conducted. These data give no evidence of hybrid inviability.

A control and hybrid male from cross (1), Table 4.8(a)(i) were artifically crossed to a western female collected in amplexus near Gawler, S.A. This female was also crossed to her amplectant male. Results are given in Table 4.8(a)(ii). The F_1 hybrid male was fertile and produced the same frequency of abnormal embryoes as both control males.

ii) Pseudo-natural hybrid matings.

The frequency of abnormal embryoes and other non-developing eggs was also scored in hybrid egg masses resulting from what I will henceforth refer to as, pseudo-natural matings.

Cages similar to those secribed in section 4.2 1) b) were set up containing (a) Male and female western L.tasmaniensis, (b) southern males and females, (c) southern males and western females and (d) southern females and western males. In cages (c) and (d) any fertilized eggs produced had to result from hybrid matings; the other cages acted as controls.

Pseudo-natural hybridisation results are given in Table 4.8(b). No eggs were ever produced in any cages containing southern females and consequently the expected frequency of abnormalities in pure southern egg masses is unknown. Lacking these data it is impossible to determine the cause of the lowered frequency of normal embryoes in southern male, western female hybrid egg masses. These results may indicate significant hybrid inviability but equally may represent a level of hybrid debility intermediate between that found in pure western and pure southern egg masses produced under these conditions.

iii) Success and time to metamorphosis.

These parameters were scored in tadpoles (controls and hybrids)

(a) Artificial Hybridisations

(i)

Female	Male crossed to	
Western	Western	Southern
1.	95.83 (24)	96.00 (25)
2.	44.44 (9)	27.27 (11)
3.	94.12 (51)	95.83 (48)
4.	71.43 (7)	83.33 (18)

(ii) Backcross, southern/western hybrid to western female.

Amplectant male	Control male	Hybrid male
68.25 (63)	66.66 (30)	67.39 (46)

(b) Pseudo-natural hybridisations.

Western male, western female	Southern male, western female
55.55 (117)	63.38 (71)
90.56 (286)	57.5 (40)
83.89 (329)	73.33 (240)
97.02 (335)	

Table 4.8 Results of various hybrid combinations of the southern and western call races. Data given are the percentage of eggs developing into morphologically normal larvae. Number in brackets is the base for the percentage. In (a) (ii), the female was collected in amplexus in the field. Control male was from the control cross of the artificial hybridisation which produced the hybrid male.

resulting from one artificial hybridisation, in the backcross of a known F_1 hybrid and in several groups of hybrid and pure western tadpoles from pseudo-natural hybridisations. All tadpoles from artificial hybridisations were raised but only fifteen tadpoles per egg mass for pseudo-natural matings.

All tadpoles were raised similarly in aged tap water in plastic trays (30 x 50 x 10 cm deep, water depth about 8 cm). They were fed boiled lettuce and flakes of high protein breakfast cereal (Kellogg's Special K; Littlejohn, Watson and Loftus-Hills 1971). Tadpoles from artificial hybridisation were raised at room temperatures and under natural lighting, whilst others were raised in heated rooms (20 - 25°C) and generally under artificial illumination.

Western call race frogs used in all crosses (artificial and pseudo-natural) came from localities near Adelaide either in the Mt. Lofty ranges or on the Adelaide plains. Southern frogs were from near Kingston or from the Mia-Mia, 2 km east of Tilley Swamp (Figure 4.1).

Data on success to metamorphosis, etc. are given in Tables 4.9 to 4.11. In artificial matings both F_1 and backcross hybrids are as likely to metamorphose as pure western tadpoles from control crosses (Table 4.9). However, in pseudo-natural matings hybrids did not complete metamorphosis or transformed into abnormal frogs (Table 4.9). F_1 and backcross hybrids from artificial matings took the same time to reach metamorphosis as western controls (Table 4.10 (1)) but in pseudo-natural matings hybrids took longer though they were larger (Table 4.11).

The only clear evidence of hybrid inviability comes from the metamorphic failure of pseudo-natural hybrids, but this is contradicted by the data from artificial crosses. Presumably, the particular environment of pseudo-natural hybrids (eggs laid in the open in galvanised iron cages was the major difference, section 4.2 1) b)) contained some factor which produced the abnormalities. I cannot say whether

	Died before metamorphosis	Metamorphosed
(1) Artificial hybridisations		
(i) Pure call race cross		
Control	6	17 (0)
Experimental (S male, W female)	9	15 (0)
	$\text{Chi}^2 = 0.704$ n.s.	
(ii) Backcross		
Control	13	18 (0)
Experimental S/W male, W female	6	14 (0)
(2) Pseudo-natural hybridisations.		
W male, W female	5	10 (0)
S male, W female	4	11 (10)
S male, W female	3	12 (12)

Table 4.9 Numbers of tadpoles resorbing the tail in various hybrid combinations of the southern and western call races. Brackets contain the number of frogs which were abnormal or died during metamorphosis. Death was usually caused by incomplete eruption of the fore-limbs. (W western, S southern, n.s. not significant)

	Mean	S.E.	N	
(1) Artificial hybridisations				
i. Pure call race cross				
W male, W female	206.8	8.45	17	$t_{30} = 1.12$ n.s.
S male, W female	225.5	15.21	15	
ii. Backcross				
W male, W female	195.5	6.72	14	$t_{30} = 0.91$ n.s.
S/W male, W female	183.5	10.43	18	
(2) Pseudo-natural hybridisations				
W male, W female	141.7]	3.88	10	
S male, W female	162.3 ⎤	3.77	11	$F_{2,30} = 8.03$ **
S male, W female	162.5 ⎦	4.48	12	

Table 4.10 Time (days) to metamorphosis for tadpoles from various hybrid combinations of the western and southern call races. Square brackets contain means not significantly different at 5% level as determined by the Student-Newman-Keuls test. S southern, W western, n.s. not significant, ** p less than .01, N sample size.

Pseudo-natural hybridisations

	Mean	S.E.	N	
W male, W female	18.32]	0.314	10	
S male, W female	20.23 ⎤	0.491	11	$F_{2,30} = 5.8$ **
S male, W female	20.11 ⎦	0.447	12	

Table 4.11 Snout vent lengths at metamorphosis (mm) of frogs resulting from pseudo-natural hybridisations. Symbols etc. as in Table 4.10.

this factor occurs in nature but I do know that susceptibility is not confined to southern, western hybrids. Pure northern tadpoles raised from eggs laid in the pseudo-natural hybridisation cages also had difficulty in metamorphosing.

I cannot resolve the contradiction between the results of the two sorts of hybridisation but the failure of pure northern tadpoles inclines me to the view that the inviability of pseudo-natural metamorphlings is essentially an artefact. This view is supported by Murphy (1965) who observed similar abnormalities in metamorphosing Rana palustris. He found that high frequencies of abnormalities were correlated with heavy infestations of spiruroid nematode larvae. Murphy did not establish that the presence of nematodes caused the abnormalities but suggested this might be so. Murphy also offered another unproven explanation; that the abnormalities were the result of some particular genetic constitution. Murphy's first theory complements my inference about southern, western call race hybrids: nematode infestations could constitute an intermittently-acting environmental component.

b) Behaviour in amplexus and egg mass form.

Variations in egg mass form may result from morphological or behavioural differences between the frogs producing them (Rabb 1973). If two taxa have different behaviours in amplexus e.g. in the timing of, or the position held when eggs and sperm are released then none or only a portion of the eggs laid by hybrid pairs might be fertilised. There would thus be gamete wastage by both individuals.

Fletcher (1889), Harrison (1922), Parker (1940) and A.A. Martin (1967) all describe the spawn of L.tasmaniensis similarly, "...white frothy looking more or less circular patches, ... deposited in the water;" (Fletcher 1889).

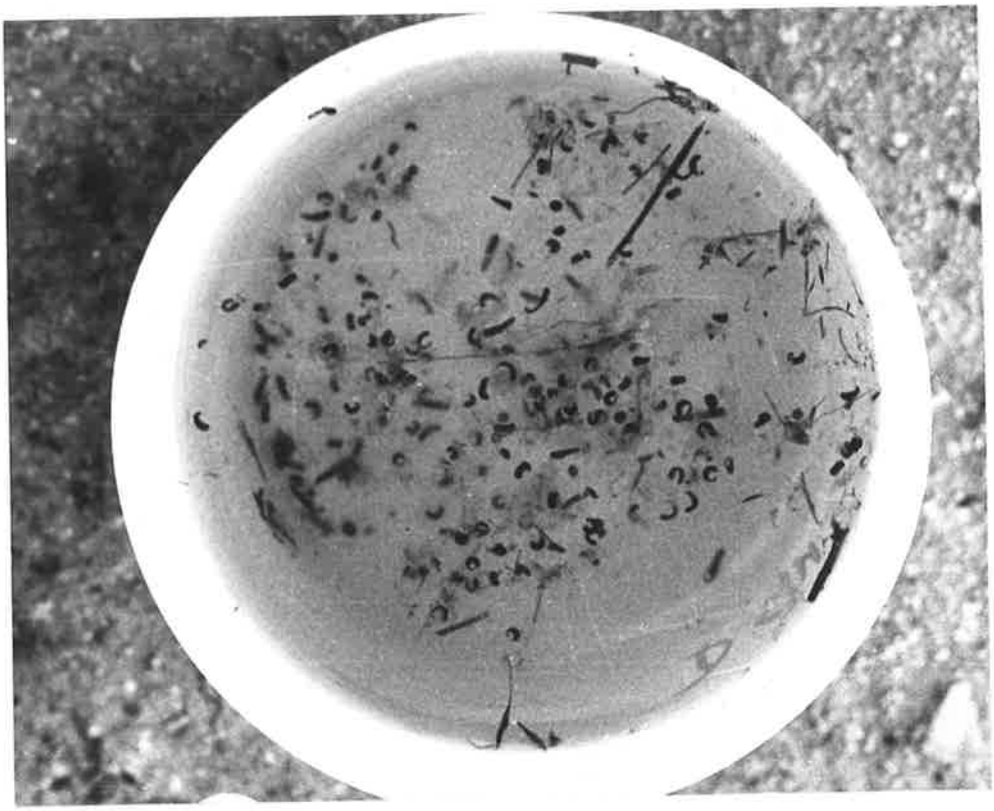
Egg masses seen at call sites of western males at Magrath Flat and Petherick Road in 1971 fit this description, as does the spawn produced in laboratory cages populated by western call race frogs. Eggs produced by southern call race frogs in laboratory cages were in a non-frothy, jellied egg mass (Figure 4.7). Similar spawn was found in areas where dense choruses of southern males were heard in the field. Namely, at Kingston; 17 km W.N.W. and 9 km S.W. of Millicent; in the Tilley Swamp 4.1 km N.E. of Banff and at the southern end of sympatry, 24.0 km N.N.E. of Kingston. Tadpoles from such egg masses, collected at Kingston, transformed into L.tasmaniensis proving the laboratory produced masses were not abnormal (cf. Fletcher (1889) who reported frothy spawn in the field but non-frothy when eggs were deposited in the laboratory).

Southern females collected at and near Kingston generally lacked flanges on the first two fingers (Table 4.12; Figure 4.8). This alone may be the cause of non-frothy egg masses in the southern call race as Parker (1940) asserted that flanges on the fingers of female L.tasmaniensis improved the efficiency of froth production. However, I have not observed the southern call race depositing eggs in the field. It may be that in south-eastern South Australia behaviour in amplexus of southern L.tasmaniensis is very different from that in the western call race.

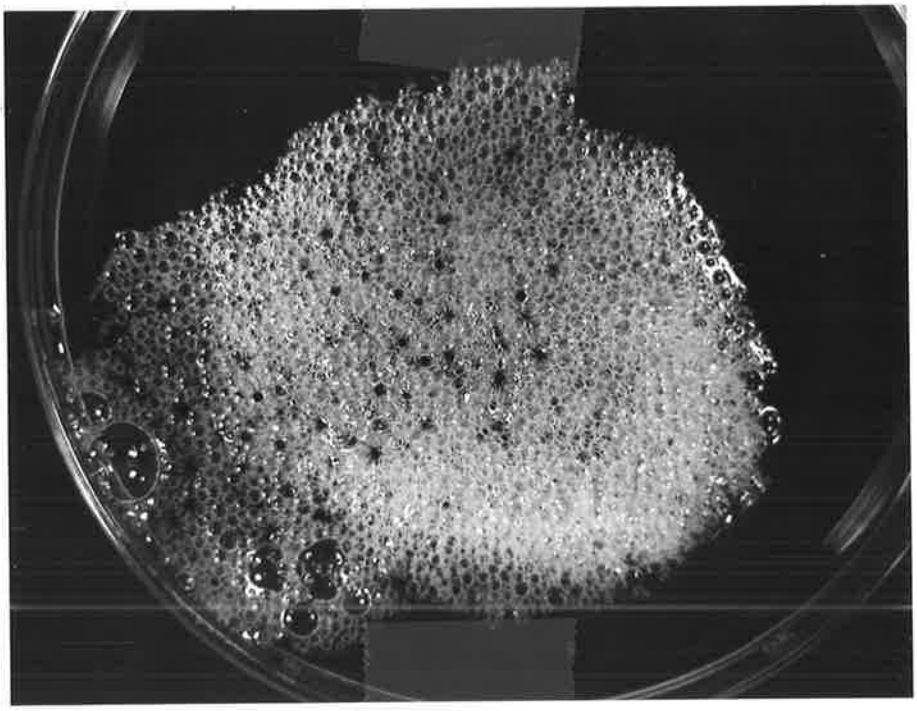
The results of pseudo-natural hybridisations suggest that this is not so. Southern males can successfully fertilise eggs from western females, though I do not know whether the converse applied (Table 4.7).

I would be foolish to conclude from these observations that the very obvious differences between southern and western egg masses is likely to cause gamete wastage in hybrid matings. Thus, again, I have no specific evidence of postmating isolation between the southern and western call races.

Figure 4.7 Egg masses of the southern (a) and western (b) call races both at about 4/5 natural size.



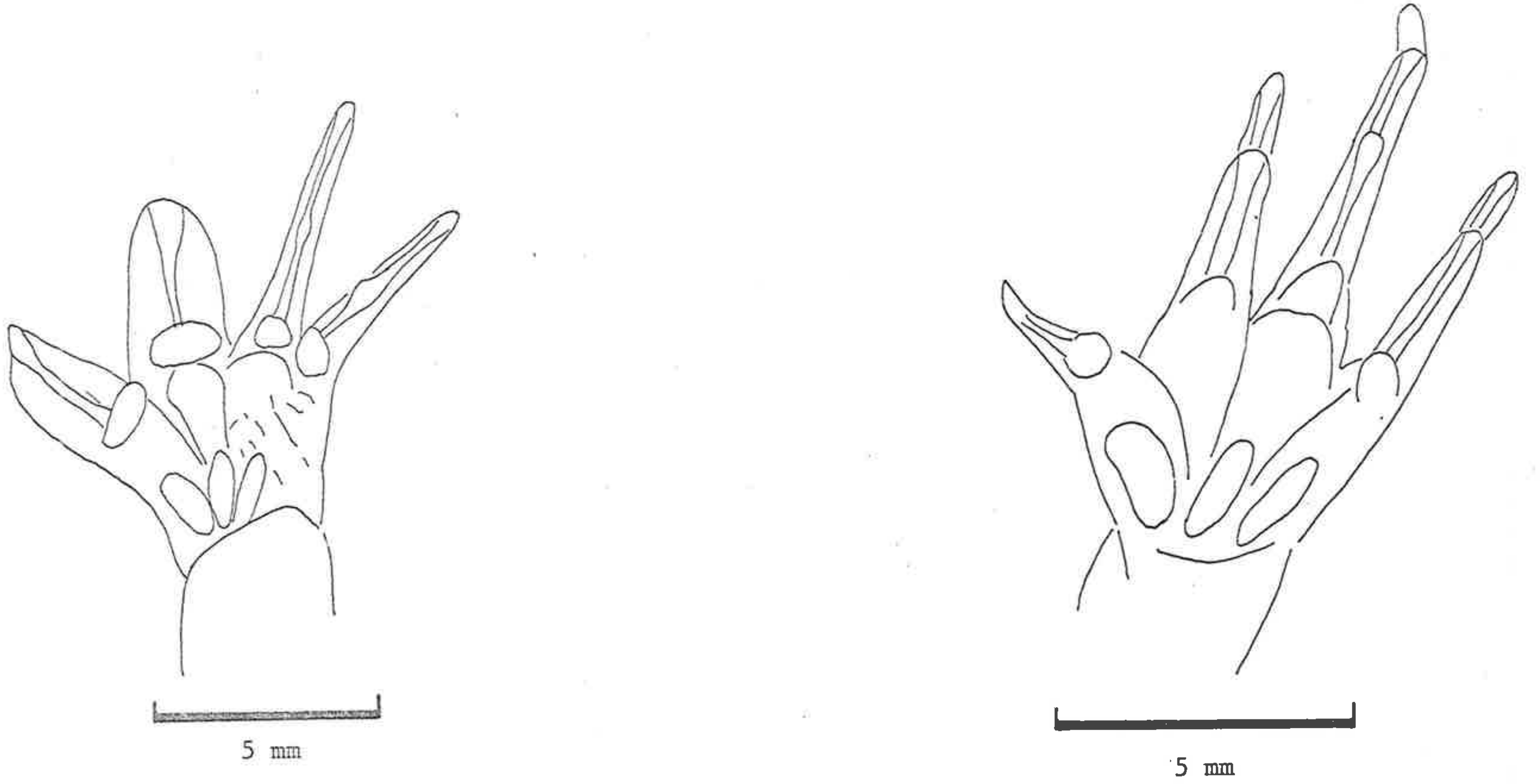
(a) Southern egg mass from Kingston, South Australia.



(b) Western egg mass from Dutton, South Australia.

Locality	No Flange	Slight Flange	Well Developed Flange	Total
Southern				
16 - 32 km S.E. of Kingston	16	1	0	17
2.7 km S.E. of Kingston	5	0	1	6
Mingbool (S.A. Museum collection, Reg. No. 5072)	24	4	2	30
Western				
3.5 km N. of Dutton	0	0	8	8
3.3 km N. of Mount Mary	0	0	9	9

Table 4.12 Numbers of southern and western females showing various degrees of finger flange development. Frogs other than in the S.A. Museum collection were scored immediately after capture but were then used in experiments and were not necessarily preserved for future reference.



(a) Left hand of western female from 3.3 km north of Mount Mary.

(b) Left hand of southern female from south of Kingston.

Figure 4.8 Flanged (a) and non-flanged (b) fingers of female <u>L.tasmaniensis</u>. Both females were collected during the breeding season.

3) Geographic distribution and limits on the ranges of the southern and western call races.

a) Distribution.

Figure 4.2 gives detailed data on the distribution of both call races along the Coorong. Data were collected from 1971 to 1975 on night traverses during the late winter and spring.

The western call race was not detected in the swamp system running north, south through Tilley Swamp or elsewhere, except in swamps on the eastern edge of the Coorong lagoon (Figures 4.1 and 4.2). I have one report of western L.tasmaniensis from the Younghusband peninsula, opposite Magrath Flat (W.Jones, personal communication). Although this record is probably correct, I have not included it on Figure 4.2 as I have no way of verifying it. North of Banff and Salt Creek fresh water swamps are rare and generally small. This is reflected in the paucity of L.tasmaniensis records in that area. This lack of data is not due to inadequate sampling as on night traverses in September 1971 stops were made every 6 - 10 km from Magrath Flat to Kingston. Of the three years when I worked intensively on the Coorong, 1971 was the wettest (Table 4.13) and probably the most favourable for collecting distribution data.

This sort of distribution may result in western L.tasmaniensis in the area of sympatry being isolated from possible gene flow from the north and thus constitute a situation where reinforcement is particularly likely (Littlejohn and Watson 1973; Hall and Selander 1973).

b) Limits on ranges, tadpole transplant experiments.

The total area of sympatry is small relative to the known distributions of the pure call races. This may be a consequence of some subtle environmental effect; may result from some interaction between the races (Jaeger 1970, 1971a, 1971b, 1972; Smyth 1973) or may be because the western and southern call types have only recently contacted. Walker (1974) stated that recent overlaps between closely related forms were areas where reinforcement might most profitably be sought. From his experience with soniferous arthropods, Walker felt that biased geneflow out of the region of sympatry rapidly obscured differences produced by reinforcement.

In an attempt to understand the causes of the small area of sympatry I planned to transplant western tadpoles south and east of their present range and southern tadpoles north. Only the latter half of this experiment was done as western tadpoles from appropriate localities were not available.

The experiment involved a simple transplant of hatched southern tadpoles from Kingston to two points north of the known range of this call type in South Australia. Two control cages were at Kingston and two experimental cages at each of Woods Well and Magrath Flat.

The rationale for this design is as follows. If tadpoles survived and grew at Woods Well and Magrath Flat I would conclude there was no severe environmental bar to the survival of the southern call race at either site. I would then have to look for other explanations of the northern limit of the range of the southern call race. Alternatively if tadpoles did not grow I would conclude there was some bar to development, e.g. the absence of a specific compound required for growth (cf. Neauport 1973). This latter conclusion should be qualified as failure might also be produced by inadequate experimental technique, e.g. poor cage design, too high a density of transplanted tadpoles. The control cages at Kingston were guards against these sorts of possibilities.

The cages were a rectangular, fibreglass fly-wire bag, 137 x 107 x 46 cm deep. This sac was supported by a plastic coated wire mesh frame. The whole set-up, bag plus support was covered with a close fitting fly-wire lid. The fly wire mesh was square and 0.16 mm on a side. Cages were weighted down with pieces of brick and stones from the ponds they were placed in. Each cage was placed in water 25 - 30 cm deep and thus contained about 450 l of water. 100 tadpoles were put in each cage giving a tadpole density of .22 tadpoles per litre.

This density approximates that estimated for hatchling southern call race tadpoles in a shallow, rectangular pool at Kingston. On July 20, 1973 all (i.e. 17) the L.tasmaniensis egg masses in the pool were collected. The volume of water in the pool was estimated by taking length, breadth and several depth measurements. The egg masses were brought to Adelaide and the number of live tadpoles was counted a few days later. The resultant density estimate, 0.25 tadpoles per litre was probably an underestimate of the density which would actually have occurred as many embryoes died in transit. I do not know the cause of these deaths but it may have been oxygen deprivation. The eggs were carried in closed plastic bags but on other trips there were few embryonic deaths in southern egg masses carried in open plastic containers.

Tadpoles used in the transplant experiments came from the survivors of the spawn collected at Kingston on July 20, 1973. Tadpoles were allocated to cages in a quasi-random fashion. All tadpoles surviving from the original seventeen egg masses were put in a single container. The water in this was mixed vigorously and tadpoles removed with a small net until a total of twenty five were obtained. These were then placed in circa .25 l of water in a small plastic container. Twenty four such lots were selected and then randomly assigned to cages and localities. Cages were set up in the field and tadpoles put in them 21 days after the egg masses were collected. They were then checked regularly until metamorphlings appeared and then about fortnightly.

Frogs metamorphosed at Magrath Flat and Woods Well but not at Kingston (Table 4.14). At Kingston all tadpoles died long before they reached a size where metamorphosis seemed likely. The first metamorphlings appeared at both Woods Well and Magrath Flat on 10.xi.1973, i.e. 113 days after they were collected as eggs, and the last were collected on 23.xi.73. On this latter date one of the cages at Magrath Flat still contained 25 tadpoles but these either died or escaped before the cages were visited again. The corner of this cage may have been submerged between 23.xi.73 and 10.xii.73 (the next visit).

Because it was possible that tadpoles escaped at both Woods Well and Magrath Flat (see above and Table 4.14) in 1973 I visited both localities during the spring of 1974. No southern calls were heard at either place.

These transplant experiments have clearly shown that southern *L.tasmaniensis* tadpoles can grow and metamorphose well north of their present range in South Australia. However, this does not necessarily mean that the southern call race could survive and reproduce in such areas.

c) Limits on ranges, environmental changes.

Along the Coorong there are no marked changes in vegetation (Specht 1972), climate (S.A. Year Book 1975) or soils (Northcote 1960) correlated with the range limits of either the southern or western call race. This reinforces the conclusion from the tadpole transplant experiment that there is no environmental brake to increasing the area where the southern and western call races are sympatric.

4) Discussion.

In sections 4.2 3) a) and b) I proffered reasons why the Coorong contact between the southern and western call races might be particularly likely to give evidence of reinforcement. These reasons were, because

	1971	1972	1973
Meningie	594	466	517
Kingston	689	460	626

Table 4.13 Total rainfall in mm at Meningie and Kingston for the years 1971 to 1973. Data were supplied by the Bureau of Meteorology, Adelaide.

Kingston	a. 0		b. 0	
Woods Well	a. 6	(13.41, 0.342)	b. 2	
Magrath Flat	a. 49	(18.75, 0.024)	b. 31	(17.36, 0.029)

Table 4.14 Number of metamorphosing frogs from an input of 100 tadpoles per cage. There were two cages (a. and b.) at each site. Numbers in brackets are mean snout vent lengths (mm) followed by the mean's standard error. Both Woods Well cages were overturned several times. Magrath Flat b. held 25 tadpoles on 23.xi.73 but 0 tadpoles or frogs on 10.xii.73.

the contact may be recent (cf. Walker 1974) and because the potential for gene flow from allopatric to sympatric populations was low, particularly in the western call race.

Despite this I found no good evidence of reinforcement of calls or of any of the other potential premating isolating mechanisms I investigated (section 4.2 1)). There may be several reasons for this.

First, and perhaps most likely, differences in call structure, male calling site and the possible earlier start to breeding in the southern call race (section 4.2 1)) together constitute an effective barrage of premating isolating mechanisms extant when the races first contacted. I know that the split in call structures is at least 8 - 10,000 years old (section 4.1) and the occurrence of differences in calling site and breeding time outside the overlap zone augurs similarly for them.

Second, although inter-call race hybridisation may occur at an appreciable frequency (1/22 frogs recorded in sympatry was a hybrid) the lack of postmating isolation (section 4.2 2)) means there is no selective force promoting reinforcement. If this is so, significant introgression and ultimately fusion of the two races might occur. However, if a high frequency of hybridisation is a prerequisite for that result then there might not be fusion as hybrids were probably considerably over represented in my call sample. Virtually all the frogs calling at Petherick Road Corner (site 4, Table 4.1) were recorded but at the second sympatric site (3, Table 4.1) only three frogs, including the hybrid, were recorded. These three individuals constituted only a very small proportion of all the L.tasmaniensis calling at the site and not surprisingly I made extra efforts to record the hybrid. Therefore my sympatric call sample is not unbiased and the real frequency of hybrids in nature may be much lower than would be predicted from my data.

There were no pure southern *L.tasmaniensis* calling at site 3 on the night the hybrid was recorded. If southern frogs are usually rare there, the hybrid may be the result of an unique, isolated failure of normally effective premating isolating mechanisms and might therefore be of little consequence. Nelson (1971) found a female *Rana sylvatica* amplexed and ovipositing with a male *R.pipiens* at a site where males of the former, but not the latter species are rare. At the time the pair was found many *R.pipiens* were calling but *R.sylvatica* males had not been heard for some time. Nelson argued that the *R.sylvatica* female hybridised only because no appropriate mate was available.

Third, the contact between the southern and western call races is very recent and if there has been any reinforcement of premating isolating mechanisms my analyses did not detect it. The only reason to believe this argument is that before 1940 the lower river Murray and lakes Albert and Alexandrina at the river mouth may have constituted an important barrier to the south-eastern dispersal of the western call race. These water bodies were subject to periods of high salinity which in part was caused by the intrusion of sea water (Interstate Royal Commission Report 1902; Stephens 1966). In February 1940 barrages preventing the movement of sea water into the lakes were completed (Stephens 1966) and the usually fresher nature of the waters since then may have reduced any importance they had as barriers to dispersal.

One difficulty with the above argument is the lone record of western *L.tasmaniensis* from the Younghusband peninsula, near Magrath Flat (section 4.2 3) a)). Throughout its length this peninsula is separated from the mainland by waters at least as saline as the sea (Noye 1975) suggesting that high salinity water does not inhibit movement of the western call race. However, freshwater soaks are common along the Younghusband peninsula (Noye 1975) and it may be that after dispersing south along the eastern side of the Coorong lagoon the western call race then moved north up the western edge.

Given my present knowledge it is impossible to make accurate forecasts of the future of the Coorong contact. My speculations are these. There is probably effective premating isolation between the southern and western call races (Chapter 3; section 4.2 1)) meaning there is little chance the identity of either race will be lost through hybridisation or that reinforcement will occur. There are no obvious barriers to the expansion of the range of either call race (section 4.2 3) b) and c)). Perhaps the most likely range change that will be observed is a shift of the western call race further to the south and east. Swamps are common there and the chances of migrants establishing populations may therefore be greater than for southern frogs moving north (cf. section 4.2 3) a)). There is no reason to believe, if such range expansion does occur, that the southern and western call races would behave other than as good species (sensu Mayr 1969).

4.3 The central Victorian contact: the Southern and Northern call races.

The analysis of the northern/southern contact was conducted jointly with M.J. Littlejohn. Littlejohn had accumulated considerable distribution data prior to our joint field work in 1971, 1972 and 1973. However, much additional information was collected during those three years. Our joint analysis and interpretation of this contact has been presented elsewhere (Littlejohn and Roberts 1975) and this account is based largely on that publication. Due to the remoteness of this contact work was confined almost exclusively to an analysis of call characters. Other possible premating isolating mechanisms etc. were not studied in detail.

1) Geographic distribution.

Northern and southern calls differ most dramatically in notes per call (Chapter 2). Using this character the calls can be easily distinguished without recourse to detailed acoustical analysis. This fact was used to determine the distribution of the two call races in central Victoria by noting calls heard on road traverses during the breeding season. Localities were scored as pure southern when only single note calls were heard, as pure northern when only multiple note calls were heard and as intermediate when both multiple and single note calls were heard in a long uninterrupted calling sequence from an individual frog. Frogs producing only single or only multiple noted calls were also heard at many intermediate localities.

Categorisation of localities was, however, subject to some cautions. Multiple note calls have occasionally been heard from southern males but such calls are of much lower intensity than normal calls and appear to differ in temporal and spectral qualities. They may have a territorial function (A.J. Brook, pers. comm.). In the northern call race males sometimes start calling with one or two single note calls then change to exclusively multiple note calls. Single note calls are only produced again at the start of a new calling sequence after an interruption.

Distribution data are presented in Figure 4.10. Intermediate localities occur in a broad band oriented north west to south east across north central Victoria. The population of the southern call race in the upper Goulburn River valley (near Alexandra, Figures 4.9 and 4.10) appears to be isolated from the main distribution by forests and intermediate populations.

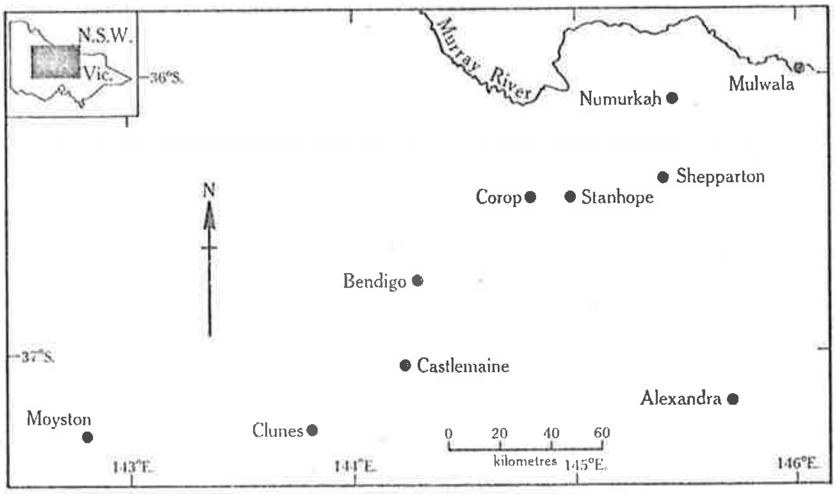


Figure 4.9 Principal localities mentioned in section 4.3

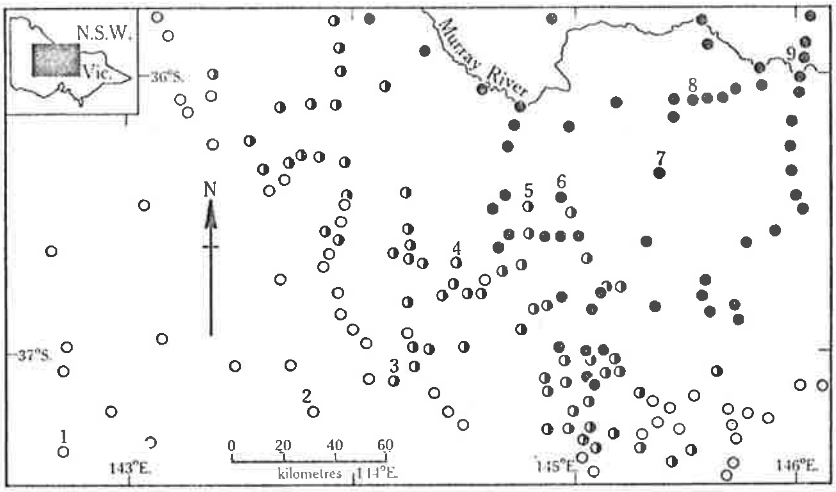


Figure 4.10 Distribution of call races and intergrade zones in north central Victoria and location of the recording transect (sites 1 - 9). Open circles, southern call race; closed circles, northern; half closed intergrade localities.

2) Call structures.

Calls of 115 males from nine localities were subject to detailed analysis. The recording stations fall on a line roughly perpendicular to the long axis of the main distribution of intermediate localities (Figure 4.10, Table 4.15). Calls were analysed as in Chapter 2. Where the last call contained a single note but an earlier call included two or more notes, the latter call was also analysed and the average values used. Note structures in single-noted and multiple-noted calls of the same individual were virtually identical. No correction or analysis of temperature effects was done as the overall range of water temperatures was small (Table 4.15). Also, previous investigation had shown that only one character studied (note repetition rate) was affected by temperature changes (Chapter 2).

Data on variation in note duration and notes per call across the recorded transect are given in Figure 4.11. In this figure notes per call are presented as realised classes of different combination of calls. In this way, the presence or absence of single note calls is apparent. Note repetition rate and dominant frequency data are given in Figure 4.12 and Figure 4.13.

The two most distinctive call components, note duration and notes per call show a slow and relatively smooth change across the intermediate localities. Multiple note calls are present in very low frequency at station 3 and, similarly single note calls at station 5. Note duration variation follows a similar pattern. Dominant frequency shows little change across the transect except that values are relatively high at Moyston. In Chapter 2, I showed that note repetition rate was linearly related to water temperature in South Australian populations of the northern call race. I would expect a similar relationship in northern Victorian populations and at stations 6, 7, 8 and 9, despite the small range of water temperatures this appears to be so.

Locality	Map and Grid reference	Recording date	Recorder	Number of frogs recorded	Temperature range (^{o}C)
1. 10.0 km S.W. of Moyston, Vic.	SJ 54-8 562391	10.x.73	J.D.R. & M.J.L.	18	14.1 - 17.0
2. 7,1 km N.N.E. of Clunes, Vic.	SJ 54-8 187405	3.xi.71	M.J.L.	11	14.4 - 15.8
3. 6.8 km S.S.W. of Castlemaine, Vic.	SJ 55-5 223420	19.ix.71	J.D.R. & M.J.L.	11	12.3 - 14.3
4. 13.8 km N.E. of Bendigo, Vic.	SJ 55-1 244473	9.x.72	J.D.R. & M.J.L.	15	15.0 - 17.5
5. 1.6 km S. of Corop, Vic.	SJ 55-1 282499	4.xi.71	M.J.L.	14	16.2 - 17.1
6. 4.3 km W. of Stanhope, Vic.	SJ 55-1 296501	18.x.71	J.D.R. & M.J.L.	10	15.4 - 17.0
7. 3.7 km N.N.W. of Shepparton, Vic.	SJ 55-1 340515	8.x.72	J.D.R. & M.J.L.	14	16.4 - 18.0
8. 7.0 km E. of Numurkah, Vic.	SJ 55-2 353545	8.x.72	J.D.R. & M.J.L.	9	14.2 - 16.4
9. 1.3 km N.N.E. of Mulwala, N.S.W.	SI 55-14 401561	29.viii.69	M.J.L.	13	16.7 - 17.7

Table 4.15 Details of recording localities etc. for the central Victorian transect. Map and grid references are for Edition 1, Series R 502 maps, scale 1:250,000, Department of National Development, Canberra.

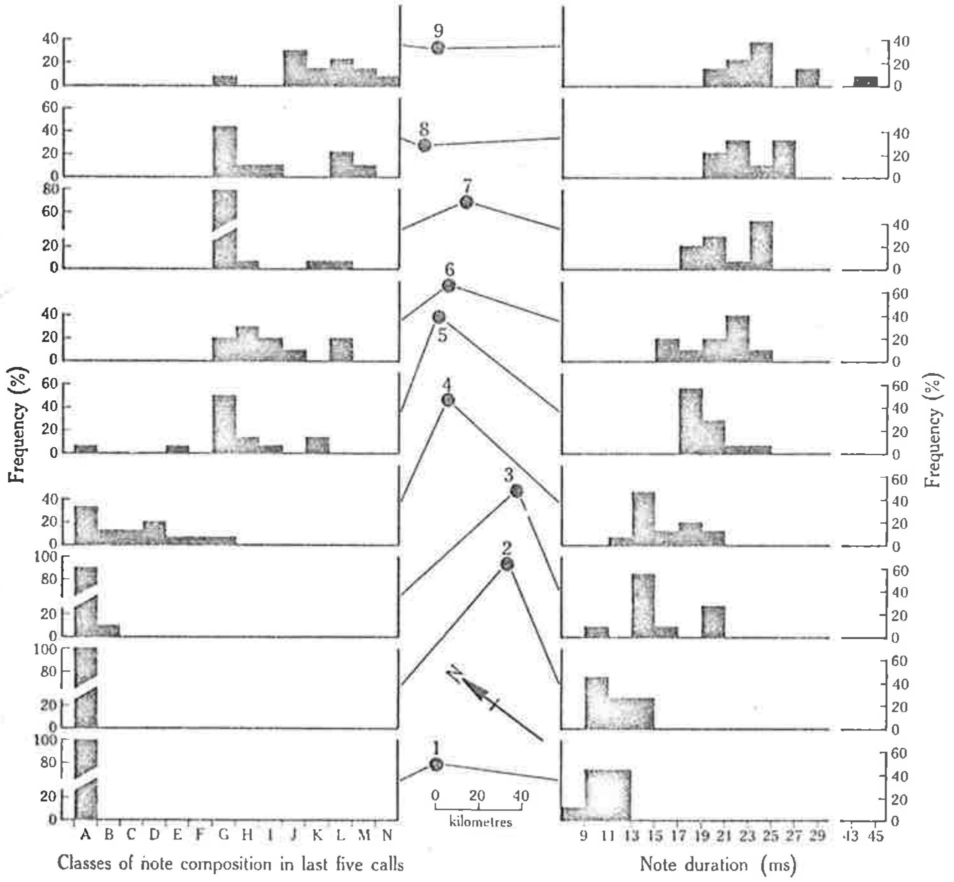


Figure 4.11 Histograms showing geographic variation in notes per call and note duration along the recording transect (sites 1 to 9 on Figure 4.10). Explanation of classes of note composition. A, all five calls of one note; B, four of one note, one of two notes; C, three of one note, two of two notes; D, two of one note, three of two notes; E, one of one note, four of two notes; F, one of one note, two of two notes, two of three notes; G, five of two notes; H, four of two notes, one of three notes; I, three of two notes, two of three notes; J, two of two notes, three of three notes; K, one of two notes, four of three notes; L, five of three notes; M, four of three notes, one of four notes; N, five of four notes.

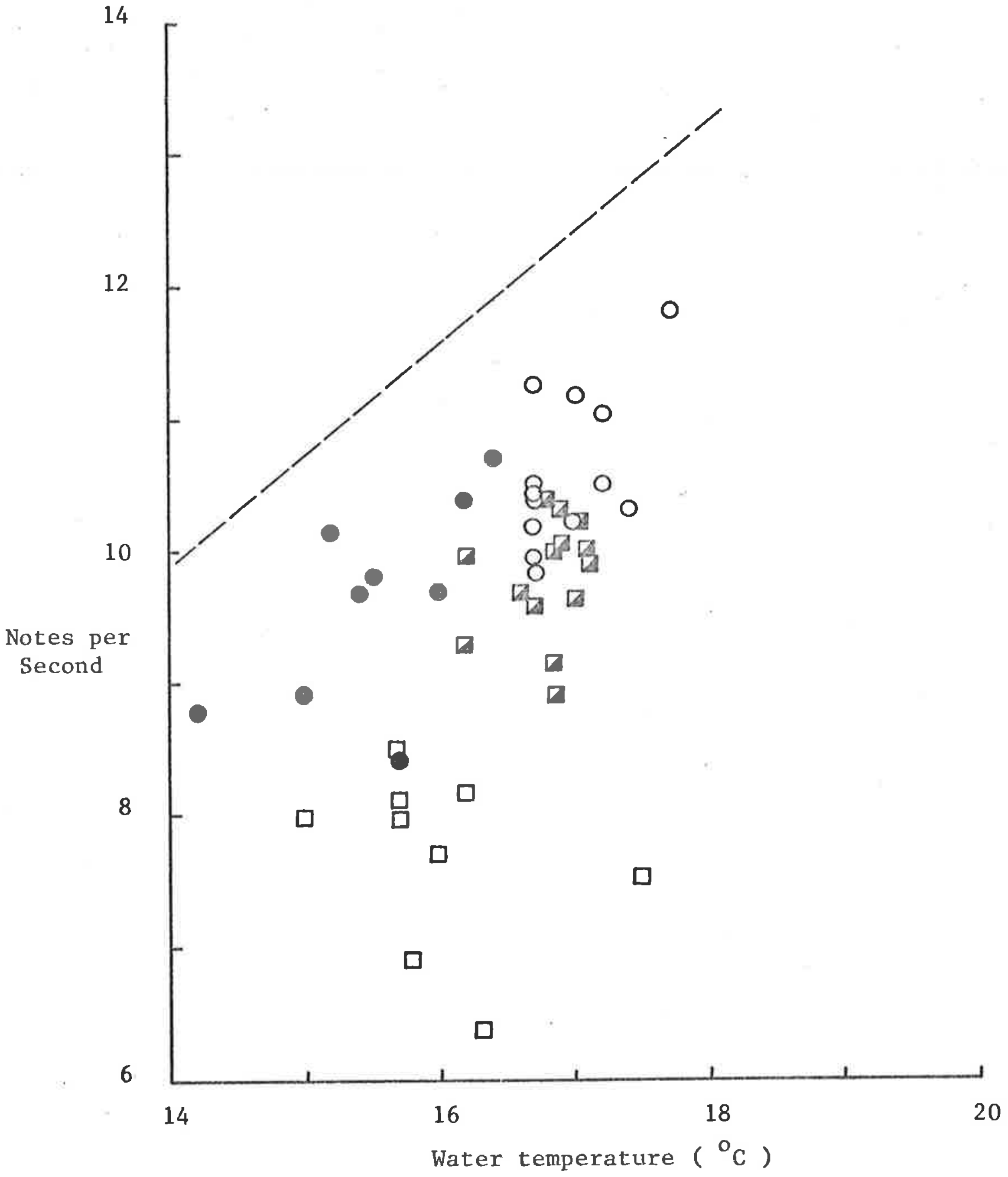


Figure 4.12 Plot of note repetition rate against water temperature for localities in and near the main intergrade zone. Dashed line is the best fit regression line for comparable data from northern call race populations in South Australia.

Station	9	○	Station	5	◩
Station	8	●	Station	4	□

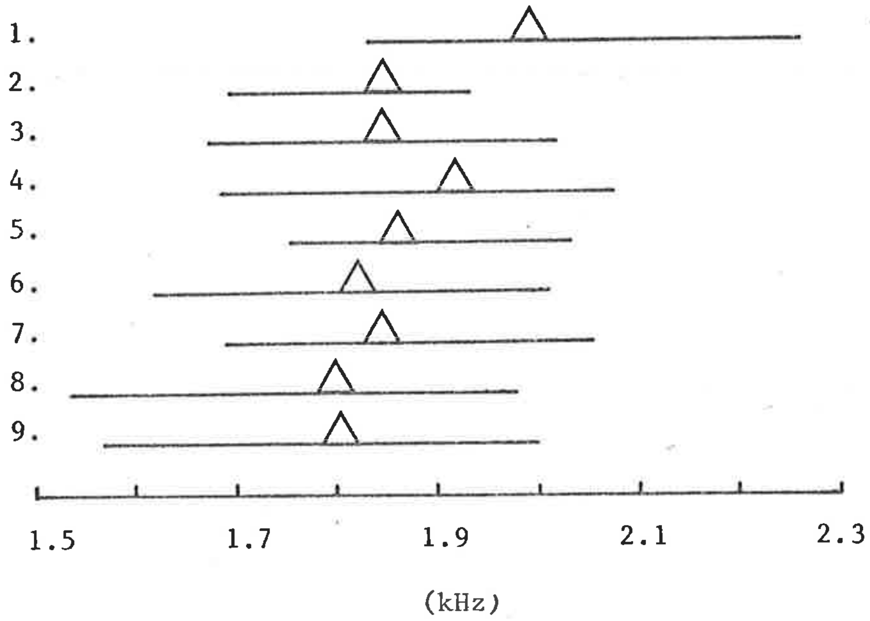


Figure 4.13 Means (triangles) and ranges (solid lines) of dominant frequency values at stations 1 to 9.

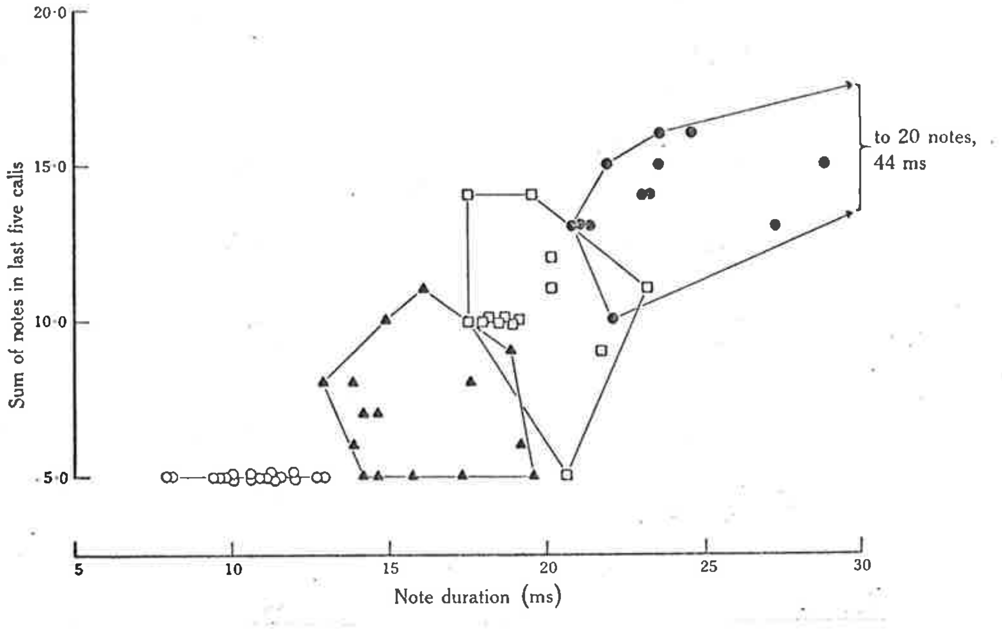


Figure 4.14 Scatter diagrams of values of sum of number of notes in last five calls and note duration, in individuals in two parental and two intergrade samples. Open circle, station 1 (southern); triangle, station 4 (intergrade); square, station 5 (intergrade); closed circle, station 9 (northern). See table 4.15 and Figure 4.10 for details.

However, at stations 3, 4 and 5 some or all values are considerably lower than I would have predicted (Figure 4.12). This decrease in note repetition rate does not seem to be correlated on an intra-locality basis with any of the call components investigated, but particularly not with notes per call. Explanations for these data will be given later (section 4.3 4)).

3) Artificial Hybridisations.

Two artificial hybridisations were conducted using techniques as outlined earlier. Only northern females were used, one coming from Swan Hill in north eastern Victoria and the other from Morgan, South Australia. These females were crossed to southern males from near Kingston, S.A. No control male from Morgan was available at the time the crosses were conducted so a northern male from Mannum and another from Blanchetown were used instead. Results are given (as the percentage of cleaving eggs developing into normal larvae) in Table 4.16. These data give no indication of hybrid inviability. I did not attempt to raise any of these tadpoles (hybrid or other) to metamorphosis.

4) Discussion.

Analyses of call recordings across the main zone of interaction between the call races indicates a gradual transition over a distance of 90 to 135 km. Stations 3 and 5 gave evidence of both call races, but at stations 2 and 6 only the southern and northern call races were found respectively. Within the intermediate localities the various call components seem to associate independently (Figure 4.14), suggesting that genes giving the southern and northern call phenotypes are freely mixed. It is unlikely that individuals of both pure call races occur together in significant numbers, if at all, at any station across the

Cross	Control	Experimental
1. *	(a) 94.51 (91)	94.64 (149)
	(b) 90.28 (72)	
2. **	96.00 (75)	(a) 86.0 (200)
		(b) 96.55 (29)

Table 4.16 Results of artificial hybridisations of northern females and southern males. Data are expressed as the percentage of cleaving eggs developing into normal larvae. Number in brackets is the base for the percentage.
* 2 control males (see text) ** eggs from one female were fertilised by sperm from two southern males.

transect. The interaction is thus similar to what Short (1969) has described as a true hybrid zone and what Woodruff (1973) would call an intergradation. Such a zone would most likely result from hybridisation following contact of already differentiated taxa (though see Rising 1970).

Calls of the southern and northern call races of L.tasmaniensis are as different as those of many sympatric, synchronic anuran species (e.g. Littlejohn 1959) and might be expected to act as efficient premating isolating mechanisms. If reinforcement had generated differences as large as these I would expect hybrids to be rare or absent. This is totally contrary to my observations and I would therefore contend that reinforcement has not contributed to the differentiation of these call races. The high levels of genetic compatability observed in the few artificial hybridisations conducted are a further argument against reinforcement.

Note repetition rate was the only call character investigated where values changed at intermediate localities relative to values in pure populations (Figure 4.12). These data are not contrary to the appearance expected if reinforcement had occurred but a plausible alternative explanation can be offered. The putative western/southern hybrid recorded on the Coorong, S.A. had a similarly reduced note repetition rate value (Table 4.3). The low note repetition rates at the three intermediate localities may result from mixing of analagous genes from the northern and southern call races rather than reinforcement.

Littlejohn and Martin (1964) and Littlejohn (1967) provide a reasonable scheme for the allopatric divergence and recontact of these two call races. They assumed that at some time in the past the range of L.tasmaniensis was split into two isolates; one on the southern Australian mainland and the other on Tasmania. Call structures in the two isolates then diverged. From 20,000 to 12,000 years ago world sea level was more than 60 m lower than at present (Milliman and Emery 1968) and the resultant Bassian land bridge would have allowed the southern call race (the Tasmanian isolate) to colonize the adjacent mainland and ultimately contact the northern call race.

now ? / I will not digress slightly to discuss the nature and history of this intergrade zone.

I noted earlier that an isolate of the southern call race exists on the upper Goulburn river near Alexandra. Mainland isolates of this call race also occur on the southern Monaro plains in south eastern New South Wales (Littlejohn 1967) and in the Benambra - Omeo basin in eastern Victoria. At several localities in north eastern Victoria (2.1 km and 10.9 km south east of Myrtleford, 2.6 km north west of Porepunkah, 15.5 km north north west of Cudgewa, 14.0 km north west and 2.4 km east of Burrowye) individuals with calls similar to those of intermediates from the main zone of interaction have been heard but only in association with calls of the northern call race (M.J. Littlejohn, unpublished observations; A.A. Martin, unpublished observations). These findings indicate the southern call race was once more extensively distributed through south eastern Australia, particularly on the northern side of the Great Dividing Range.

The main contact between the southern and northern call races was presumably initiated several thousand years ago (see earlier in this discussion) and since then has probably shifted in a south westerly direction as is evidenced by the southern call race isolate in the upper Goulburn River valley and the relict populations of putative hybrids in northern Victoria. Note repetition rates in northern call race populations in northern Victoria and southern New South Wales are slightly lower than values for this race in South Australia (Figure 4.12) and this may be further evidence of southern call race contamination (cf. earlier comments in this discussion on the effects of hybridisation on note repetition rate). However, in the absence of data from appropriate populations remote from the interaction this latter suggestion is speculative.

Although there is some evidence that the southern and northern call races are highly compatible genetically, the relative narrowness of the intergrade coupled with its presumed age indicate that unrestricted introgression has not occurred. The preponderance of hybrid and recombination products (Figure 4.14) suggests they are fitter than either parental form within the zone, which at present lies at some balance point along a subtle selective gradient or ecocline. The nature of such gradient is obscure for the zone's position is not obviously correlated with gross changes in soils (Northcote 1960, 1962), climate (Leeper 1955) or vegetation (Victorian Year Book 1962).

Unless the selective gradient or ecocline changes in the future the present arrangement of hybrid and pure populations would seem to be a stable one. The intermediate populations separating pure southern and northern L.tasmaniensis preclude the direct interaction of the call races and the evolution of efficient premating isolating mechanisms. This process would and would have been further inhibited by the high viability of hybrids.

Factors which might have caused allopatric divergence of these two call races will be discussed elsewhere in this thesis (Chapter 5). There seems to be no evidence that reinforcement, resulting from an interaction of the present southern and northern call races or their progenitors, has contributed to their present forms.

4.4 The Murray plains contact: the western and northern call races.

As with the Coorong contact the major aims of this study were two. First, to determine whether calls, or any other character capable of acting as a premating isolating mechanism, showed evidence of reinforcement. Second, to determine the nature of the interaction between the western and northern call races and to make some prediction about the future form of populations in contact areas.

This section is split into four parts. 1) discusses characters which might promote premating isolation between the northern and western call races and also includes an assessment of the nature of the contact based on call and some other data. Part 2) covers postmating isolating mechanisms, and 3) considers factors that might limit the range of both call races in areas where they meet. Finally, part 4) is a general discussion.

1) Premating isolating mechanisms and the nature of the interaction between the western and northern call races.

a) Call component values and reinforcement.

Detailed distributions of the call races between the Mount Lofty ranges and the river Murray were determined by noting call types heard on road traverses. As noted in Chapter 2, I felt that subjective judgements of call structures could reliably distinguish these two call races. Particular locations were scored as either pure western, pure northern, mixed or doubtfully mixed. The last category contained only two locations which were revisited several times with no change of status. Many spots, close to mixed localities, scored as pure, either western or northern, on the initial visit, were revisited on one or more occasions as a check. Only one change was made; from pure western to mixed. The total distribution data are presented in Figures 4.16 and 4.17.

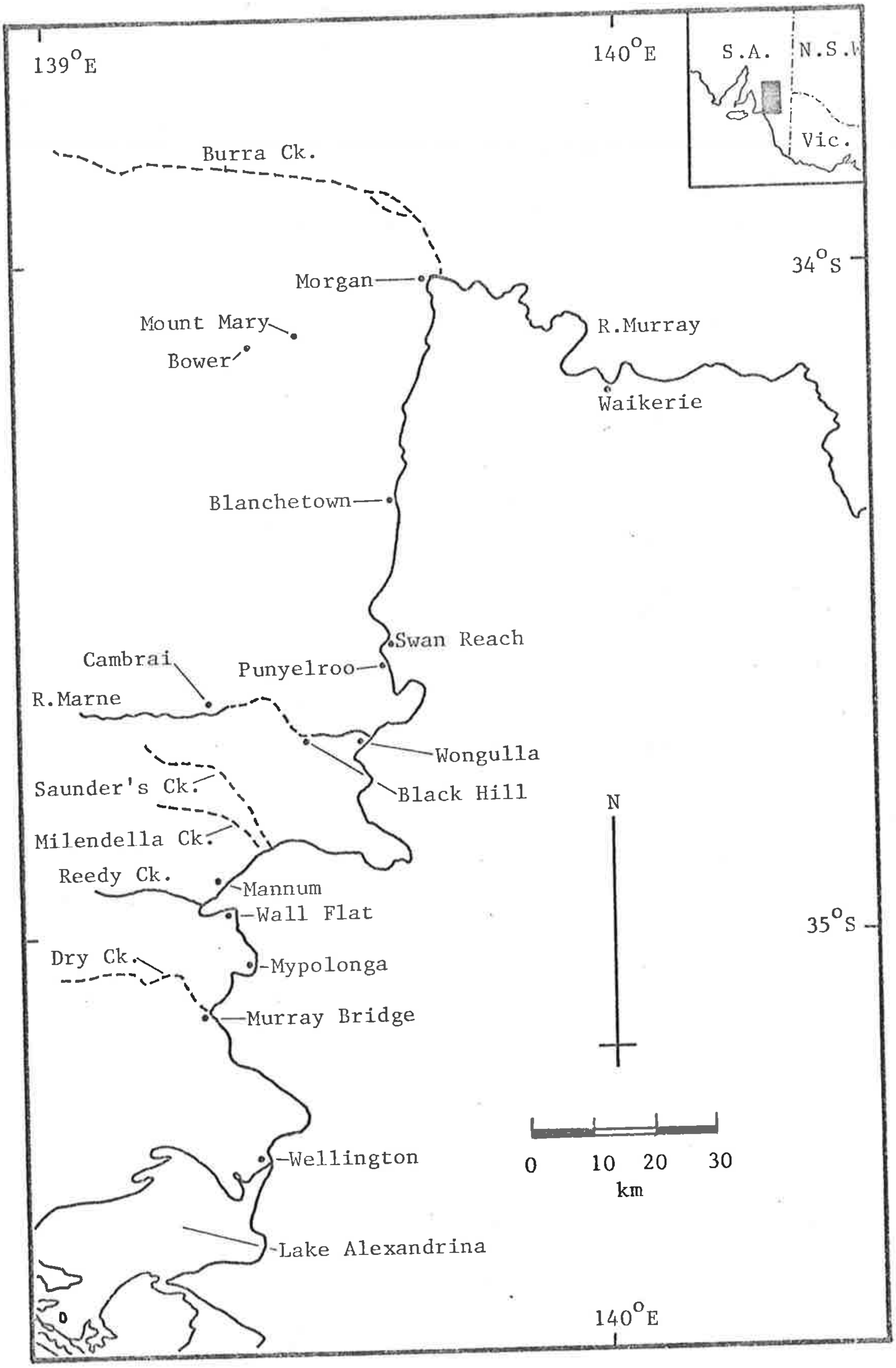


Figure 4.15 Principal localities, rivers, creeks etc. mentioned in section 4.4.

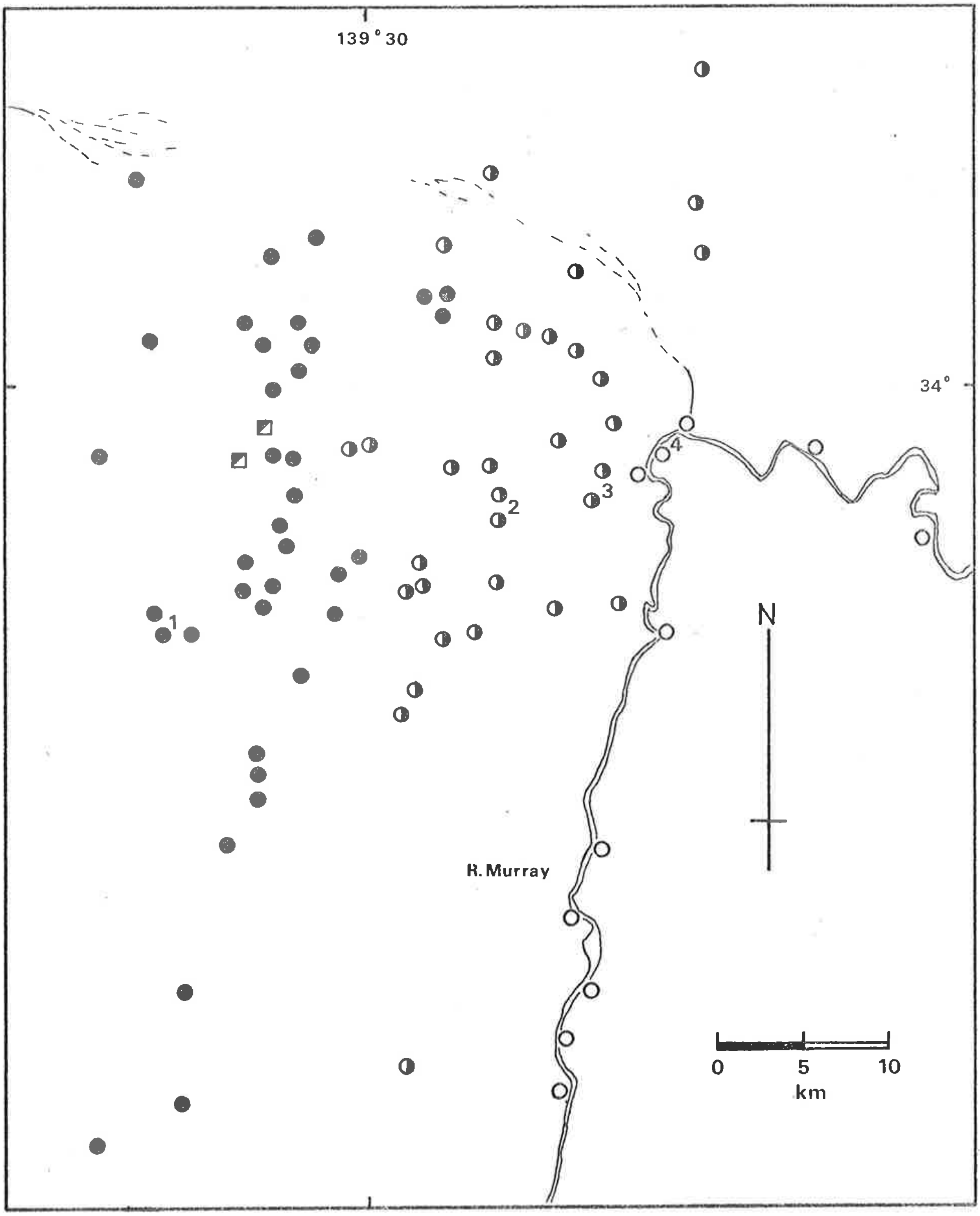


Figure 4.16 Distribution of L.tasmaniensis in the Morgan area. Open circles, northern call race; closed, western; half filled, mixed; half filled square, doubtfully mixed. 1 to 4 are recording sites listed in Table 4.16.

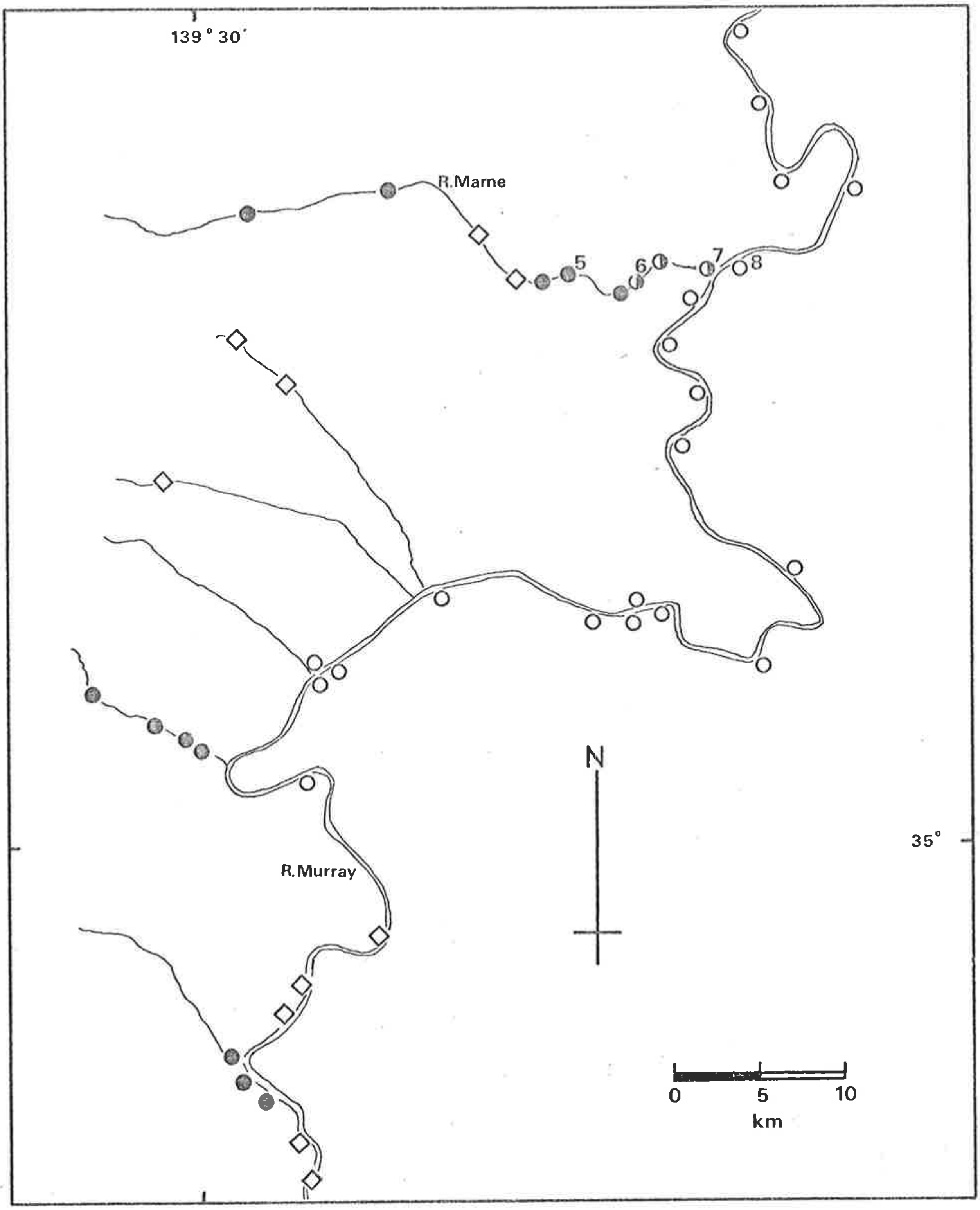


Figure 4.17 Distribution of L.tasmaniensis on the lower R.Murray in South Australia. Symbols as in Fig. 4.16 with the addition that diamonds are for sites where no L.tasmaniensis have been seen or heard. Numbers are recording sites listed in Table 4.16.

There are two zones of sympatry. One extending west from Morgan to just east of Mount Mary and the other spreading west from Wongulla along the Marne river (see Figure 4.15 for principal localities). Calls were recorded at locations in and close to both overlap areas; on the Marne in 1972 and near Morgan in 1973. Details of recording sites, numbers of males recorded etc. are given in Table 4.17. Field recording and subsequent oscillogram analysis were conducted as in Chapter 2. However, statistical treatment of the resultant call parameters was rather different.

I investigated only four call characters: note repetition rate, dominant frequency, average notes per call and pulse repetition rate. The data were collected over a wide range of water temperatures but this was ignored except in the analysis of note repetition rate (Chapter 3, Table 2.3).

Temperature effects on note repetition rate were corrected for but without making the assumptions inherent in traditional methods (Chapter 2).

I demonstrated in Chapter 2 that fitted lines for the regression of note repetition rate on water temperature were parallel, though of different elevations in the northern and western call races. I divided the space between these lines into three by drawing two more lines parallel to each other and to the northern and western reference lines, and equidistant from each other and the reference lines (Figure 4.18(a)). Each individual note repetition rate value was plotted against its water temperature and then assigned a note repetition rate score from 0 to 4 according to where it fell (Figure 4.18(a)). Classes 0 and 1 contained nearly all northern reference (i.e. Big Bend and Blanchetown data); 3 and 4 most western reference (Gawler and Murray Bridge) data, and 2, intermediate values. Values falling on lines were put into group 2 or the group closest to 2. For example, if a point fell on

Table 4.17 Details of recording localities etc. for contacts between the western and northern call races. Map and grid references are to Edition 1, Series R 502 maps, scale 1:250,000, Department of National Development, Canberra. Sites 1 to 4 are referred to in the text as the Morgan transect, sites 5 to 8 as the Marne transect.

Locality	Map and Grid reference	Recording date	Number of frogs recorded
1. 1.2 km S.E. of Bower	SI 54-9 236783	26 & 27.x.73	19
2. 9.6 km S.W. of Morgan (Eba)	SI 54-10 256791	3.iii.73 8.iii.73 * 26.ix.73	2 5 8
3. 4.3 km S.W. of Morgan	SI 54-10 263793	25.ix.73	16
4. Morgan, east bank of Murray river	SI 54-10 267796	7.viii.73 22.viii.73 28.x.73	4 6 4
5. 2.8 km E. of Black Hill	SI 54-9 247714	11.ix.72 16.xi.72	7 6
6. 3.8 km W. of Wongulla (Marne Ford)	SI 54-10 252713	17.xi.71 17.ix.72 27.ix.72	1 5 15
7. Wongulla, Marne river mouth	SI 54-10 257714	17.xi.71 4.ix.72 24.ix.72 28.ix.72	1 1 9 14
8. Kroehn's Landing	SI 54-10 259715	28.ix.71	20

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* Recordings on 8.iii.73 were actually made 1.4 km due north of the designated site.

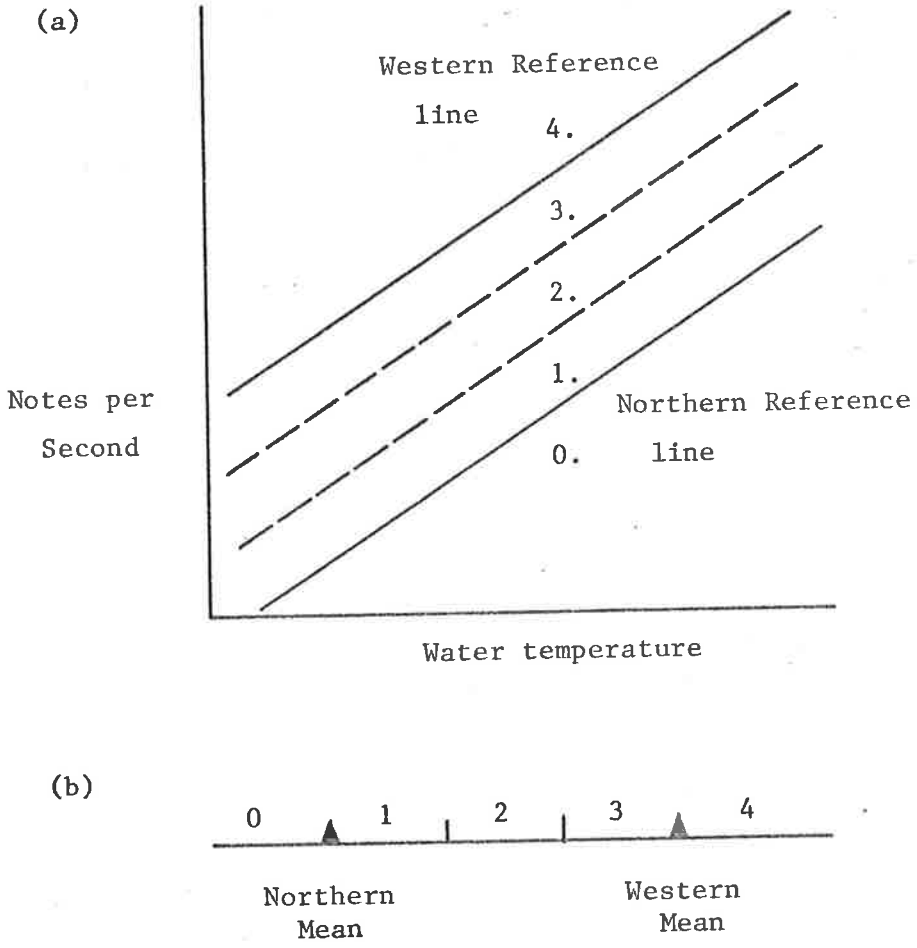


Figure 4.18 (a) Graphical arrangement used to separate note repetition rate data into five categories. The two dashed lines are equidistant from each other and the two reference lines. Equations for the reference lines are given in Table 2.5.
(b) Graphical arrangement used for separating other call components into five categories analagous to note repetition rate scores.

the line separating groups 0 and 1 it was scored as 1, not 0.

Histogra~~m~~ms illustrating call component values from the eight recording sites and the western and northern reference samples are given in Figure 4.19(a) to (d).

The most distinctive character is note repetition rate score. Using only this, I would designate the Kroehn's Landing (site 8, Table 4.17) and Morgan (site 4, Table 4.17) samples as pure northern, and the Black Hill (site 5, Table 4.17) and Bower (site 1, Table 4.17) samples pure western. At stations 2, 3, 6 and 7 (see Table 4.17), contrarily, many frogs had note repetition rate scores of 2. The other call components showed a similar, though less clear cut, trend, with either intermediate values at sites 2, 3, 6 and 7 or values characteristic of both call races.

None of the call components showed shifts, either individually or on a population basis, that could be interpreted as resulting from reinforcement. The only peculiarities worthy of note in this context are some high notes per call averages at Kroehn's Landing (locality 8) and one frog with a very low dominant frequency at Morgan (locality 4; actual value was 847 Hz). In the analysed call of the latter individual dominant frequency was measured in the loudest, apparently distortion free, pulse in the second note. Subsequent pulses in this note had a much higher carrier frequency (circa 1600 Hz). Analagous pulses in other notes of the same call and in other calls were similar to that giving the 847 Hz value. However, some were subtly distorted. I may not have detected distortion which is actually present in the pulse I analysed. The high notes per call cannot be explained away as artefacts, but equally are unlikely to have resulted from reinforcement as they increase rather than decrease the similarity of western and northern calls.

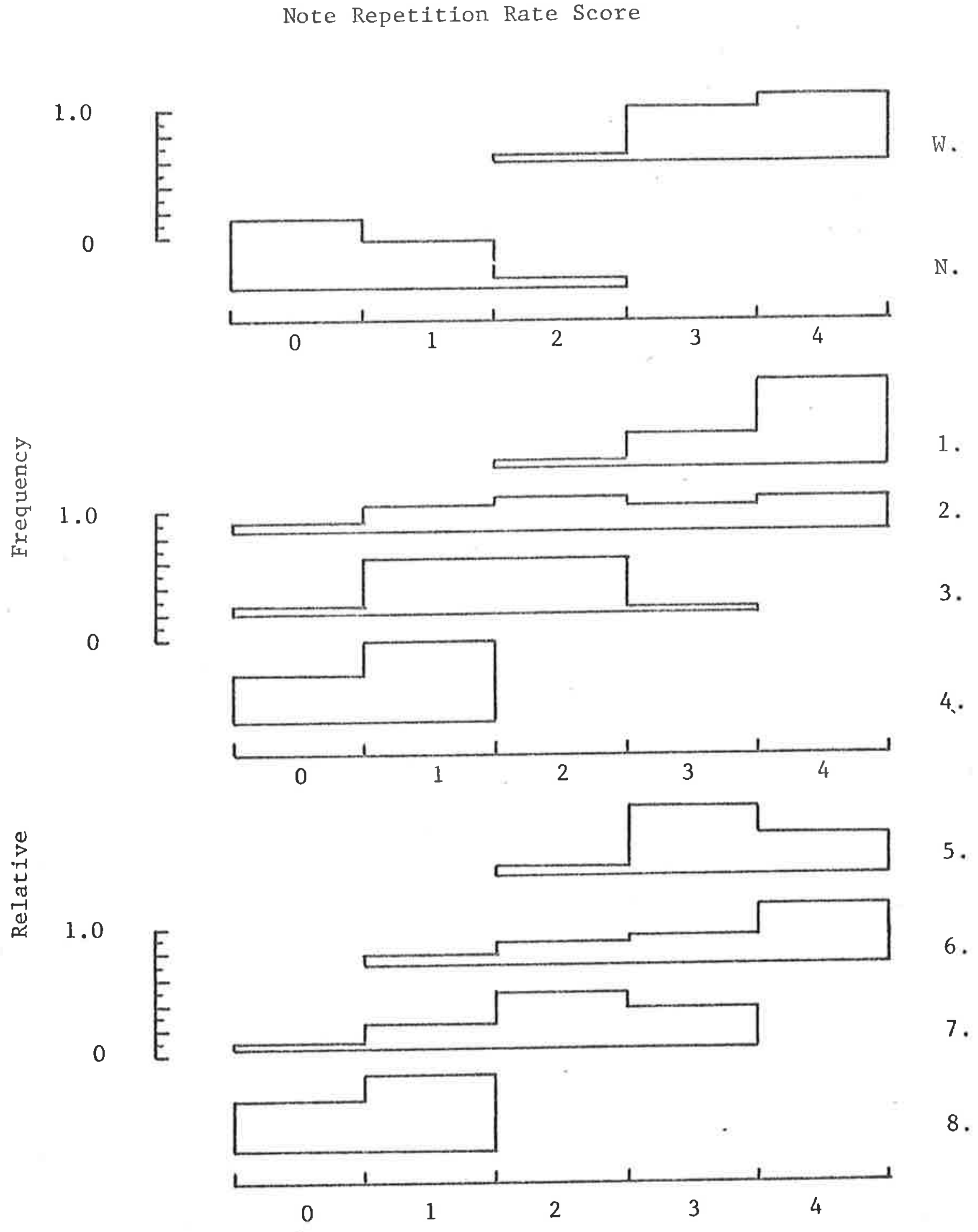


Figure 4.19 (a) Distribution of note repetition rate scores at recording sites 1. to 8. and in the western (W.) and northern(N.) reference samples. Data are given as relative frequencies in each class. Sample sizes, see Table 4. 16.

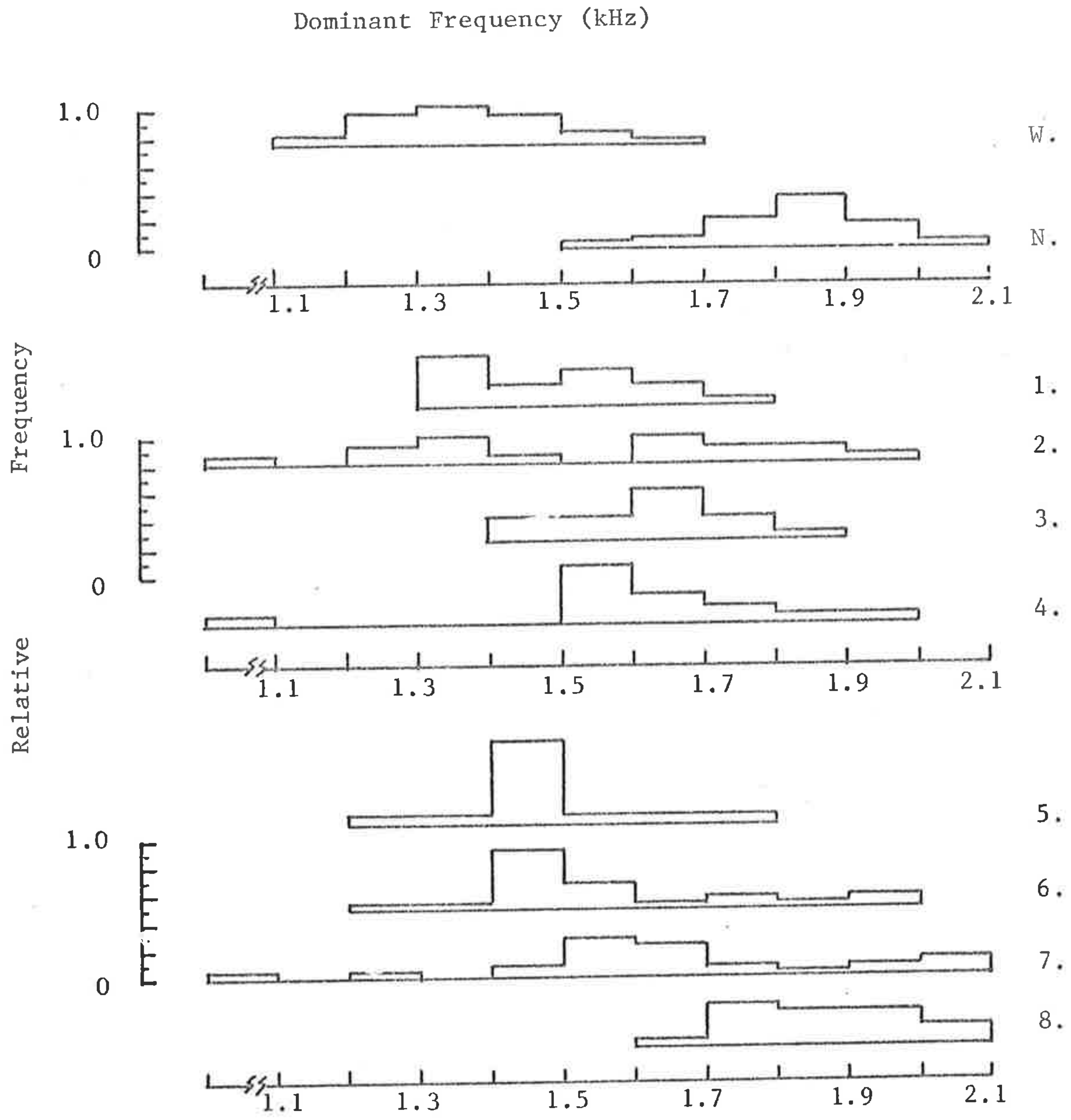


Figure 4.19 (b) as for (a) except that data is dominant frequency. The lowest class contains all data less than 1.1 kHz.

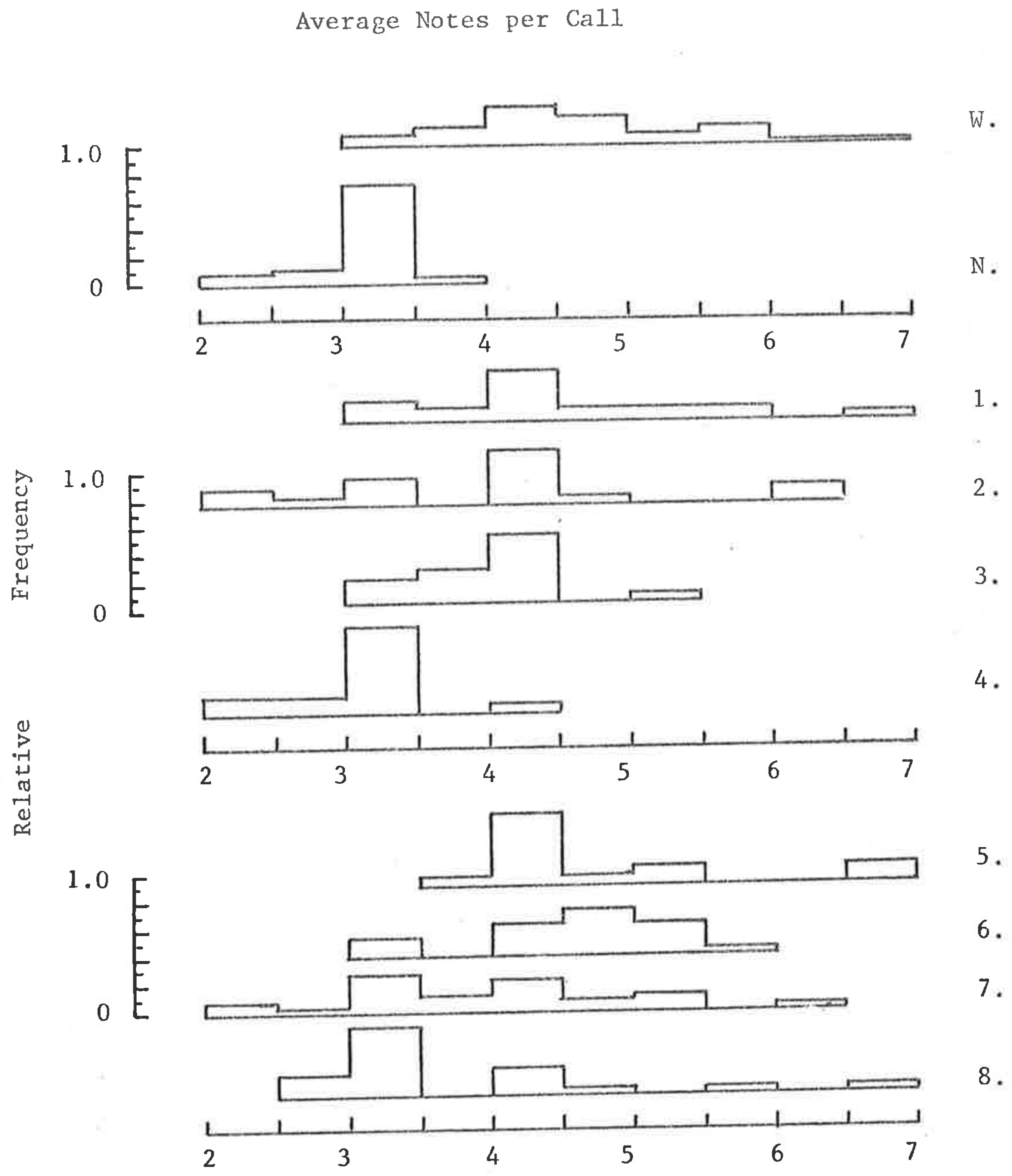


Figure 4.19 (c) as for (a) except that data ~~is~~ are average notes per call.

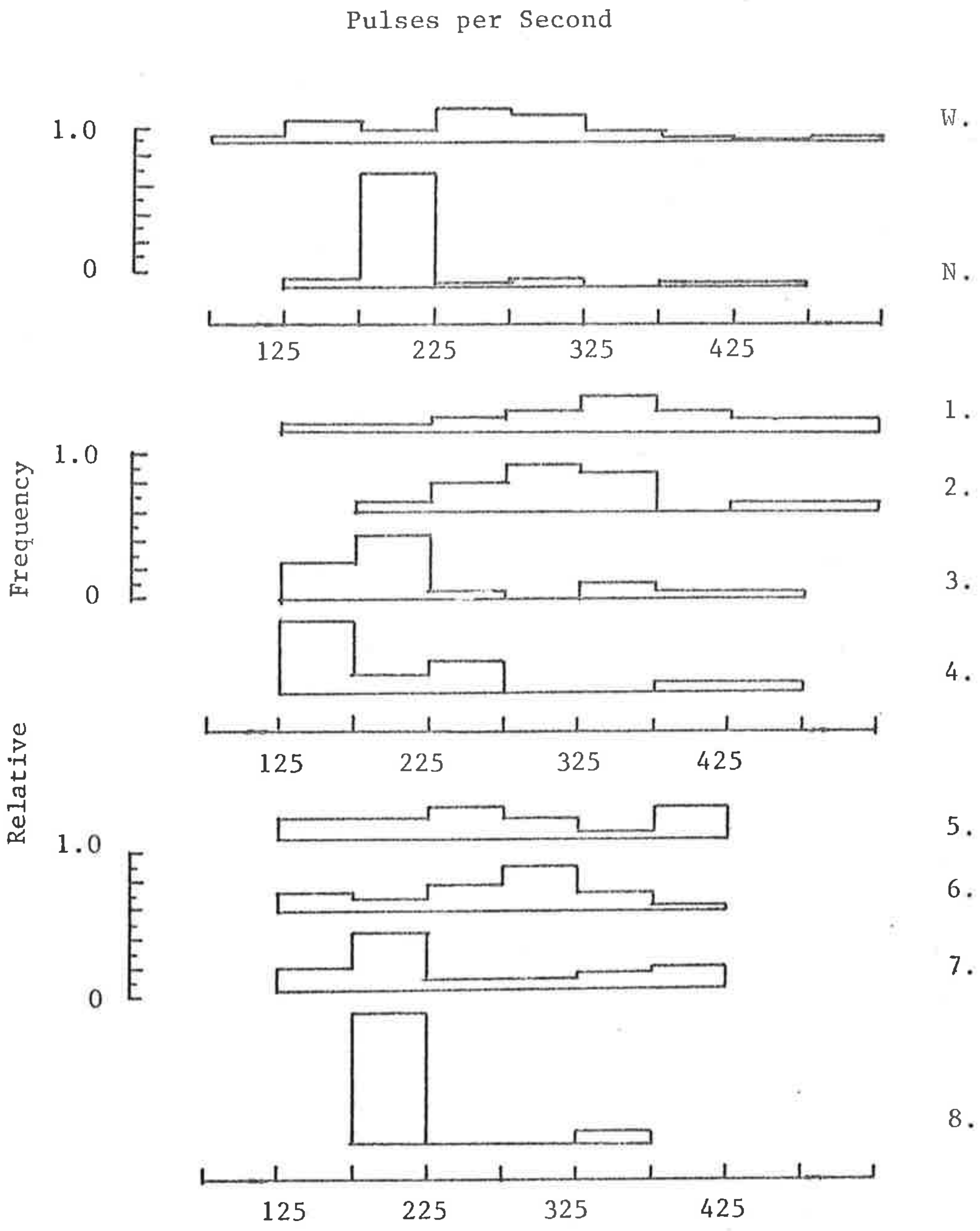


Figure 4.19 (d) as for (a) except that data ~~is~~ are pulses per second. The lowest and highest classes contain all data below 125 and above 475 pulses per second respectively.

b) The interpretation of intermediate call values.

In an attempt to clarify the meaning of intermediate call component values I constructed a call index. Note repetition rate data has already been sorted into five categories, and an analagous process was used to rate other call components on a 0 to 4 scale. For each call character the range between the northern and western reference sample means was divided into three equal segments. The resultant five categories were labelled 0 to 4 as for note repetition rate (see Figure 4.18(b)). The category scores for all call components were then summed for each individual to give a call index value. Extreme northern call types would be expected to score 0 and extreme western 16. The distribution of call index scores in the western and northern reference samples and in the samples from recording sites 1 to 8 are given in Figure 4.20. Values from the two reference samples overlap slightly but the means and modes are quite different (Western mean, 12.41; mode 12. Northern mean 2.56; mode 1).

Call index scores at stations 1 and 5 are very similar to those in the western reference sample except for one individual with a score of 6 at Bower (station 1). At stations 4 and 8 call index distributions are very like that in the northern reference sample. At stations 2 and 6 most call index values fall within the western range, though a few at both localities might be considered intermediate or northern. At station 3 most values are in the range 5 to 8, i.e. either intermediate or at the extremes of western or northern values but some fall well within the western and northern reference distributions. At Wongulla (station 7) the situation is slightly different with most values falling in the northern distribution, and possible intermediates being closer to the bulk of the western reference sample. In none of these samples is there any tendency to segregate into two distinct groups, nor is the relative frequency of extreme northern and western

Call Index Scores

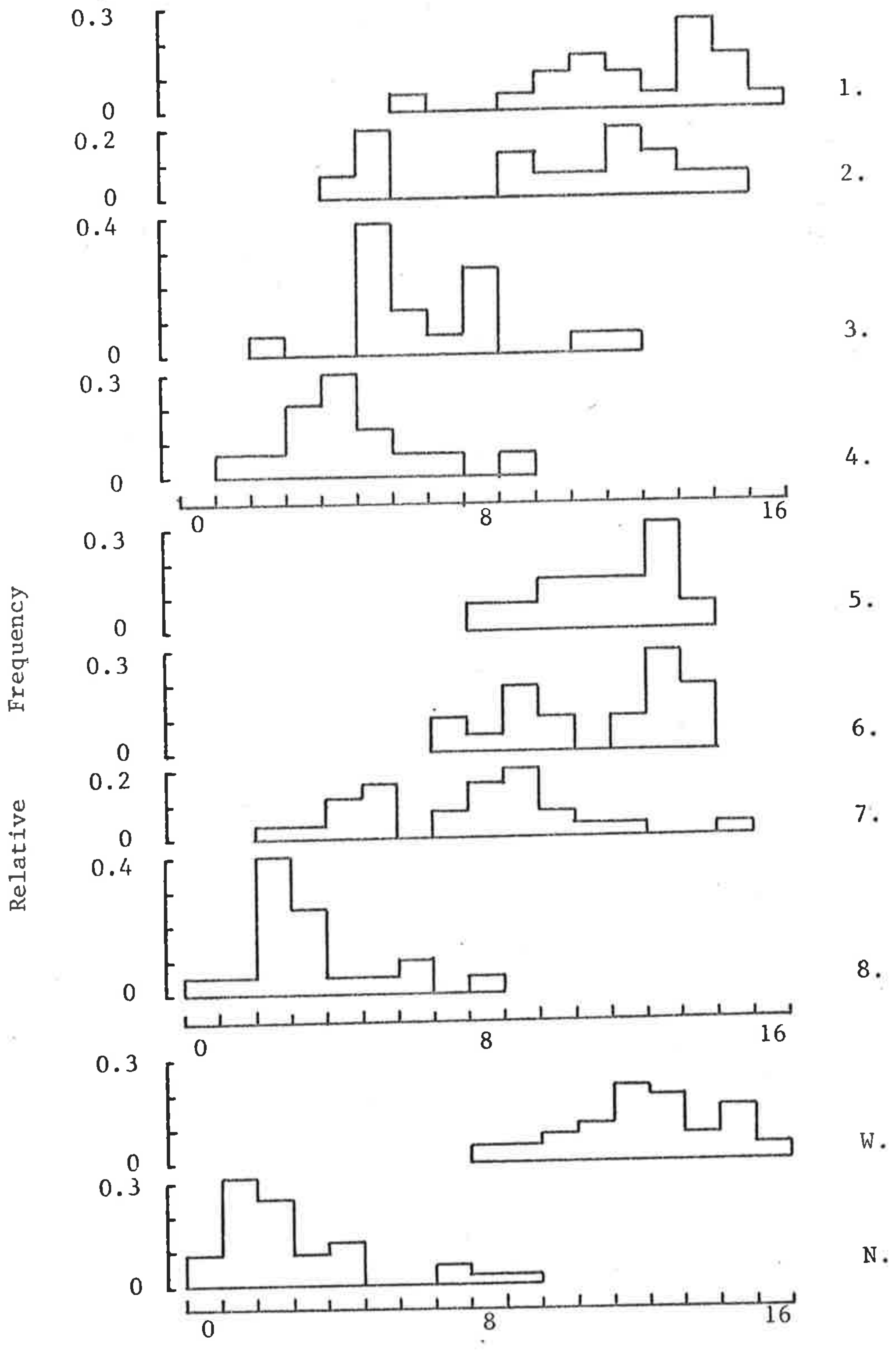


Figure 4.20 Distribution of call indeces (expressed as relative frequencies) for recording sites 1. to 8. and the western (W.) and northern (N.) reference samples. Samples sizes, see Tables 4.16 and 2.2.

phenotypes particularly high. Thus I see no evidence that reinforcement has affected calls in either of these overlap areas.

As apparently intermediate and pure calls of both races occur in both contact areas, the situation is consonant with what Short (1969) described as overlap with hybridisation, and what Woodruff (1973) would probably term parapatric hybridisation. The call index above could also be used as an hybrid index (cf. A.A. Martin 1973; Littlejohn and Watson 1973) and the nature of hybrids, etc. discussed. However, it lacks discriminatory power as there is marked overlap of notes per call and pulse repetition rate values in the two call races.

Accordingly I determined the frequency of intermediate calls by using the most distinct call components, dominant frequency and note repetition rate score. The western and northern reference sample data were plotted and maximum area polygons containing each reference data set were drawn as in Figure 4.21. This results in two non overlapping areas. Calls from the contact areas were then plotted and scored as western if they fell within the western reference polygon or its leftward extension (Figure 4.21) and as northern on a similar basis. All other calls were classified as intermediate. Table 4.18 contains the results of this analysis. Mixed populations of northern, western and intermediate animals were recorded at sites 2, 3, 6 and 7; 1 and 5 western plus intermediates; at 4 northern and intermediates and at 8 only northern.

As a check on the validity of these findings I also analysed call data from three presumed pure western populations remote from known northern populations (Table 4.19).

It is clear that the polygons in Figure 4.21 are not absolutely reliable as a test for distinguishing pure western and northern L.tasmaniensis calls. From the data in Table 4.19 I would expect pure western populations to contain some frogs with call characteristics

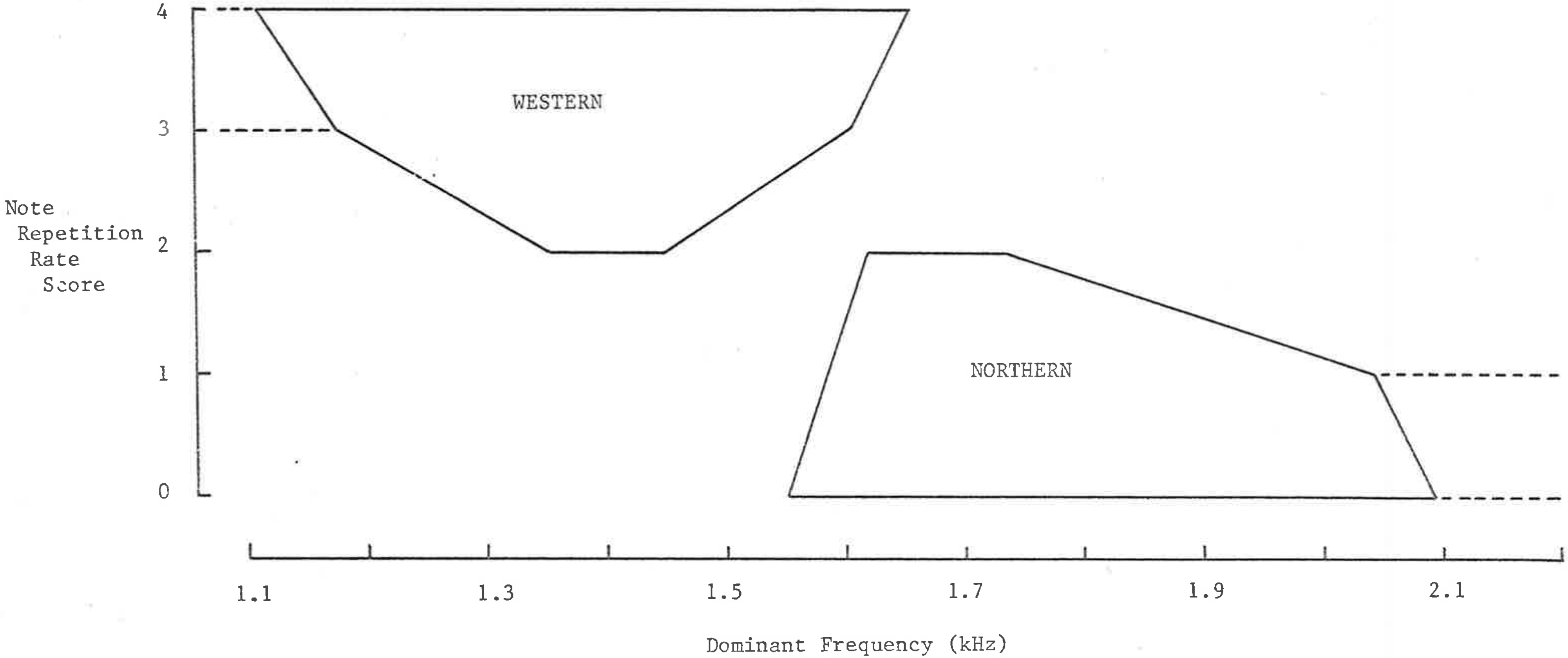


Figure 4.21 Diagram used for separating western, northern and hybrid calls for data collected on the Morgan and Marne transects. The two polygons contain all the western and northern reference sample data respectively. Calls were scored as pure western if they fell within the western polygon or its leftward extension indicated by dashed lines. Similarly for pure northern calls. All other calls were scored as hybrid.

Locality	Western	Intermediate	Northern
1.	16	3	0
2.	4	7	4
3.	1	6	8
4.	0	6	8
5.	11	2	0
6.	11	9	1
7.	3	14	8
8.	0	0	20

Table 4.18 Frequencies of western, northern and intermediate calls at recording sites 1 to 8 (see Table 4.17) scored using Figure 4.21.

Population	W	I	N	Distance to nearest northern population
Magrath Flat	14	1	0	100 km
Petherick Road	13	2	0	170 km
Muratchina Dam	14	0	0	360 km

Table 4.19 Numbers of northern, western and intermediate calls in three pure western populations remote from known northern call race populations. Calls were scored as western (W), northern (N), or intermediate (I) with Figure 4.21.

intermediate between the average values in the northern and western call races.

The intermediate calls in the Bower and Black Hill samples (sites 1 and 5, Table 4.18) are thus not unexpected if these are pure western populations. I will treat these samples as such in this thesis rather than as western populations which have incorporated some northern call race genes.

I do not have sufficient northern samples to conduct a similar analysis for this call race. The validity of the score of intermediates at Morgan and Kroehn's Landing (sites 4 and 8, Table 4.18) might therefore be open to question.

c) Calls of known hybrids.

As the high frequency of intermediate calls in the Morgan and Marne contact areas may result from inter-call race hybridisation, I attempted to collect call data from a series of artificially-produced hybrids. Although quite a number of hybrids were raised to metamorphosis successfully, the majority died soon afterwards of unknown causes. Calls of one male were recorded on three occasions and of another (possibly the same individual) once. Calls of one male from the control cross conducted with the hybridisation were also recorded once. The hybridisation was of a western female to a northern male.

Most of these recordings are of poor quality due to background interference from air-conditioning machinery and because of severe echoing in the small containers the frogs were calling from. Note repetition rate and notes per call could be scored reliably, though I put little faith in the other call component values estimated in Table 4.20 (a). Table 4.20(b) contains call index values and the results of graphical scoring (using Figure 4.21) for the same calls.

Over a range of water temperatures the hybrids consistently had note repetition rate scores of 2 (cf. Figure 4.18(a)), with all values

(a)

Frog, date	Dominant frequency (Hz)	Pulses per second	Notes per second rating	Average notes per call	Water temperature (°C)
Hybrid 1, 27.viii.72	1453	420.76	2	4.0 (4 4 3 6 3)	20.5
Hybrid 2, 23.xi.72	1444	328.76	2	3.0 (3 3 3 3 3)	18.7
18.xii.72	1757	707.91	2	2.2 (2 3 2 2 2)	18.2
18.xii.72	1502	731.62	2	2.4 (2 3 2 3 2)	17.5
Western control 18.xii.72	1598	723.38	3	3.0 (3 3 3 3 3)	19.0

(b)	Hybrid 1	Hybrid 2	Hybrid 2	Hybrid 2	Control Western
(1)	11	9	7	9	9
(2)	I	W	I	I	W

Table 4.20 (a) Values of call components in known hybrids and a western control male from the same cross. Numbers in brackets are notes per call in the last five recorded calls. Hybrid 2 was recorded on three occassions.
(b) (1) Call index values for Hybrids 1 and 2 and the western male;
(2) Categorisation of hybrid and control male calls using Figure 4.21. W western, I intermediate.

falling in the middle of the two range. The control male had a note repetition rate score of three which was just below the western reference line. The western control and hybrid males were raised in adjacent containers and were only exposed to each other's calls. Despite this, there were differences in note repetition rate suggesting the form of this call component is genetically determined, not learnt.

The notes per call data are less easily interpreted, but the control male did not make two-note calls whilst one of the hybrids did. Hybrid one, however, had an average typical of the western call race, though there was considerable variability between calls (Table 4.20(a)).

In conclusion, although the data from known hybrids are few they do not contradict the following thesis: Namely, the intermediate call component values in the western, northern call race overlap zones have resulted from hybridisation between these two forms.

d) Egg size and number of eggs per egg mass.

In an attempt to find corroborative evidence for the hypothesis that the western, northern contacts were areas of overlap with hybridisation I wanted to examine some character other than calls. I selected egg size and number of eggs per egg mass, as superficial examination suggested these differed in the two call races. Data were only collected on the Morgan transect as egg masses were very hard to find on the Marne transect.

i) Eggs per egg mass.

Counts were performed on masses collected at recording stations 1 to 4 (Table 4.17) on various dates in 1973 and 1974. Eggs were taken to the laboratory in open plastic containers and allowed to hatch. The numbers of tadpoles and of dead or unhatched eggs were then counted. I tried to collect eggs synchronously at all four locations, though this was not always possible. Once, when there were no eggs at Bower, eggs were collected at Mount Mary (7.8 km E.N.E. of Bower) instead. Data were analysed by anova, with a posteriori comparisons amongst means by the Student-Newman-Keuls test. Mean egg numbers and ranges are given in Table 4.21(a), and details of the anova in Table 4.21(b).

Means and ranges at Bower (station 1) and Eba (2) were virtually identical. However, at the other two localities, means were much higher and ranges broader. At Morgan (4) there were two very low values and one high value, but with all other data clustered closely around the mean. In contrast, at station 3, data were spread fairly uniformly over the whole range (Figure 4.22).

If the above data are to be used as indicators of hybridisation, then it is important to establish that each egg mass represents the complete egg output of a single female at a particular ovulation. I am fairly certain that this is so as counts of eggs in the oviducts of females dissected after ovulation induction in the laboratory gave numbers comparable to field data. The only exceptions ~~is that~~ are two northern females which gave slightly lower values than the mean for Morgan (Table 4.21 (c)).

Commonly, during periods of peak breeding in both the western and northern call races, froth patches which look like normal egg masses are found. These patches have few (1 - 5) or no eggs. Such "pseudo" egg masses were excluded from my analysis as they presumably represent attempts at mating by already mated or non-ovulated females. I think that the latter is the more likely explanation, as when I was trying to induce ovulation artifically it was often preceded by copious jelly production and the expulsion of a few eggs long before the bulk of eggs had moved into the oviduct. I did not keep detailed records of the frequency of "eggless" egg masses but they seemed about equally common in both call races.

(a)

Locality	Mean	Range	Number of egg masses counted
1.	355.8 ⎤	189 - 724	21
2.	436.5 ⎦	59 - 837	15
3.	782.9]	72 - 2369	15
4.	1217.0]	43 - 2402	12

(b)

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	3	6618589.5	2206196.5	14.83 ***
Within localities	59	8776991.9	148762.6	

(c)

Eggs per ovulation, Western females	Eggs per ovulation, Northern females
438	499
521	465
	1056

Table 4.21 (a) Mean number of eggs per egg mass from recording stations 1 to 4 on the Morgan transect. Means not significantly different at the 5% level are enclosed by square brackets.

(b) Anova on data for which means are given in (a) above.

(c) Numbers of eggs produced in a single ovulation by various females used in artificial hybridisations. All mature eggs in the ovary were shed.

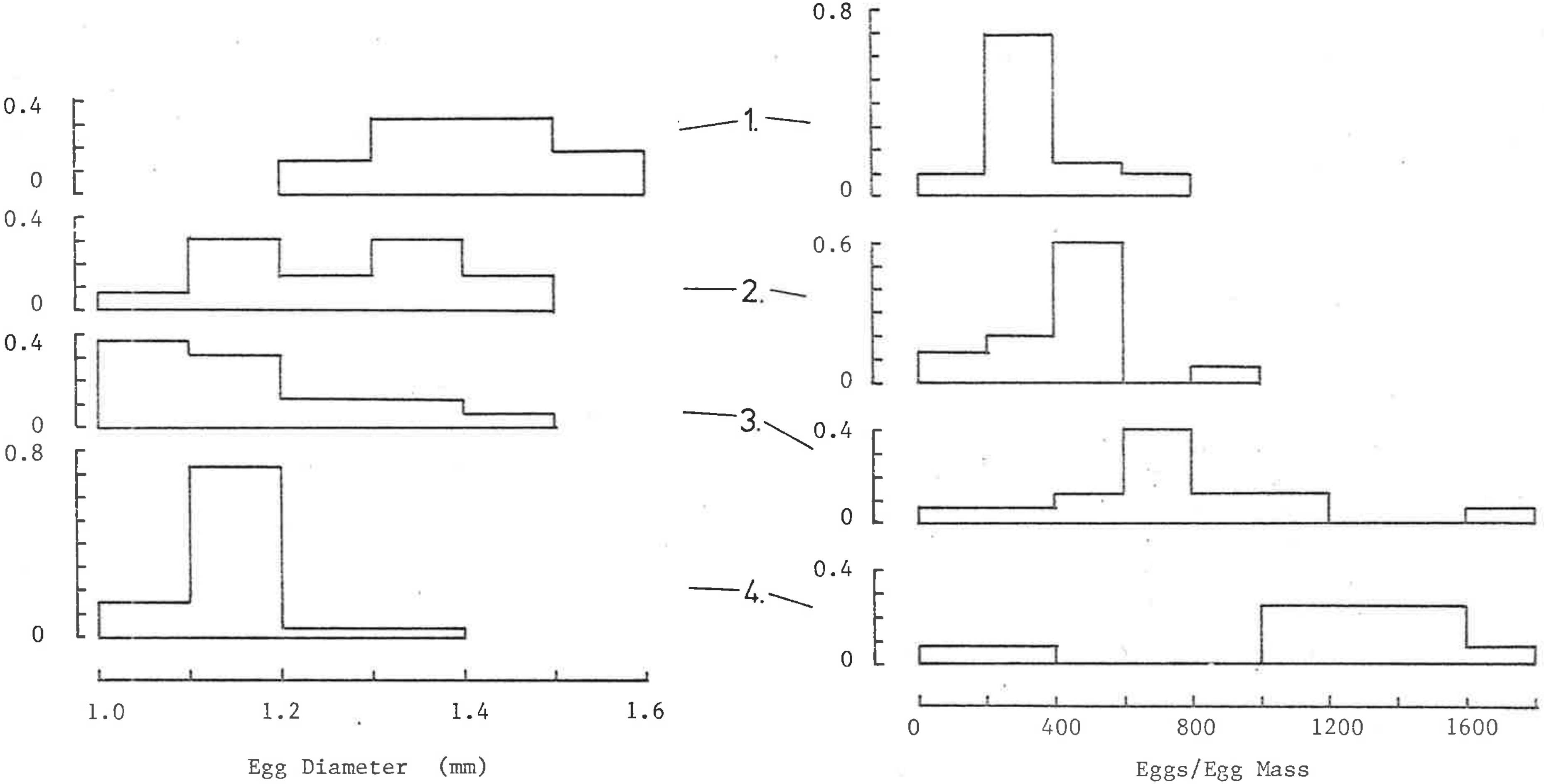


Figure 4.22 Egg diameter and eggs per egg mass data from recording sites 1. to 4. on the Morgan transect. Sample sizes are given in Tables 4.20 (eggs/egg mass) and 4.21 (egg diameter). For both sets of data in this figure the values drawn are relative frequencies in each class. For eggs/egg mass the highest class contains all values above 1600.

ii) Egg size.

Portions were taken from egg masses in the field and immediately preserved in tadpole fixative (Tyler 1966). Ten eggs from this sample were subsequently measured with a calibrated micrometer eyepiece in a binocular microscope. The maximum dimension of the egg (yolk plus vitelline membrane) was taken irrespective of whether the fundamental shape was spherical (the usual situation) or slightly ovoid. All measured eggs were pre gastrula, though other than this I did not standardise the stage of development. I have no evidence that <u>L.tasmaniensis</u> eggs change shape before gastrulation, nor did the data reveal any correlation between size and developmental state.

Some eggs measured came from masses collected at Bower and Morgan on 27.x.1973, but the majority were from collections made at Mount Mary (in lieu of Bower, see section 4.4 1) d) i)) and at recording stations 2, 3 and 4 on 23.iv and 25.iv.1974. Data were analysed with a nested anova and the results of this are given in Table 4.22. The distribution of mean egg diameter/egg mass for each of the four localities is given in Figure 4.22.

The anova showed that, despite significant differences between egg masses within localities, there was additional significant heterogeneity between the four sites. Figure 4.22 suggests that this is largely caused by eggs at locality 4 being smaller than those at 1. Data from localities 2 and 3 overlap the other two samples. Overall mean diameters for the four samples are as follows: station 1, 1.41 mm; 2, 1.26 mm; 3, 1.17 mm; 4, 1.15 mm.

The data on egg size and egg number, although both show some overlap between localities 1 and 4, demonstrate additional differences between in the northern and western call races. The distribution of values of these two variables at the four sites sampled on the Morgan transect are consistent with the information from call analysis. If localities 1

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	3	8.3263	2.7754	29.45*** @
Among egg masses within localities	65	6.1202	0.0942	29.44***
Within egg masses	621	2.0026	0.0032	

Table 4.22 Nested anova on egg diameter data from the Morgan transect. Data from Bower and Mt. Mary were combined. Sample sizes, recording site 1, 21; 2, 13; 3, 16; 4, 19. *** p less than .001. @ based on an amended among egg masses within localities mean square (Sokal and Rholf 1969, page 274).

Call race	Mean egg diameter (mm)	Mean egg number	Yolk volume (mm^3)
Western	1.41	355.8	522.23
Northern	1.15	1217.0	969.13

Table 4.23 Yolk volume estimates for egg masses of the northern and western call races.

and 4 are considered pure western and northern respectively then station 2 (Eba) gave evidence of both call types plus intermediates, and station 3 likewise but with a preponderance of northern like phenotypes.

An interesting consequence of these differences is that the committed energy expended per reproductive event may be much greater larger in northern females. Table 4.23 gives estimates of the volume of yolk material per egg mass for the two call races. The estimates were made by assuming eggs were spherical and that mean egg number and diameter at Bower and Morgan were representative of for the two call races. More realistic estimates of yolk volume could be made if egg size and number information were both collected from single egg masses. I have some data in this form but insufficient to justify detailed analysis.

e) Egg mass form and behaviour in amplexus.

Both call races have a foamy egg mass as has been repeatedly described for eastern Australian <u>L.tasmaniensis</u> (see section 4.2 2) b)). I did not make any direct observation of amplexus in either call race or in hybrid pairs. However, in cages set up for "pseudo-natural" hybridisations, viable egg masses were produced in western and northern control cages, and in the cage containing with northern females and western males, but not in the reciprocal hybrid combination. I cannot comment on the significance of this latter result. There are no morphological differences which might reflect differences in oviposition or amplexus behaviour.

f) Calling sites of males.

Males of both races call in still water, and if sufficient cover is available they are generally well hidden. Western and northern males will call from exposed positions if no suitable alternative is available. There was no segregation of calling sites as was observed between the western and southern call races on the Coorong.

g) Calling and breeding seasons.

Throughout 1972, transects, similar to those described for the Coorong contact (section 4.2 1) b)), were made along the Marne river and the lower river Murray. The transects were made in a single night and localities visited on most trips included Cambrai, recording sites 5 to 7 and occasionally 8, Punyelroo, the Reedy creek mouth area south west of Mannum, Mypolonga and Murray Bridge (see Figure 4.15). In 1973 and 1974 irregular visits were made to sites on the Morgan recording transect.

Several 24 h periods of observation on calling activity were made at the Marne Ford (recording site 6) in 1972. There were no differences between data from these and that obtained on the short stops involved in the transect.

Murray Bridge data on calling activity has already been given in Figure 4.6. Information from the Marne and Morgan transects is presented in Figure 4.23. Observations made at the other localities visited on these surveys agree closely with the patterns in Figure 4.23.

Both call races start calling in late August or early September and continue through to December. Calling may occur at very low levels in mid to late summer (January to March) but peak activities generally only follow heavy rain.

Eggs were seen in various populations of both call races throughout the calling period but insufficient detailed data were collected to detect small differences in the time of breeding initiation or cessation. There was no obvious segregation.

Both call races may call throughout the night and day. Daylight calling occurred most often during periods of peak breeding in the early spring (September) and after rain in the summer. Overnight observations on calling activity were made at Murray Bridge (western

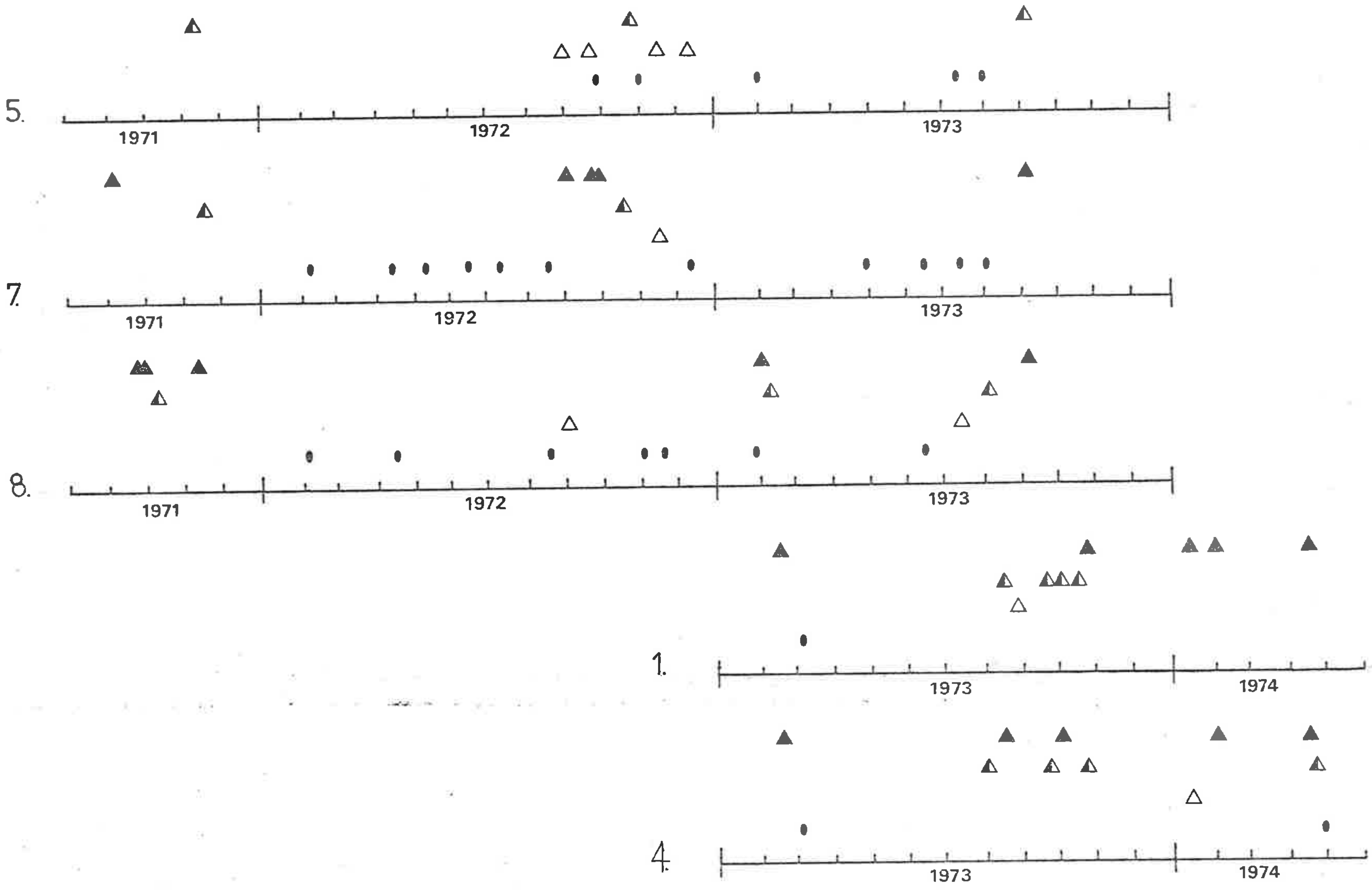


Figure 4.23 Level of calling activity at three sites on the Marne transect and two at Morgan. Numbers refer to recording localities listed in Table 4.16. Symbols are as in Figure 4.6 with closed ovals representing no calling and closed triangles maximum calling activity.

call race) and Kroehn's Landing (northern) while field recording in 1971, at the Marne Ford (recording site 6, mixed population) and Wongulla (mixed population) similarly in 1972 and at Bower (western) and Morgan (northern) in 1973.

Observations on calling and breeding were also made on laboratory populations of both call races. Frogs were caged as in the southern, western study (section 4.2 1) b)). Northern frogs came from the River Murray in South Australia, chiefly from Blanchetown and locations just north of Wongulla. The western frogs were the same as those referred to in the southern, western study. Observations were made from May 1972 to February 1973. Data were collected as in section 4.2 1) b).

First western calls were heard on 14.viii.72 and the first northern on 15.ix.72. Last calls were on 12.xii.72 and 30.i.72 for the western and northern call races respectively. Northern eggs were found on various dates from 5.ix.72 to 21.i.73, and western eggs from 4.ix.72 to 8.xii.72. The laboratory results are thus consistent with observations made in the field.

2) Post-mating isolating mechanisms.

Post-mating isolation between the western and northern call races was investigated by artifical and pseudo-natural hybridisations as described earlier (section 4.2 2)). Progeny from one hybrid cross were raised, but their size at metamorphosis was not recorded.

The zones of interaction contain many animals which have intermediate calls and I have suggested elsewhere that these individuals may have resulted from hybridisation. If this is so, then some proportion of naturally-produced egg masses should have involved either crossing of the pure call races or back or inter-crossing of hybrids. If hybrid inviability exists and is detectable as embryonic failure then this could be should be observed in field collected egg masses. Observations on egg masses have been made in other studies of natural hybridisation and evidence of hybrid debility obtained (Volpe 1960; Watson 1972; Littlejohn, Watson and Loftus-Hills 1971). In all these studies field data correlated well with information from artificial hybridisations.

I counted the frequency of abnormal larvae in the egg masses used in getting eggs per egg mass data. Details of collection and subsequent treatment of these masses was given in section 4.4 1) d). Egg masses from the Morgan series of recording sites only were analysed. After hatching, fifteen to twenty larvae at a time were put in a plastic petri dish and scored for abnormalities whilst viewed through a binocular dissecting microscope. All tadpoles from any egg mass were inspected. Data were expressed as the percentage of all eggs which produced normal, live larvae. This means that any eggs stuck on top of the egg mass and dying from desiccation were scored as abnormal, as were any eggs or tadpoles which died of other causes before counting. Thus, for example, if any egg mass, or portion of an egg mass, suffered oxygen deprivation from the way it was transported or raised, then the true frequency of abnormal larvae would be inflated. I suspect that a proportion of eggs and larvae in some masses died unnaturally, though I cannot prove this.

The results of all methods of hybrid inviability analysis are given in Tables 4.24 to 4.26 and Figure 4.24.

Reciprocal hybridisations were done but neither cross gave consistent evidence of hybrid abnormality (Table 4.24(1)). Field collected egg masses from the Morgan transect gave a similar picture (Figure 4.24, Table 4.25). Very few truly abnormal tadpoles were found in field-collected spawn. The majority scored in this class were dead but apparently normal embryoes or hatchling tadpoles. There was no pattern of one particular type of abnormality recurring in many different egg masses.

(1)

(a) Western female, northern male.

	Control	Experimental	Difference
1.	100 (3)	100 (1)	0
2.	95.83 (24)	89.58 (48)	6.25
3.	44.44 (9)	44.44 (27)	0
4.	94.12 (51)	92.31 (39)	1.81
5.	71.43 (7)	100 (4)	- 28.57

(b) Northern female, western male.

	Control	Experimental	Difference
*1.	(i) 94.51 (91)	97.17 (106)	- 2.66
	(ii) 90.28 (72)		- 6.89
**2.	96.00 (75)	(i) 94.62 (130)	1.38
		(ii) 95.00 (20)	1.00

(2)

Western control	Northern control	Western male, northern female
55.55 (117)	2.51 (199)	40.99 (605)
90.56 (286)	97.55 (693)	43.28 (263)
83.39 (329)	18.55 (221)	1.99 (201)
97.02 (335)	85.81 (310)	51.72 (29)
	1.02 (196)	

Table 4.24 (1) Results of various hybrid combinations of the northern and western call races (artificial hybridisations).

(2) Results of pseudo-natural hybridisations of the northern and western call races.

In (1) data are the percentage of cleaving eggs developing into normal tadpoles. In (2) data are the percentage of all eggs producing normal tadpoles. In both cases the number in brackets is the base for the percentage. In (2) western frogs were from various locations on the eastern side of the Mt. Lofty Ranges, northern from sites on the Murray river. In (2) nearly all abnormals were severely oedematous.

* No control male from same site so crossed to two northern males.

** Eggs from one female fertilised by sperm from two different western males.

Variation	d.f.	Sum of Squares	Mean Square	F
Among localities	3	966.53	322.18	1.663 n.s.
Within Localities	59	11428.06	193.70	

Table 4.25 Anova on arcsine transformations of percentage of eggs developing into normal larvae in egg masses collected across the Morgan transect. The untransformed data are illustrated in Figure 4.24. n.s. : not significant

(a)

Cross	Metamorphosed	Died	Total
W female, W male	17	6	23
W female, N male	27	16	43

$\text{Chi}^2 = 0.837$, n.s.

(b)

Cross	Mean time to metamorphosis (days)
W female, W male	206.8 (8.4)
W female, N male	188.0 (5.2)

$t_{42} = 2.03$ *

Table 4.26 (a) Success to metamorphosis and (b) time (days) to metamorphosis in an artificial hybrid combination of the western and northern call races. In (b) the number in brackets is the standard error of the mean. W western, N northern, n.s. not significant, * p less than .05.

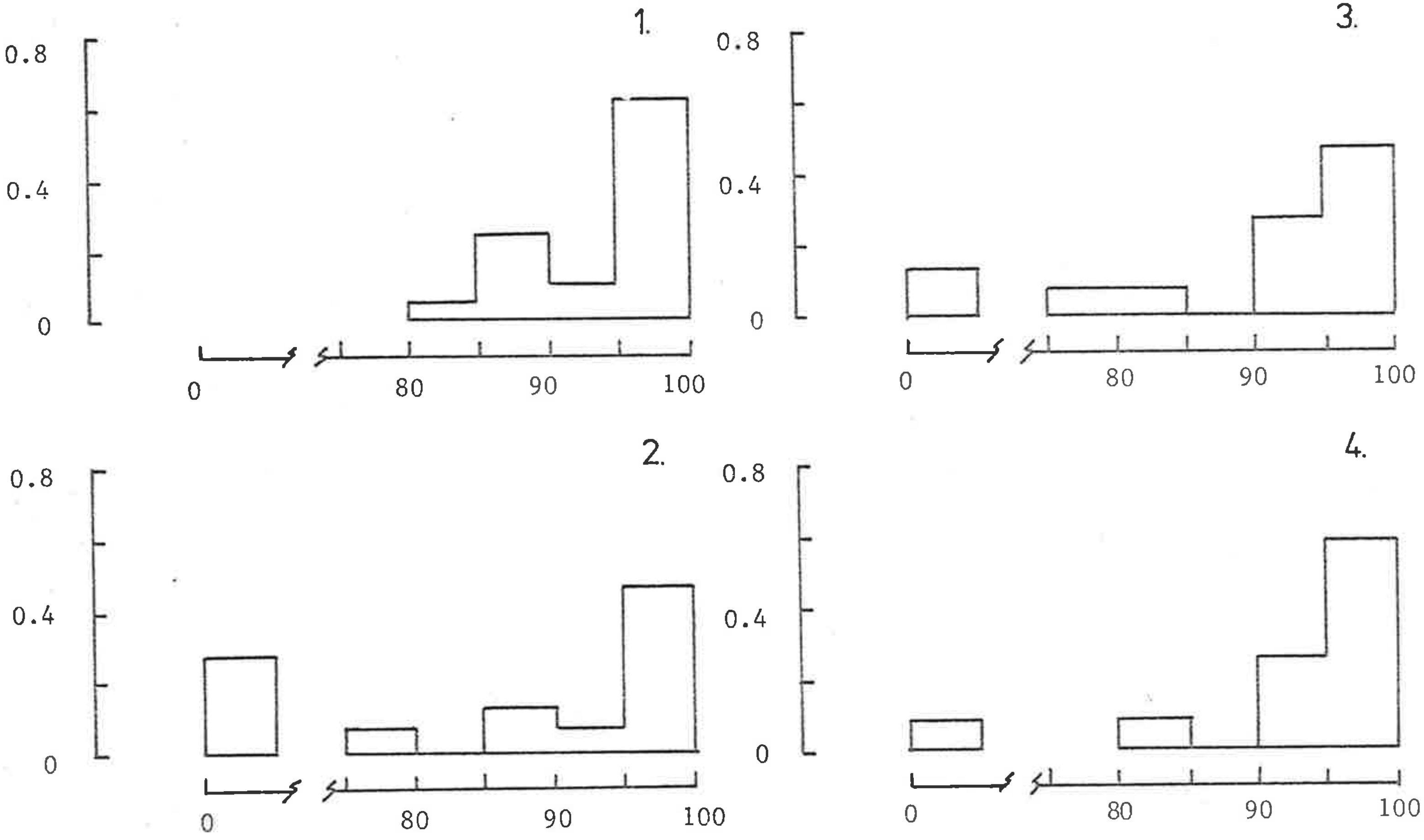


Figure 4.24 Per centage of all eggs developing into normal tadploes from egg masses collected at recording sites 1. to 4. on the Morgan transect. Data are expressed as relative frequencies. Sample sizes are, 1., 21; 2., 15; 3., 15; 4., 12. In each the lowest class contains all masses with per centages below 75.

Pseudo-natural hybridisations gave a rather different result with all hybrid egg masses having frequencies of normal tadpoles around 50% or less (Table 4.24(2)). Western control spawn was generally highly viable in contrast to northern controls where viabilities were either very high or very low. The variability in northern control data makes it impossible to assess the significance of the low probability of hybrid eggs developing into normal tadpoles.

Nearly all abnormal tadpoles from the pseudo-natural hybridisations were severely oedamatous. This form of abnormality did not occur anywhere near as frequently (relatively) in artificial hybridisations. I therefore conclude that some factor in the cages or rearing system used in pseudo-natural crosses was responsible. Clearly, I cannot say whether this factor is present in natural mixed populations of the northern and western call races of L.tasmaniensis, though the data from field collected egg masses do not suggest so.

Hybrids and western L.tasmaniensis are equally likely to metamorphose (Table 4.26(a)), but hybrids may reach metamorphosis more quickly (Table 4.26(b)). These data, however, are from only one cross and I would be unwise to generalise from them.

I did not conduct any crosses using known hybrids, but a hybrid female from cross 2,(Table 4.24(1)(a)) spontaneously ovulated when about sixteen months old and a preparation from the testes of a hybrid male gave normal-looking sperm. Hybrid males can call (section 4.4 1) c)) but no tests of call function were done. However, as western and northern females seem unable to distinguish calls of males of the two races (Chapter 3), I see no reason why hybrid calls should be ineffective.

In summary, all the above data give little evidence that hybridisation between the western and northern call races is deleterious.

3) Geographic distribution, range limiting factors and distribution changes.

a) Geographic distribution.

Detailed distribution data based on subjective auditory analysis has already been given (Figures 4.16 and 4.17). At Morgan the width of the overlap zone is about 17 km if the subjective data are used but if the Morgan and Bower populations truly contain some hybrid animals (see call data, section 4.3 1)) then the zone is much broader, at least 28 km. The Marne river overlap is obviously much narrower with a likely width of about 3 km but a possible maximum of 9 km.

There was no evidence of contact between the call races at the eastern end of Reedy creek, south west of Mannum nor did the northern race occur south of Wall Flat (the southern most record on Figure 4.17). The absence symbols on Figure 4.17 are probably most reliable for the Mypolonga area though all are similarly based. That is, a visit (or visits) has been made to each locality on a night when the western call race was heard at either Murray Bridge or Cambrai and the northern at some site on the river Murray.

The lack of L.tasmaniensis on Sanders and Milendella creeks is not surprising as both are dry channels which rarely flow except in the uppermost reaches close to the hills. The Marne river is peculiar. In winter and spring there is usually a strong flow as far as Cambrai but this disappears below ground a few kilometres east of the town. There is a defined, narrow channel right through to Black Hill but water only moves through this during floods which are short lived and follow heavy rain in the headwaters (I witnessed floods in 1971 and early 1973). 2.7 km east of Black Hill (i.e. at recording station 5) springs produce a large volume of water which results in a year round flow from there to the Marne mouth on the river Murray. The two records of no L.tasmaniensis between Cambrai and Black Hill (Figure 4.17) are thus in areas where there is usually no free water. This interruption may mean there is little or no gene flow from western populations near Cambrai to those east of Black Hill.

Except for those on the river Murray, virtually all records of L.tasmaniensis in the Morgan area are from man-made dams. However, close to Morgan, particularly north and just west, there are areas of low-lying ground where fairly extensive water bodies lay after the record rains in 1973 and 1974. Similar, smaller swamps occur elsewhere in and near the overlap zone. Dam building by man was thus not a prerequisite for the zone's formation. South of the main Morgan contact plotted in Figure 4.16 natural swamps and artificial dams are rare and this, not inadequate investigation, accounts for the lack of data from this area.

I have no records of L.tasmaniensis from east of the river Murray in South Australia and I am fairly confident this species does not occur there. I searched for frogs in this region on several occasions without success. The only free water I saw was in tanks associated with bores, and in some small roadside pools after heavy rains in February 1973. Man-made dams and areas that might act as natural swampland are extremely rare.

There are no literature reports of L.tasmaniensis from this area and no specimens in the South Australian museum collection. In north western Victoria, where climate, soils and vegetation are very similar to those east of the Murray in South Australia, the only anuran species reported by Brook (1975) were in the genus Neobatrachus. Frogs in this genus are burrowers and generally possess specific adaptations to aridity (Main 1968).

Rainfall in the Bower, Morgan area is very low (about 23.4 cm per year, Bureau of Meterology, 1966) and in various schemes for the classification of Australian climates, this region generally falls in the most arid zone (Gentilli 1972). *L.tasmaniensis* does not possess any obvious adaptations to cope with aridity. It does not burrow (cf. Main 1968), nor is it particularly capable of dealing with desiccating environments (War~~n~~burg 1965). It presumably persists because of the large numbers of moist refuges; that is, dams. I suspect, though I have no evidence, that movement between dams would therefore be restricted, except in very wet years.

Thus, as on the Marne, opportunities for gene flow into and within the contact may be minimal. Both contact zones between the western and northern call races would therefore be considered by some (Littlejohn and Watson 1973; Hall and Selander 1973), particularly likely to reveal evidence of reinforcement.

b) Determinants of range limits for the northern and western call races.

In section 4.2 3) a) I presented some possible reasons why the area of sympatry of the southern and western call races was so small. Similar arguments might be invoked to explain the limits on the ranges of the western and northern call races. However, the Murray plains contacts are different from the overlap on the Coorong in that hybrids form a large proportion of most mixed populations. One explanation which has often been offered to explain the width of hybrid belts is as follows. The hybrid zones are said to lie on an ecotone between two different environments with hybrids either better or equivalently adapted to conditions in the ecotone when compared with the parental taxa (Anderson 1948). Transplantation of representives of the pure taxa and hybrids to areas where one would predict they would do poorly, relative to the normal inhabitants, is a simple test of this hypothesis. In an attempt to determine whether the hybrid zone at Morgan lies on an ecotone I conducted a series of transplant experiments with both eggs and tadpoles.

i) Egg mass transplants.

On 27.x.73, nine egg masses were collected at Bower (recording station 1). Each was divided into four roughly equal portions. One was immediately preserved in tadpole fixative (Tyler 1966); a second was put in a terylene sack supported by a poly-eurethane foam ring (Figure 4.25(a)) and floated in water at or near the point of discovery; a third was similarly disposed but in a lagoon on the eastern bank of the river Murray at Morgan (recording station 4), and the remaining portion was brought back to the laboratory.

Nine egg masses collected at Morgan on the same day were treated the same way, except that the transplanted portion was taken to Bower.

Rising floodwaters at Morgan disrupted most cages there, particularly those with western eggs, and data from this site are therefore unreliable. There was no interference at Bower. Results from both sites are given in Table 4.27. The frequency of dead or abnormal tadpoles is much higher in northern eggs at Bower than western controls but the significance of this cannot be assessed due to the failure of the Morgan end of the experiment. Continued flooding during 1973 and again in 1974 meant these experiments could not be repeated.

ii) Tadpole transplants.

1. Fixed cages.

In this experiment cages with no artificial lid or bottom (Fig. 4.25 (b)) were set up in dams at Bower and Eba (recording site 2) and in a lagoon at Morgan. At each site, two cages held western, two cages northern, and one cage hybrid tadpoles. There were initially twenty tadpoles per cage for the western and northern cages, but only seven or six for the hybrids. Hybrid tadpoles were from a pseudo-natural hybridisation of a western male to a northern female. Pure call race tadpoles were the survivors of egg mass transplants plus others collected on the same date. Within each group tadpole sizes were homogeneous

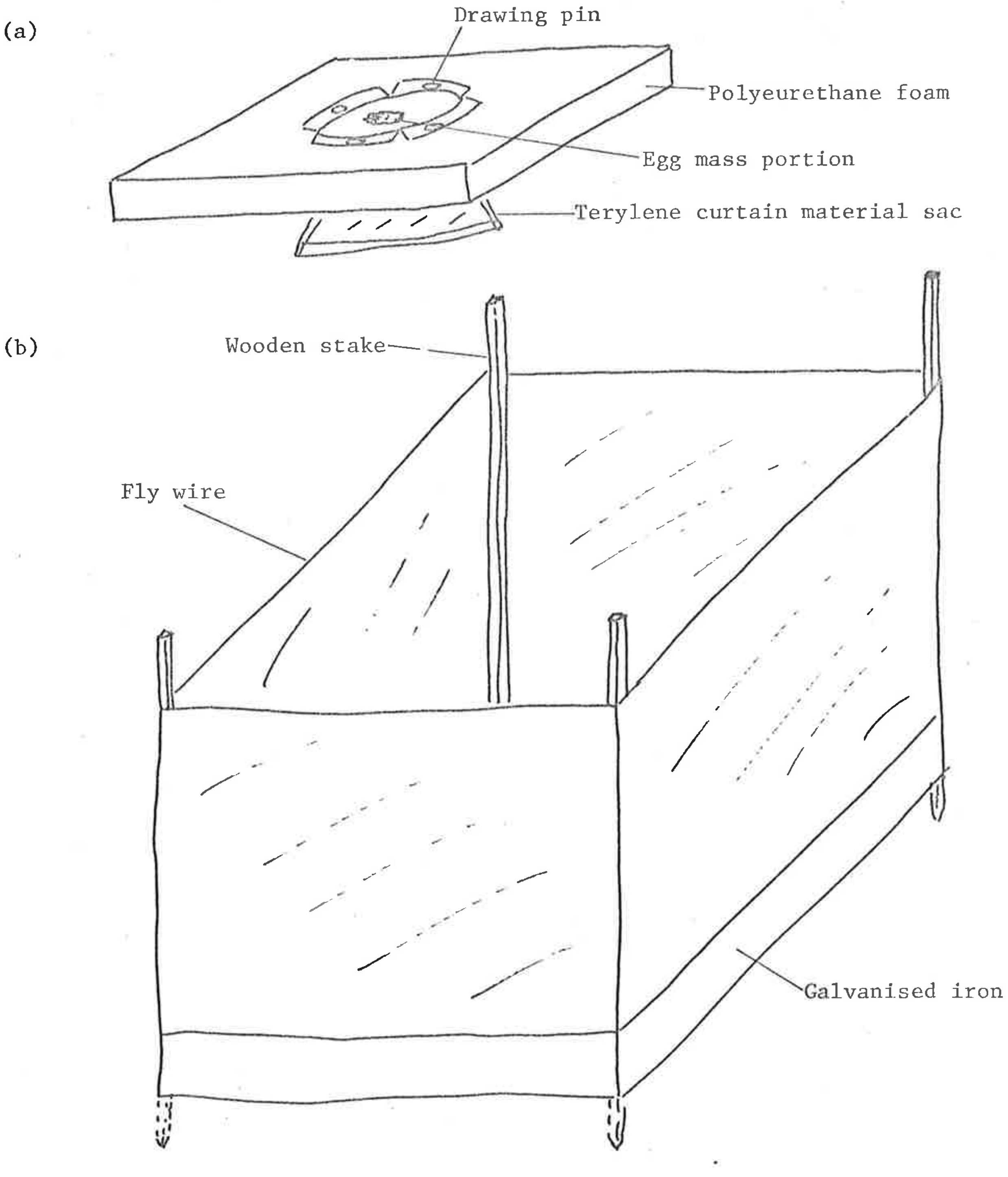


Figure 4.25 (a) Apparatus used to enclose egg mass portions in transplant experiments reported in section 4.4 (3).
(b) Fixed cages used in tadpole transplant experiments described in section 4.4(3).

		Egg mass portion location		Developmental Stage
		Bower	Morgan	
Western eggs	1.	0 (151)	lost	18
	2.	0 (44)	0 (1)	10
	3.	0 (15)	lost	20
	4.	0 (38)	0 (2)	19
	5.	0 (69)	lost	20
	6.	0.92 (38)	lost	18
	7.	0 (86)	0 (14)	10
	8.	0 (49)	lost	9
	9.	0 (42)	lost	7
Northern eggs	1.	0.4 (10)	lost	11
	2.	0 (15)	lost	18 - 19
	3.	0.4 (10)	0 (17)	18
	4.	0 (46)	0 (18)	12 - 13
	5.	0 (128)	lost	12
	6.	0.17 (29)	0 (24)	12
	7.	0.06 (35)	0 (1)	12
	8.	0.16 (45)	0 (46)	17 - 18
	9.	0.24 (33)	0 (3)	12

Table 4.27 Proportion of eggs dying or developing abnormally in egg mass transplant experiments in the Morgan area. Numbers in brackets formed the denominators of the proportions. Developmental stage after Gosner (1960).

(Table 4.28).

After waiting some time for the water levels to stabilise at Morgan, following flooding, tadpoles were eventually put in cages on 8.xii.73. However, water levels fell even further after this date and cages at Morgan were dry within one month. Cages at Eba also dried up. At Bower heavy summer rains had the reverse effect and all cages were eventually completely covered by water. This experiment was thus a complete failure.

2. Floating cages.

To overcome water level fluctuation problems the transplant experiment was tried again, but this time floating cages were used. The cages were those used in analagous experiments on the Coorong (section 4.2 3) b)), but instead of resting on the pond bottom, the cages were buoyed up with large blocks of polyeurethane foam. Only western tadpoles were available when this experiment was started on 25.ii.74. Fifty tadpoles were put in each cage and the survivors counted on 11.iv.74. Results are given in Table 4.29.

The majority, if not all, of the tadpoles in Eba 2 probably entered the cage through the mesh after hatching from egg masses laid around the cage edge. Tadpoles I placed in the cage were too large to pass through and escapes cannot account for the results. Metamorphlings did not escape from these cages during the Coorong experiments, and anyway the maximum tadpole age of 75 days is much less than the minimum time to metamorphosis that I observed in laboratory culture (123 days) or the larval life span estimates for <u>L.tasmaniensis</u> given by Moore (1961) and Littlejohn and Martin (1974) (both suggest 4 - 5 months).

The failure of all transplant experiments means I can only speculate about the presence of ecotones.

c) Vegetation, soils and climate as potential limiting factors.

The vegetation (Specht 1972), soils (Northcote 1960), and presumably

Western	Northern	Hybrid
$F_{5,113} = 0.37$ n.s.	$F_{5,114} = 2.97$ *	$F_{2,19} = 0.08$ n.s.

Table 4.28 F ratios from anova on body length data for tadpoles released in the fixed cage transplant experiments. n.s. not significant, * p between .05 and .01.

Cage	Number of tadpoles	Number of frogs
Bower 1	0	0
Bower 2	0	0
Eba 1 *	0	0
Eba 2	153	0
Morgan 1	0	0
Morgan 2	0	0

Table 4.29 Numbers of frogs and tadpoles found in floating cages on 11.iv.74. * cage overturned.

therefore micro-climate in the actual Murray river valley, are very different from those on the plains extending westwards. These changes are probably irrelevant, though, as the overlap zone is on the plain. There is no environment I would consider intermediate between the plain and the river valley.

Running roughly east west through Morgan is a major north south vegetation change. To the south is, "open scrub" dominated by mallee (Eucalyptus socialis and E.gracilis), and to the north, "open woodland" dominated by either black oak (Casuarina cristata) or sandalwood (Myoporum platycarpum) (Specht 1972). These two vegetation communities are quite distinct and the change from one to the other occurs rapidly. Some faunal changes are associated with the boundary (Smyth 1973; Bull and Smyth 1973), and as part of a study on one of these R.D. Sharrad (Adelaide College of Advanced Education) has charted the distribution of the two floral communities in some detail. Sharrad supplied Figure 4.26 and if this is consulted in conjunction with Figure 4.16 it is obvious that the overlap between the western and northern call races traverses the vegetation boundary at right angles.

Sharrad (pers. comm.) has also noted soil changes in this area. Mallee is found on shallow sandy soils underlain with limestone, and black oak on desert loams with less limestone and higher clay contents (Northcote 1960, discusses these soil types in more detail but does not map them as distinct entities as he is concerned with soil associations).

I could not see any changes in either soils or vegetation along the Marne that are associated with the position of the present overlap of the northern and western call races nor have any been documented in the literature. Vegetation along the Marne river is similar to that described for the river Murray by Specht (1972).

Detailed climatic studies on the lower Murray (Bureau of Meteorology 1955) gave no evidence of major climatic shifts associated with the

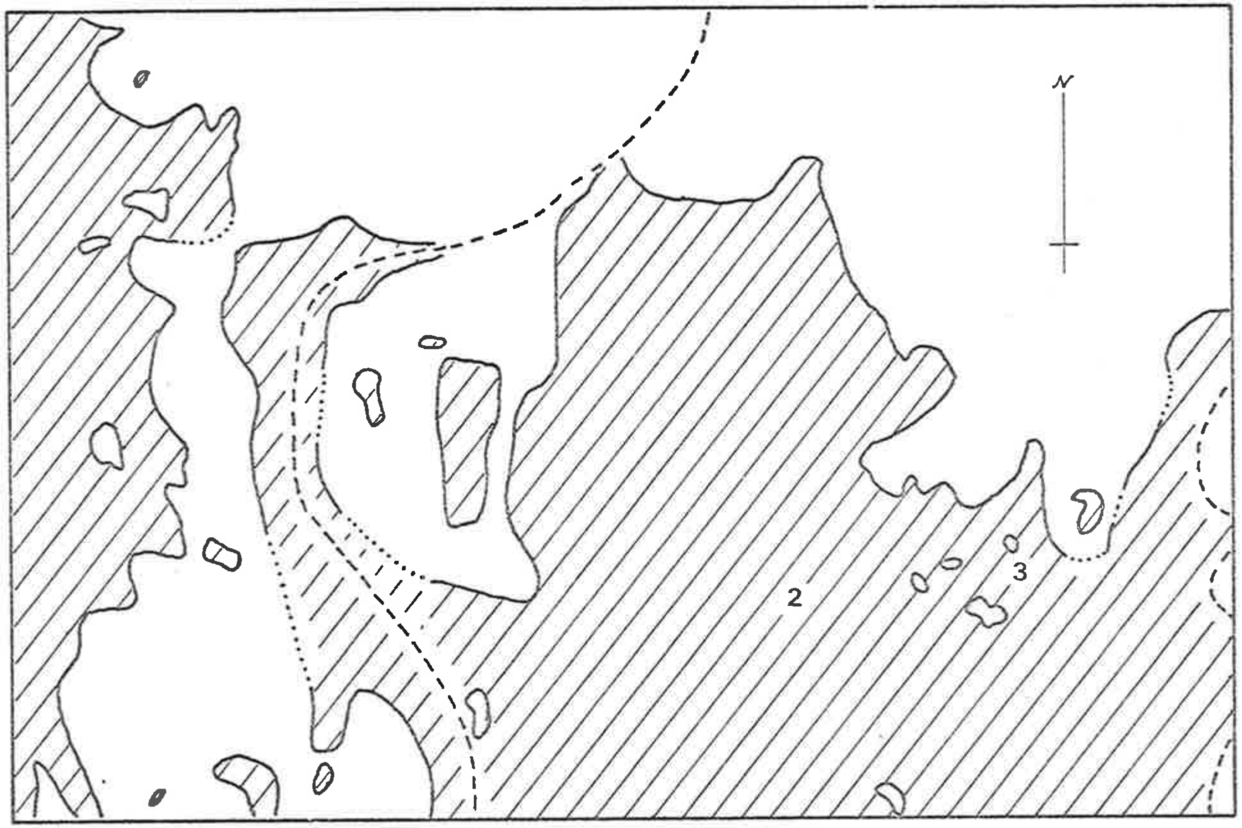


Figure 4.26 Distribution of open scrub (malle; shaded area) and open woodland (black oak, sandalwood; blank) in the Morgan area. The numbers 2 and 3 indicate the position of recording sites listed in Table 4.16. Dashed lines indicate the approximate hybrid zone edge (cf. Fig. 4.16) and dotted lines indicate areas where the vegetation boundary is unclear either because natural vegetation has been cleared or because the area is in-accessible.

position of the overlap on the river Marne. Comparable work has not been done at Morgan but there is little topographic relief likely to have significant effects on climate.

d) Salinity as a limiting factor.

The concentration of various cations (potassium, calcium and magnesium) can affect the growth and differentiation of tadpoles of the frog, Discoglossus pictus (Neauport 1973; Bellec and Stolkowski 1964). This means it is conceivable that differences in water chemistry may constitute subtle environmental gradients influencing the position of the western northern call race overlap zones.

I did not conduct any detailed analysis of the nature or quantities of specific dissolved substances in any waters in or near the contact zones. However, salinity determinations were made on several occasions from 1973 to 1975, either in the field or on samples brought to the laboratory, using an Autolab, Hamon temperature salinity bridge.

Water was sampled at the eight major recording sites and in the Reedy creek south west of Mannum (Figure 4.15). At most locations readings of water were taken at places where L.tasmaniensis eggs are likely to be laid. At recording sites 5 and 6 samples were taken on the downstream side of a culvert through which all water from upstream flowed. I collected data on the Marne transect six times, near Morgan on three occasions and from the Reedy creek twice. Samples were also taken at Murray Bridge in the main stream and in the swamps where western L.tasmaniensis breed.

Main stream samples from the Murray always gave salinity values less than 1 part per thousand as did those from most Murray swamps. However, data from swamps at Murray Bridge ranged from less than 1 to more than 4 parts per thousand while water from shallow lagoons near Mannum and Swan Reach gave values between 1 and 2 parts per thousand. Salinities at two locations on Reedy creek (8 km W.S.W. of Mannum and

11 km W. of Mannum) were consistently high at 3 to over 7 parts per thousand, but all samples were taken in mid summer when flow is minimal and evaporation is probably greatest. The Marne river also gave high readings of between 1 and 2 parts per thousand at recording site 5 and increasing to 2 to 3 parts per thousand at sites 6 and 7. In 1973 and 1974 there was considerable spread of Murray water (recognisable by its turbidity and colour) into the Marne mouth, and on these occasions salinities were the same as those in the Murray's main stream.

At Morgan there was no pattern of salinity variation consistent with that on the Marne or Reedy creek. Values in water samples from dams (recording sites 1 - 3 plus others throughout the overlap) never exceeded 1 part per thousand. However, these data were taken in 1973 and 1974, years of record or near-record rainfall, and it is possible that salinities are higher in drier years.

Although salinity is a rather crude measure of water quality my data show clear differences between the Morgan and Marne river overlap zones. The higher salinities on the Marne (or some factor contributing to them) may be limiting the range of the northern call race and/or spread of northern genes into western populations. Salinity would therefore constitute a relatively subtle ecotone or environmental disjunction causing the Marne overlap to be much narrower than that at Morgan.

However, there are some difficulties with this argument. The northern call race exists over a considerable length of the Marne and has thus demonstrated its ability to cope with high salinities. Secondly, the data I collected suggested that salinities increased downstream from Black Hill, and the northern call race has thus already successfully traversed the major hurdle to westward movement.

However, water at the Marne mouth is not always of relatively high salinity. During floods on the river Murray (as in 1973 and 1974) water from this stream spreads into the Marne mouth area. Even so, Murray water does not spread far upstream, about 1.4 km measured along the stream bed, and northern *L.tasmaniensis* have spread at least 6.2 km along the Marne river. Influxes of low salinity water at the Marne mouth are therefore not very likely to have facilitated westward movements by the northern call race along the river Marne.

Without data from the Morgan area in more normal years, without a detailed experimental investigation of salinity effects on *L.tasmaniensis*, and in the face of the arguments I have put in the paragraph above, I think it is unwise to conclude that salinity tolerance differences between the northern and western call races affect the nature or position of the present contact zones. I have no evidence that any other sorts of ecotones exist.

e) Changes in the distribution of the northern call race.

Until 9.ix.74 the known southern limit of the northern call race on the river Murray in South Australia was Wall Flat (Figure 4.17) where a few animals were heard calling on 16.ix.72. The only certain occurrence of *L.tasmaniensis* on the Murray, below Wall Flat, was at Murray Bridge where the western call race was known from a swamp below the abbatoirs (just north of the road bridge over the river) and from a long swamp extending from the southern side of the road bridge (behind Sturt Reserve) to opposite the north west end of Long Island. This information is from my own observations during 1971, 1972 and 1973 and is supported by observations by M.J. Littlejohn at Sturt Reserve and by one specimen of *L.tasmaniensis* in the S.A. Museum collection, collected at Long Island (Register number 4403).

This is the only *L.tasmaniensis* from the river Murray in the S.A. Museum collection, though other large species common along the river (*Litoria aurea* and L *peroni*, *Limnodynastes dumerili* and L *fletcheri*) are represented by specimens from a number of localities. There are no literature reports of L.tasmaniensis from specific sites on the lower Murray (Moore 1961), but Littlejohn (1967, Figure 6.5) showed L.tasmaniensis as ranging right along the Murray and west to the coast. However, this distribution was inferred from records of L.tasmaniensis on the upper Murray and Murray Bridge and was made before the northern and western call races were distinguished.

On the night of 9.ix.74 I made a traverse from Wellington to Reedy creek (Figure 4.27). Calls of northern L.tasmaniensis were heard at the localities listed in Table 4.30. In each case only a few individuals were calling. The northern call race had thus apparently extended its range southwards by at least 40 km. This movement, if it is such, occurred during years when there was extensive flooding in the spring which may have aided the downstream dispersal of either eggs, adults or tadpoles.

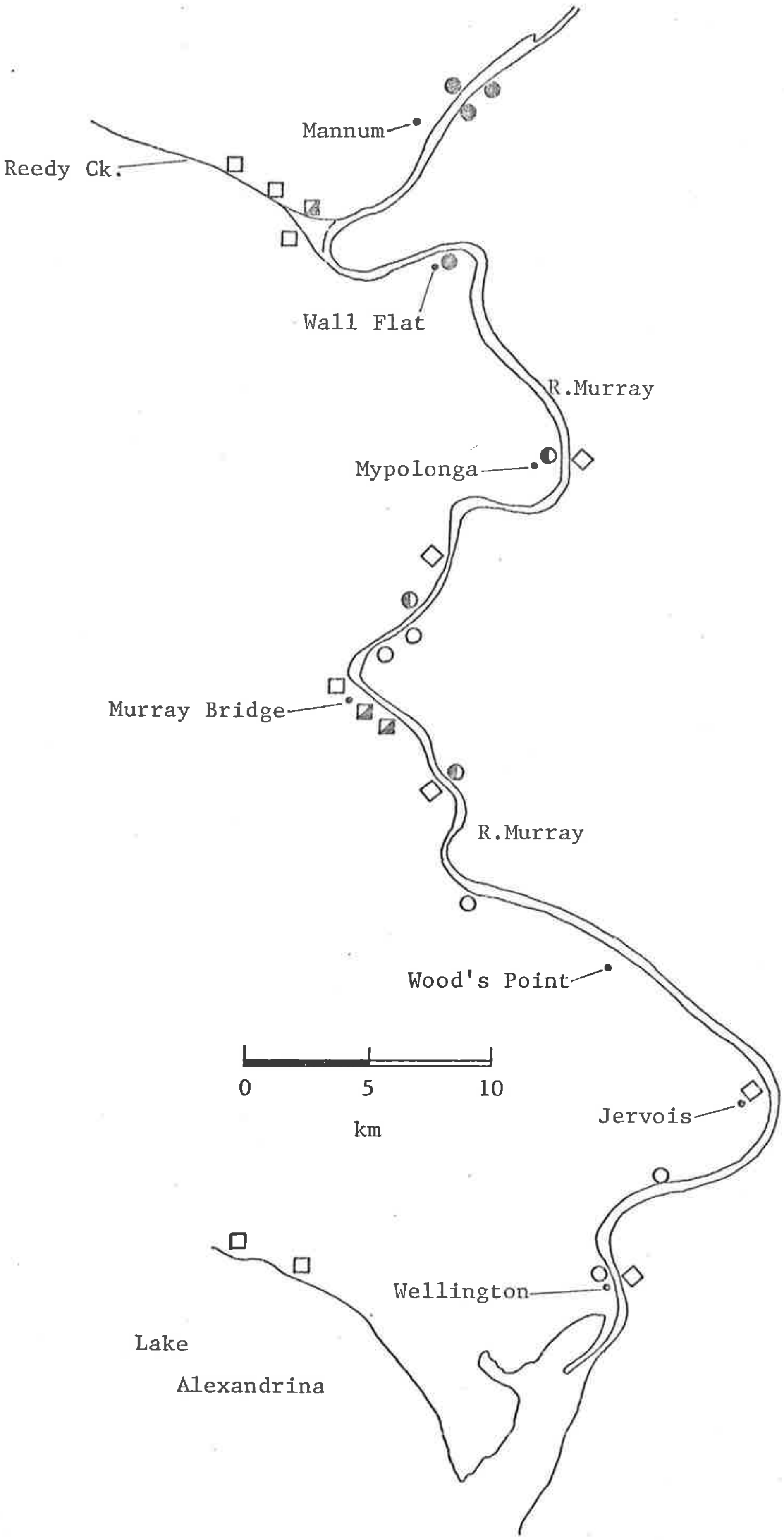
However, several alternatives to the above interpretation should be mentioned. It may be that prior to 1974 I had not done sufficient investigation to detect the northern call race. This could be true in the Wellington area but is not so for the region between Mannum and Murray Bridge. Details of visits to most of the spots I went to on 9.ix.74 are given in Table 4.31. In other areas, the northern and western races call approximately synchronously (section 4.4 1) g)). Thus I would expect nights when either or both western and northern calls were heard to be occasions when the northern call race might be expected to vocalise on the lower Murray. For localities 6, 10 and 11 in Table 4.31 the number of visits on such nights is large. These data are a strong argument against the notion that I sampled inadequately on the lower Murray.

A second argument against dispersal is the large numbers of individuals involved and the total distance moved. The minimum distance from Wall Flat to Wellington is about 42 km, but the distance along the river

Figure 4.27 Distribution of the western and northern call races of *L.tasmaniensis* on the lower river Murray in South Australia. This map includes changes observed on 9.ix.74.

Legend

□	Western call race only
●	Northern call race only
◇	No *L.tasmaniensis*
◪	Western only before 9.ix.74 but northern also present on that date
◐	No *L.tasmaniensis* before 9.ix.74 but northern present on that date.
○	Northern call race heard on 9.ix.74 but no previous visit.
•	Town


Mannum
Reedy Ck.
Wall Flat
R.Murray
Mypolonga
Murray Bridge
R.Murray
Wood's Point
0
5
10
km
Jervois
Wellington
Lake
Alexandrina

	Locality	Map and Grid reference*		
1.	1.7 km N.N.W. of Wellington	Wellington	6727-II	60904 3513
2.	5.0 km N.N.E. of Wellington	"	"	60936 3543
3.	5.7 km N.W. of Wood's Point	Mobilong	6727-I	61042 3480
4.	Swanport Reserve, 5.0 km S.E. of Murray Bridge**	"	"	61087 3462
5.	Long Island, 1.9 km S.E. of Murray bridge***	"	"	61111 3443
6.	Sturt Reserve, Murray Bridge***	"	"	61120 3433
7.	Riverglades, 3.05 km N.E. of Murray Bridge+	"	"	61146 3448
8.	Avoca Dell Reserve, 4.4 km N.E. of Murray Bridge+	"	"	61157 3457
9.	6.3 km S.W. of Mypolonga	"	"	61168 3454
10.	Mypolonga	"	"	61203 3505
11.	7.2 km S.W. of Mannum	Tepko	6728-III	61326 3389

Table 4.30 Localities where northern L.tasmaniensis were heard calling on 9.ix.74, but where there was no previous record of this call race. Unless indicated below all sites are on the western side of the Murray river.

* 1:50,000 topographic series issued by the South Australian Department of Lands (1st. Edition)

** Calls emanating from a swamp on the eastern bank, grid reference is to listening site on the western bank.

*** Northern callers in a dense chorus of western males.

\+ Eastern side of the Murray river.

Locality	No *L.tasmaniensis* calling	Western, no northern calls	Western and northern calls	Northern, no western calls
4.	-	1*	1	-
5.	-	1*	1	-
6.	6	6**	7	1
9.	-	-	2	-
10.	5	1	6	1
11.	4	3	4	1

Table 4.31 Numbers of visits to sites on the lower river Murray where the northern call race had not been heard before 9.ix.74. The numbers under the column headed locality refer to sites listed in Table 4.30. Criteria for the various categories were as follows.

No *L.tasmaniensis* calling	Calls of neither race heard anywhere on the Murray.
Western, no northern calls	Western calls heard at 6. or 11. but no northern calls at or north of Wall Flat.
Western and northern calls	Western calls at 6. or 11. and northern calls at or north of Wall Flat.
Northern, no western calls	Northern calls at or north of Wall Flat but no western calls at 6. or 11.

* includes one visit when there is no information on whether northern call race calling or not.
** " two " " " " " " " " " " " " " "

valley is much greater at close to 60 km. This may not be an impossible distance as flood induced range extensions of a few to almost 100 km have been reported for three anuran species, Scaphiopus bombifrons, Gastrophryne olivacea and Bufo cognatus (Metter, Morris and Kangas 1970). In contrast, unaided movements by Litoria ewingi introduced to New Zealand averaged only 1 km per year (Gill 1973).

The only other typically eastern Australian anuran species which did not reach to the lakes at the Murray mouth prior to 1974 was is Ranidella parinsignifera. The known southern limit for this species in South Australia before 9.ix.74 was and still is Scrubby Flat on the eastern bank of the river Murray, 7.5 km due south of Wongulla. As far as I could determine the range of this species did not change following the floods in 1973 and 1974. This could be a further indication that L.tasmaniensis did not move as one might have expected a contemporaneous downstream shift in the two species. However, there are several differences between R.parinsignifera and L.tasmaniensis which may account for why the former species did not expand its range. R.parinsignifera is much smaller as an adult ranging from 18.3 - 22.7 mm long (Littlejohn and Martin 1965b) compared with 31.7 - 39.4 mm for L.tasmaniensis (Moore 1961). The egg mass of L.tasmaniensis floats for several days before the tadpoles hatch but eggs of R.parinsignifera are probably laid singly or in small clumps on the bottom (Watson and Martin 1973; Main 1957). Larger adults may have a greater resistance to injury or fatigue when shifted around by fast flowing floodwaters. Floating egg masses may be moved relatively large distances before hatching, something which seems unlikely to happen to R.parinsignifera eggs given the way they are deposited.

The correct interpretation of these observations may never be made, but if the northern call race dies out on the lower Murray in succeeding years, or if it becomes much more abundant in areas like the swamps populated by the western call race at Murray Bridge, then the hypothesis of flood-induced range extensions is supported.

All the above argument is based on subjective assessment of calls heard in the field. In areas where there were no previous records of *L.tasmaniensis* this is less relevant, but at Murray Bridge, for example, more definite evidence would be desirable. I made several attempts to record the presumptive northern callers at Murray Bridge but all failed. The small number of calling individuals (a maximum of three in an area of about 6 ha), the overgrown nature of the swamp, and the large numbers of other anurans vocalising (notably western *L.tasmaniensis*, *Ranidella signifera* and *Litoria ewingi*) made recording particular frogs an impossible task.

4) Discussion.

My investigations of call structure and size and number of eggs suggest that at Morgan and along the Marne river the western and northern call races overlap and hybridise. However, despite the lack of effective premating isolating mechanisms (Chapter 3; section 4.4 1)), and the probable low rates of gene flow into the overlap zones (section 4.4 3) a)), I found no evidence of reinforcement of calls or any other character likely to act as a premating isolating mechanism.

The most likely reason for this is that hybrids are fully viable (section 4.4 2)), and that, therefore, the selective force needed to promote reinforcement does not exist. I have no information on the ecological tolerances or capabilities of hybrids but within the contact zones, at least, they are presumably at no disadvantage as they form a significant proportion of all mixed populations sampled (Table 4.18; Figures 4.20 and 4.22).

Outside the overlap zones I can only guess at the fate of hybrids and members of the non-resident call race. Western, northern and hybrid frogs co-exist inside the overlaps, and because there are no obvious environmental constraints on contact expansion (section 4.4 3) b)) it is tempting to suggest that the zones might broaden. If this were so, then the high genetic compatability of these two taxa might allow introgression and ultimately fusion of the western and northern call race genotypes. The nature of the resultant population is difficult to predict accurately, though there would probably be a range of phenotypes from pure western to pure northern, perhaps with a relatively high proportion of intermediate types (cf. Thaeler 1968).

If massive introgression leading to genotypic fusion is occurring between the western and northern call races then I can make some predictions about the future present forms of the zones. First, all contacts, but particularly those at Morgan and on the Marne, should increase in width and there should be a progressive loss of identity of the western and northern call races. These changes could only be established by continued monitoring of the present hybrid zones. Second, assuming approximately equal conditions in all areas, the width of any particular zone should be a function of its age with older contacts being wider.

The most obvious interpretation of the distribution of the northern call race in South Australia is that it entered that state by moving down the river Murray (section 4.4 3) c); cf. Littlejohn 1967). Downstream contacts would be younger and should, therefore, also be narrower. The Morgan hybrid zone is much wider than that on the river Marne (section 4.4 3) a)), and this is in accord with my prediction.

Otherwise there is perhaps little reason to argue that introgression is necessarily occurring in the western, northern call race contact zones. In contrast with other studies where introgression has been invoked (e.g. Martin 1972; Avise and Smith 1974), the Murray plains hybrid zones are very narrow (though this may be because they only recently formed (cf. section 4.4 3) c)).

What of other theories that have been used to explain the nature or position of hybrid zones?

Littlejohn, Watson and Loftus-Hills (1971), Watson (1972) and Thaeler (1968) have suggested that hybrid zones represent a quasi-stable situation where hybrids are at a disadvantage but that any tendency for this to promote reinforcement of premating isolating mechanisms is nullified by input of "naive" (Watson 1972) individuals from adjacent pure populations.

Key (1974) also argued that hybrid inviability may keep hybrid zones narrow, but in his model there was no mention of reinforcement. Key was concerned with explaining narrow, tension (i.e. hybrid) zones between chromosomal races of morabine grasshoppers. Individuals heterozygous for the two possible chromosome arrangements were absorbed by the recipient homozygous population and lost out because of their reduced fertility. The selection against hybrids must be severe enough to ensure that homozygotes for chromosome arrangement A do not crop up in pure B populations from interbreeding of AB heterozygotes.

Although both Watson (1972) and Key (1974) discussed examples where the position of hybrid zones was correlated with an environmental change, this is not an essential part of either model. Critical to both though, is the existence of some clear-cut hybrid inviability. In Key's cases this was manifest as reduced fertility caused by meiotic irregularities in chromosomal hybrids. In Watson's hybrid zone the debility showed as chronic anophthalmia in crosses of males of one species (*Litoria paraewingi*) to females of the other (*L.ewingi*). A similar failure occurred in analagous crosses involving hybrid males.

I have no data on the form of chromosomes in any of the call races of *L.tasmaniensis*. However, the hybridisation studies I conducted (section 4.4 2)) eliminate hybrid failures of the type observed by

Watson (1971). Clearly more information is desirable but from the available data I would not suspect that hybrid inviability is contributing to the nature or narrowness of hybrid zones between the western and northern call races.

The last theory about hybrid zones is the ecotone hypothesis that I have already discussed briefly in section 4.4 3). Many authors have claimed that the hybrid zones they studied lay on ecotones between fairly obviously different (Jackson 1973; Hunt and Selander 1973; Montanucci 1970; Yang and Selander 1968; Hagen 1967) or subtly different environments (Littlejohn, Watson and Loftus-Hills 1971; Littlejohn and Watson 1973; section 4.3 of this thesis). The hybrids were reckoned to be as well or better adapted to the ecotonal environment as representatives of the pure taxa. In the cases where subtle ecotones were suspected, various intermediate phenotypes were the sole form found in the hybrid zones. Hybrid superiority was argued as no logical alternatives were available.

In the Murray plains overlaps there is no evidence of major ecotones (section 4.4 3) b) to d)), nor any need to suspect subtle ones. Hybrids do not predominate in mixed populations, though this is clearly not the only reason one should be alert to the presence of subtle environmental changes. Hybrid superiority could only definitely be established by measuring the performance of hybrids alone and when mixed with pure western and northern frogs. The performance trials would have to be done both in and outside the known contact areas. My own unsophisticated attempts at such trials (section 4.4 3) b)) were a complete failure.

Bull (1973) was also interested in understanding the dynamics of hybrid zones, but rather than plumping for one or other extant theories he constructed a simple model which he hoped to use to predict the fate of hybrid contacts.

Bull argued that secondary contact between formerly allopatric isolates derived from a single species could have three possible outcomes; namely sympatry, introgression or parapatry. He suggested that the result in any particular case was determined by the reproductive success of individuals colonising the range of the alternate isolate. Bull attempted to model one aspect of the colonization process and derived a parameter, colonizer offspring success, which was a function of the levels of premating and postmating isolation, the numbers of colonizing individuals, and the ecological suitability of the area for colonizers.

Low colonizer offspring success, the presumed cause of parapatry, could occur when ecological suitability was 100% but only if the levels of premating and postmating isolation were relatively high (about mid range on a scale from 0 - 100). When premating and postmating isolation were minimal and ecological suitability high, then colonizer offspring success was also high and introgression was the predicted result.

However, there are some drawbacks with this model. It has only once been tested with data from natural populations and in this case it failed. <u>Ranidella insignifera</u> and <u>R.pseudinsignifera</u> hybridise and are parapatrically distributed (Littlejohn 1959; Main 1968; Bull 1973). Values of colonization rate, premating and postmating isolation levels and ecological suitability estimated by Bull and fitted to his model predicted high colonizer offspring success. One would thus have suggested introgression as the ultimate result rather than the observed stable parapatry. Bull (1973) felt that the model's failure was not caused by inbuilt errors but more likely by inaccurate estimation of relevant parameters.

The chief problem with this model is that it can only predict the likely reproductive success of a particular individual under fixed conditions at a particular point in time. It cannot deal with temporal variation in any of the input parameters. This is particularly important, as inter-taxon gene flow (cf. Wilson 1965) or the presence of hybrid animals resulting from an initial colonisation event may markedly change the performance of subsequent immigrants. Thus, although reproductive failure by colonizers may contribute to the maintenance of parapatric distributions, Bull's model cannot demonstrate this conclusively. However, despite all these possible complications it is interesting to note that this model would probably predict high colonizer offspring success in the Murray plains contacts as there is little evidence of significant pre mating or postmating isolation, colonization rates may be high (cf. distribution changes noted in section 4.4 3) e)) and ecological suitability of the alternative range may also be high.

Thus, my own prediction and that of Bull's model are coincident in suggesting introgression may be occurring between the western and northern call races. An important assumption for both these forecasts is that there are no ecological barriers to the expansion of the range of either call race. This may not be strictly true.

On the lower river Murray between Wall Flat and Wellington, western <u>L.tasmaniensis</u> have only been found at Murray Bridge (Figures 4.17 and 4.27). This may be evidence that some feature of the environment of the actual river valley precludes the persistence of this call race. If this were so there would be a significant barrier to eastwards expansion of the present contacts. However, other than habitat unsuitability, there may be several reasons why western <u>L.tasmaniensis</u> are not found extensively on the lower Murray.

Virtually all natural river swampland from Mannum to Wellington has been artifically drained, starting in 1881, and is now used almost exclusively for dairying (Williams 1974). Western call race populations may have been eliminated by destruction of appropriate habitats. I consider this an unlikely explanation, though, as some small areas of unmodified swamp still exist (notably on the eastern bank at three locations; Mypolonga (Sunnyside swamp), Swanport and just north of Murray Bridge), and western L.tasmaniensis do not occur there. These swamps are similar to those where this call race is found at Murray Bridge and to the swamps throughout this section of the Murray before drainage (cf. description of Mobilong Swamp pre cultivation, etc. in Williams 1974; particularly Williams' Fig. 72).

Since the construction of locks along the river and barrages at the Murray mouth salinity may be more consistently low. In the past salinities were occasionally quite high in lakes Albert and Alexandrina and upstream as far as Mannum, though whether this was from natural causes (drought, low river levels and inflow of seawater through the Murray mouth and thence upstream) or from irrigation practice on the upper reaches of the river, is unclear (Interstate Royal Commission report 1902; see particularly Minutes of evidence pages 20 to 33). Highest salinities occurred from late summer to midwinter and were correlated with low river levels and flow rates. With western L.tasmaniensis breeding most prolifically in spring to summer, the saline water may have had little effect on any extant populations though it is impossible to know for certain. It is possible that highly saline water occasionally decimated L.tasmaniensis on the lower Murray.

Since the completion of barrages at the Murray mouth in February 1940 salinities in the river have generally been low (Stephens 1967), and this may mean that L.tasmaniensis can now survive there (e.g. at Murray Bridge).

Finally, viable populations of the western call race might never have been established, as individuals reaching the river valley were too few numerically or too widely dispersed. I have noted elsewhere (section 4.4 3) a)) that most South Australian drainages into the river Murray flow intermittently. Thus, in the past, as now, opportunities for easterly migration by frogs of the western call race were probably limited by the amount of rainfall in the Mount Lofty ranges. Precipitation in this region has almost certainly varied considerably over the last few thousand years (cf. Dodson 1974a, 1974b; Bowler and Hamada 1971). But, for about the last one thousand years rainfall in southern Australia was roughly as it is now (Bowler and Hamada 1971; Dodson 1974b), or possibly increasing to the present from a minimum 750 - 450 years ago (Dodson 1974a). Thus streams on the eastern side of the Mount Lofty ranges probably flowed as often as, or even less frequently than they do, today. Additionally, river level fluctuations occurring before the construction of locks and other water control devices along the river Murray were much more marked (Interstate Royal Commission report 1902). This may mean that many swamps now kept permanently wet by the artificially maintained water height were often, or perhaps more realistically, occasionally dry thus reducing the chances of western immigrants surviving.

If the above explanation for the absence of western L.tasmaniensis from the lower Murray is correct, then any populations of this call race extant on the river before the northern influx were probably similar to that now found at Murray Bridge. That is, they extending over only a short section of the river and were confined to the western bank.

This would, of course, also mean the northern call race has overrun western call race populations at Morgan and the Marne mouth and is spreading west. The required advantage to the northern call race is unclear but it may be that this race is better adapted to the arid environment of the Murray plains.

Northern L.tasmaniensis produce many more eggs than western (section 4.4 1) d)), and high fecundity has often been cited as an adaptation to harsh, unpredictable (Price 1974; Salthe and Duellman 1973), or arid environments (Main 1968). I do not mean to imply that necessarily, high egg number alone gives the northern call race an advantage. Rather, the large egg number may be one obvious trait in a suite of adaptations which enable northern L.tasmaniensis to persist better in arid environments than representatives of the western call race.

I could speculate endlessly on why western L.tasmaniensis is not found throughout the length of the lower river Murray. Suffice to conclude that I have presented three reasonable possibilities, none of which involves the existence of an extant environmental barrier.

I will conclude this discussion by reiterating that I found no evidence of reinforcement in contacts between the northern and western call races. If my introgression prediction is correct, then reinforcement will not be expected, nor is it likely to have ever contributed to the differentiation of these two call races. Thus, the differences in northern and western calls must have evolved, allopatrically.

5. Geographic variation in call structure.

5.1 Introduction.

The data presented in Chapter 4 eliminated reinforcement as a cause of variation in call structure in the three modern races of the *L.tasmaniensis* complex and suggested that this process was unlikely to have contributed to the evolution of the southern, western and northern call races. In understanding the origins of this complex I am thus left with the other two theories outlined in Chapter 1; namely, adaptive change of an isolating mechanism in the face of a signal transmission difficulty or, variation in an isolating mechanism caused by adaptation in some unrelated context.

As with reinforcement, it will be difficult to prove that either of these mechanisms was definitely involved in the evolution of the *L.tasmaniensis* complex.

However, a study of geographic variation in call structure may give some clues to factors that effect *L.tasmaniensis* calls and which may previously have led to the evolution of the present call races.

Geographic variation of call structure will generally take one of three forms.

1. Population means may differ.
2. Variability of the studied parameter may be different in different populations.
3. Novel calls, that is calls with previously unencountered components, may appear in some populations.

There may be interactions or combinations of each of these effects, but they can be detected singly by standard statistical analyses. Differences in intrapopulation variability of temperature independent parameters were analysed by Bartlett's test or the F_{max} method (Sokal and Rohlf 1969, page 370). If there was no heterogeneity of sample variances, Anova were conducted to detect differences in sample means. Otherwise, differences in population location were determined by Kruksal-Wallis non-parametric analyses of variance or the parametric procedure outlined by Sokal and Rohlf (1969, page 372) for an approximate test of equality of means given sample variance heterogeneity.

As before (Chapter 2), regression lines fitted to the note repetition rate, water temperature data were compared by analysis of covariance. Novel calls were detected by inspection.

Data from ten western, five northern and five southern call race localities were analysed, with the races treated separately. The majority of these data have been analysed in various ways elsewhere in this thesis. However, there are four new western localities; Muratchina dam, Aroona valley, Chamber's Gorge and Finke Springs. Details of recording dates, numbers of frogs recorded and the geographic location of these last four sites are given in Table 5.1.

The other localities considered are as follows. Western call race: Petherick Road, Magrath Flat (see Table 4.1), Murray Bridge, Gawler (see Table 2.2), Black Hill, Bower (see Table 4.17). Northern call race: Mulwala (see Table 4.15), Morgan, Kroehn's Landing (see Table 4.17), Blanchetown and Big Bend (see Table 2.2). Southern call race: Petherick Road, Kingston (see Table 4.1), Mount Hope Drain, Moyston (see Table 2.2), Clunes (see Table 4.15).

The geographic position of all localities is given in Figure 5.1.

Summaries of call component values are given as means, ranges, standard deviations, and 95% confidence limits on the means in Figures 5.2 to 5.4. Fitted regression lines for note repetition rate, water temperature data are plotted in Figure 5.5. Results of various statistical analyses are summarised in Tables 5.2 (temperature independent variables) and 5.3 (temperature dependent variables). In discussing these data and the analyses I will deal

Locality	Map and Grid reference	Recording date	Number of frogs recorded
Muratchina Dam, 32 km N.N.E. of Kimba	SI 53-4 452939	18.ii.73	14
Aroona Valley, 28 km S.S.W. of Blinman	SH 54-13 149121	3.iv.71	18
Chamber's Gorge, 56 km N.E. of Blinman	SH 54-9 215168	5.iv.71	17
Finke Springs, 29.8 km E.N.E. of Copley	SH 54-9 162219	4.iv.71	8

Table 5.1 Details of recording localities for western populations not given elsewhere in this thesis. Map and grid references are to Series R 502, Edition 1 maps, scale 1:250,000, Department of National Development, Canberra.

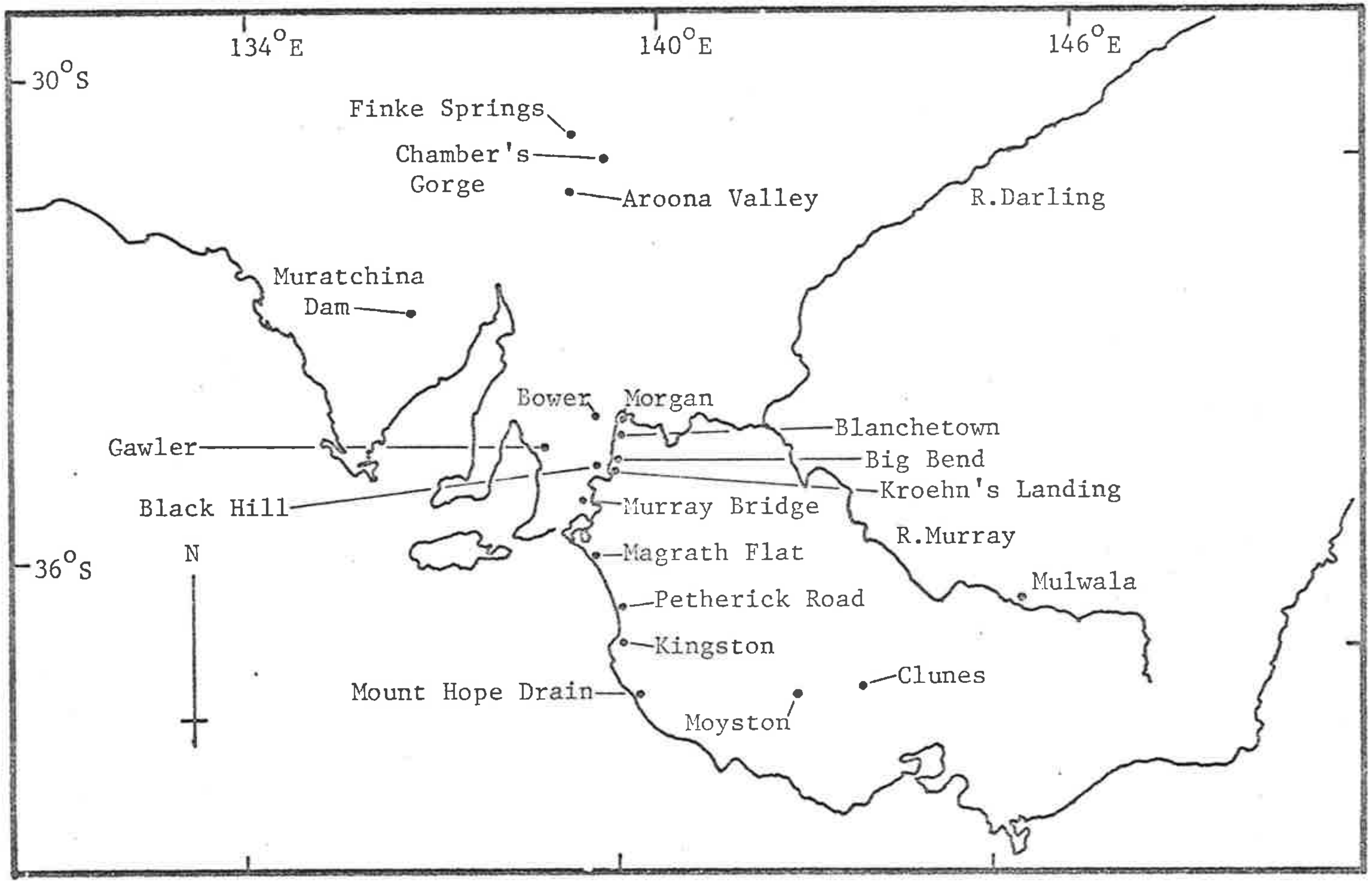


Figure 5.1 Geographic location of recording sites mentioned in the text.

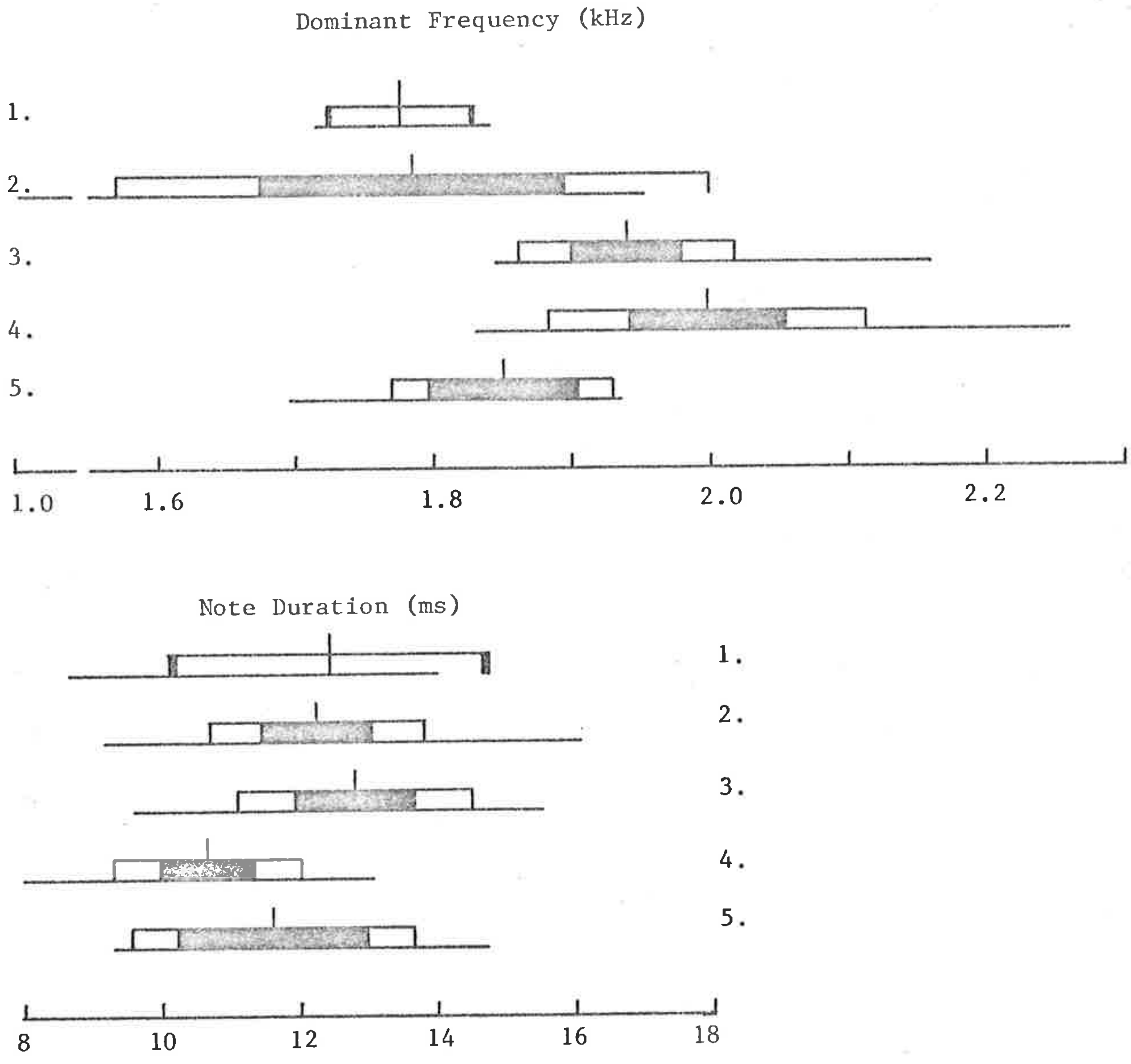


Figure 5.2 Summary of call component values in various southern call race populations, plotted as in Figure 2.4. Sites are, 1., Petherick Road; 2., Kingston; 3., Mount Hope Drain; 4., Moyston; 5., Clunes

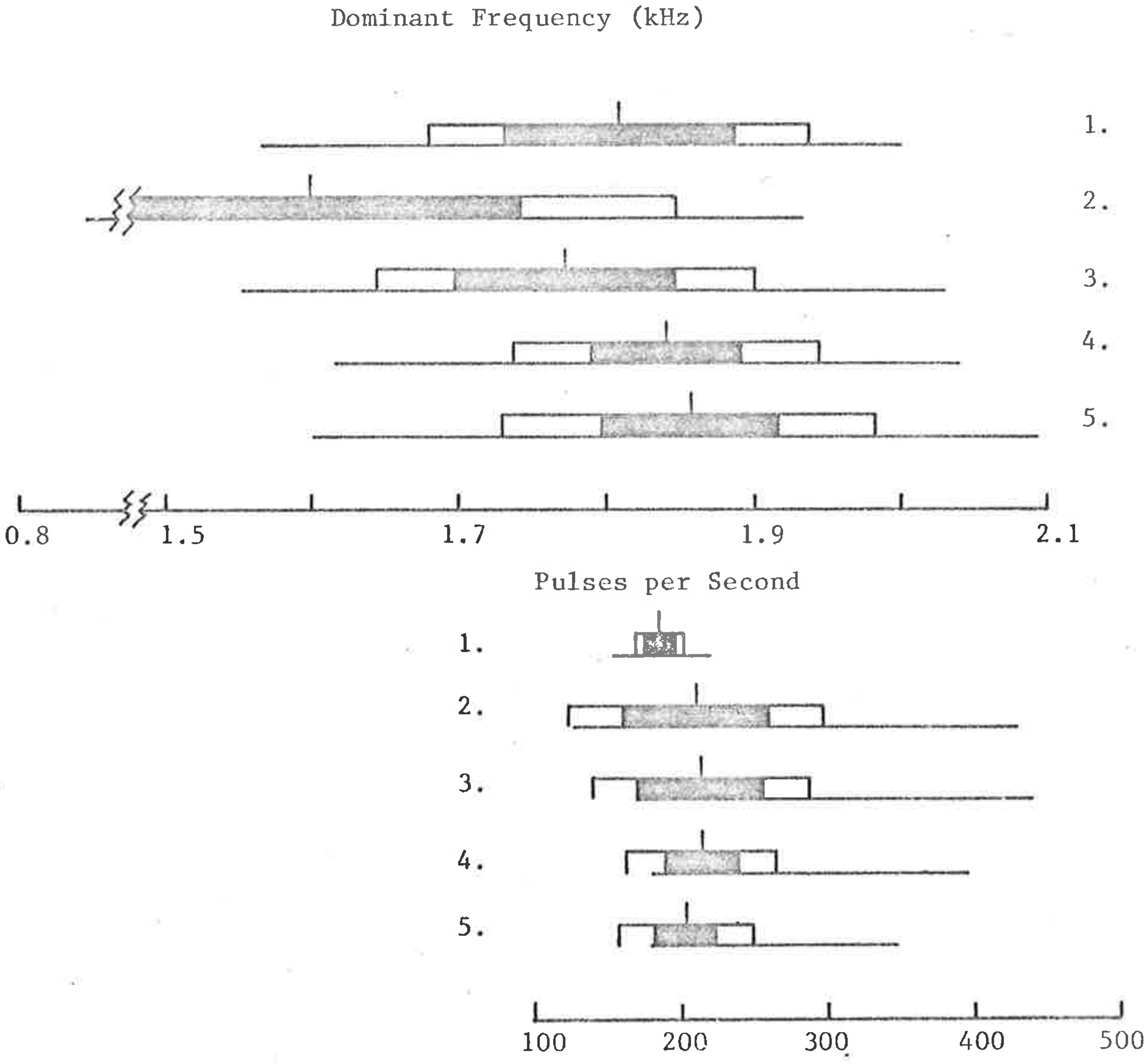
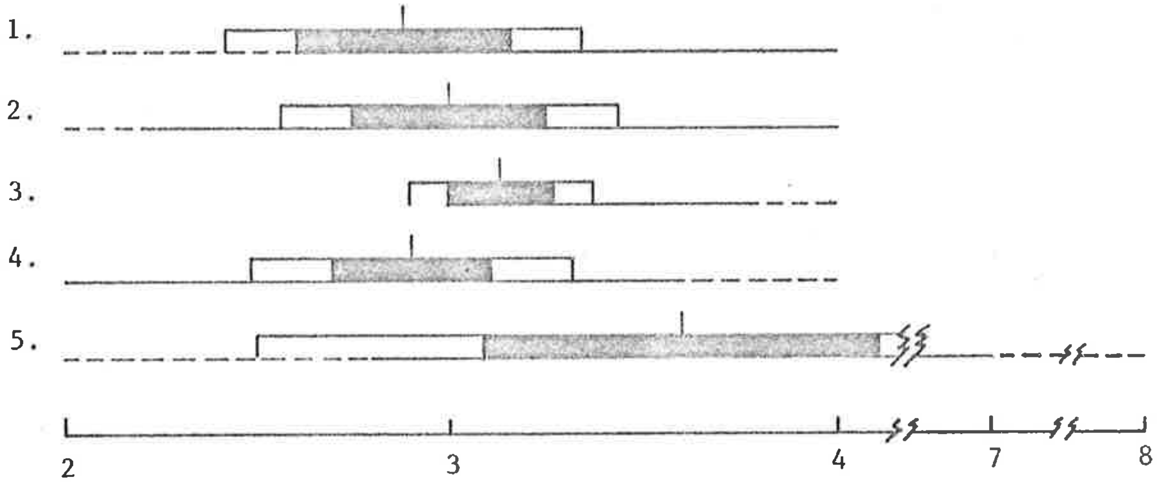


Figure 5.3 Summary of call component values in five northern call race populations, plotted as in Figure 2.4. For note duration only the mean and range are given. In notes per call, the dashed extensions on the range indicate the absolute range of notes per call values encountered rather than the range of averages. 1., Mulwala; 2., Morgan; 3., Blanchetown; 4., Big Bend; 5., Kroehn's Landing. This figure continues on next page.

Average Notes per Call



Note Duration (ms)

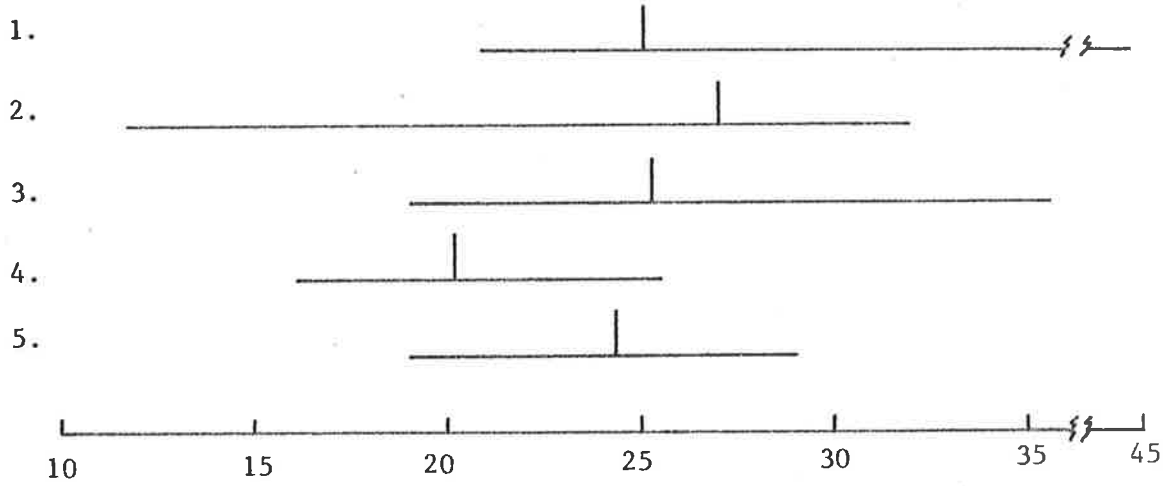
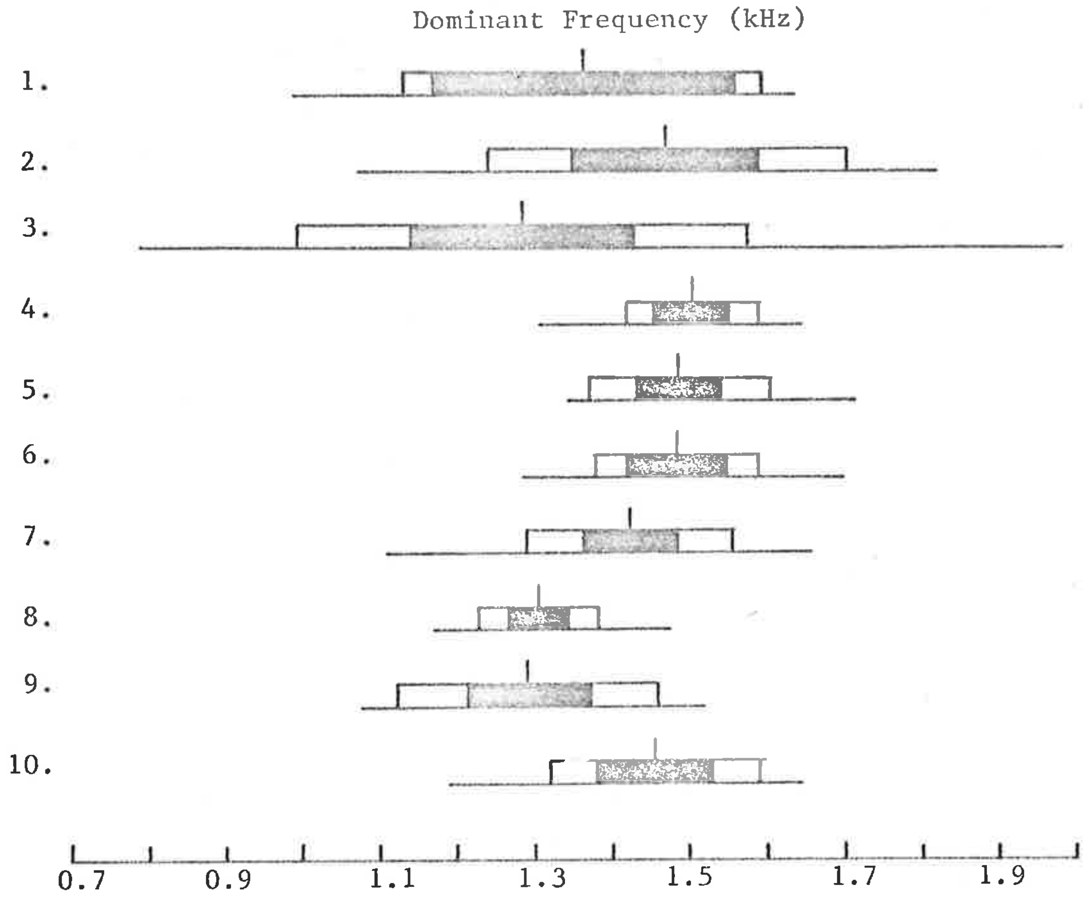


Figure 5.3 continued.



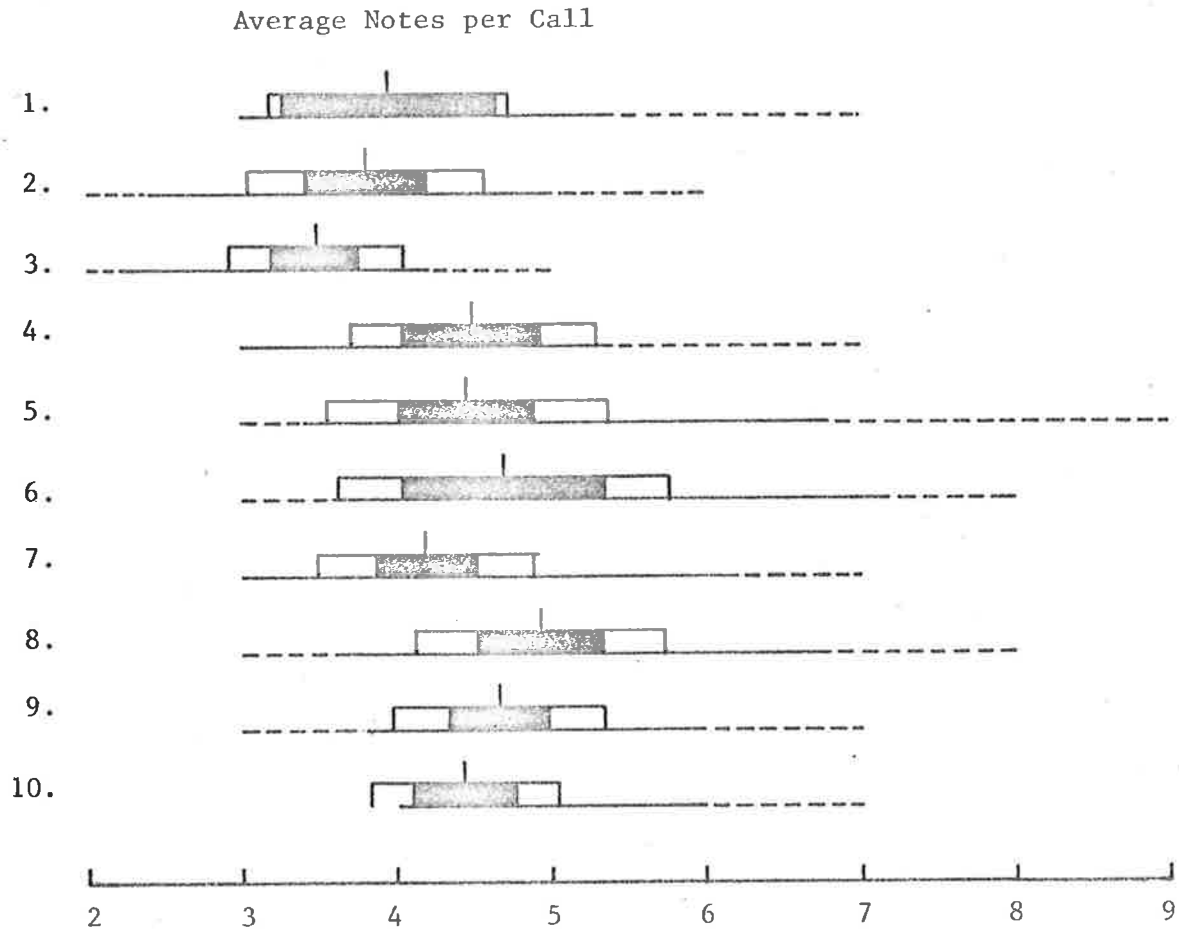
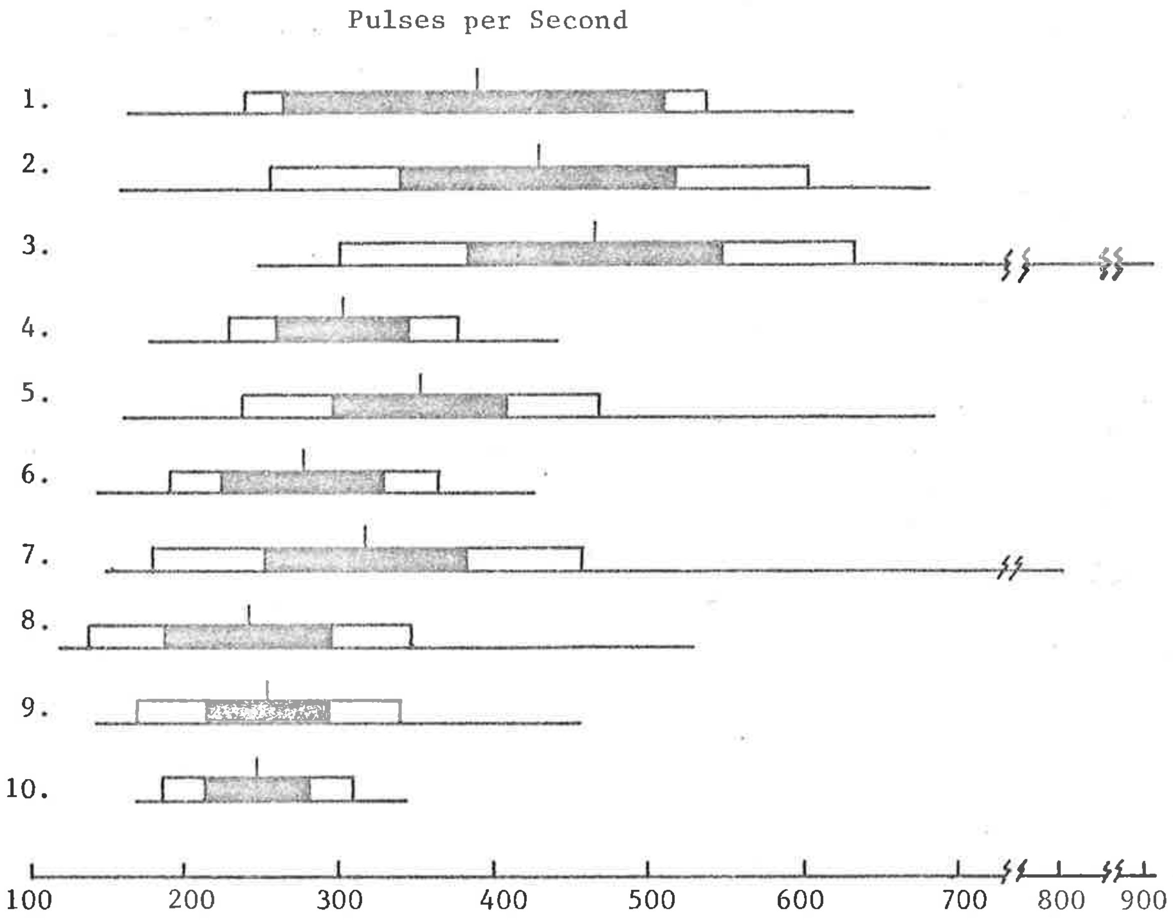


Figure 5.4 Summary of call component values in ten western call race populations, plotted as in Figure 5.3. 1, Petherick Road; 2, Magrath Flat; 3, Murray Bridge; 4, Gawler; continued next page.



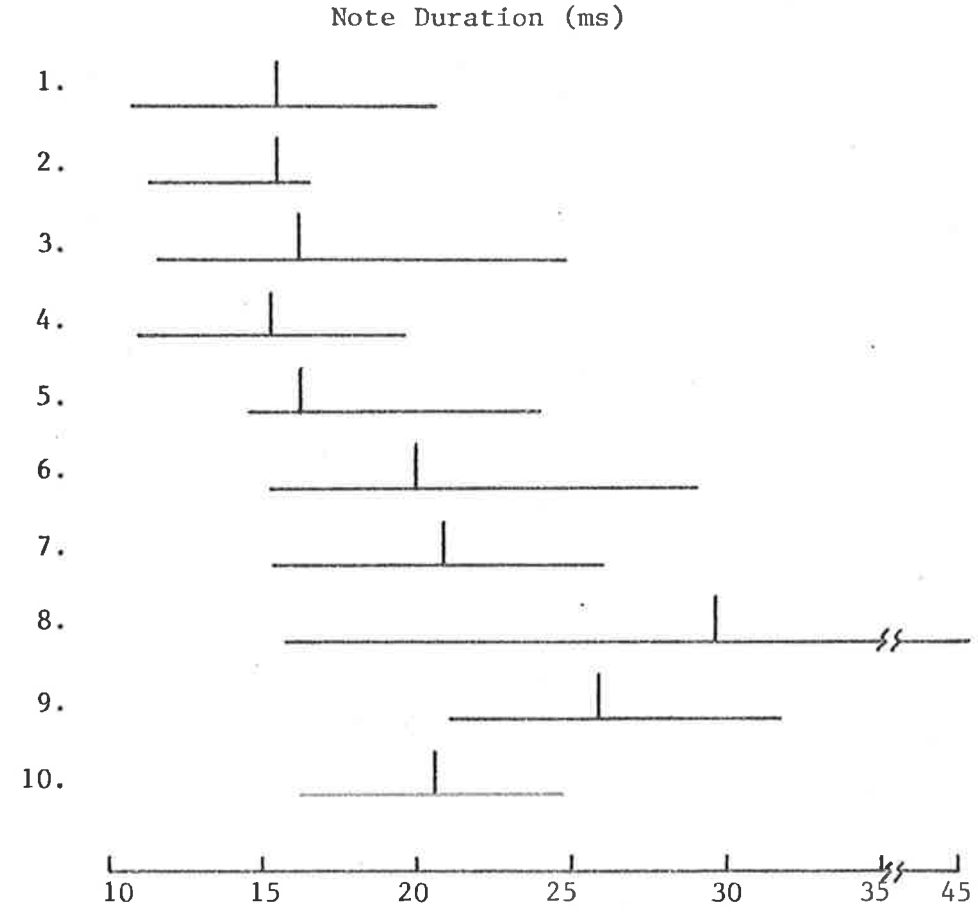


Figure 5.4 (continued) 5, Black Hill; 6, Bower; 7, Muratchina Dam; 8, Aroona Valley; 9, Chamber's Gorge; 10, Finke Springs.

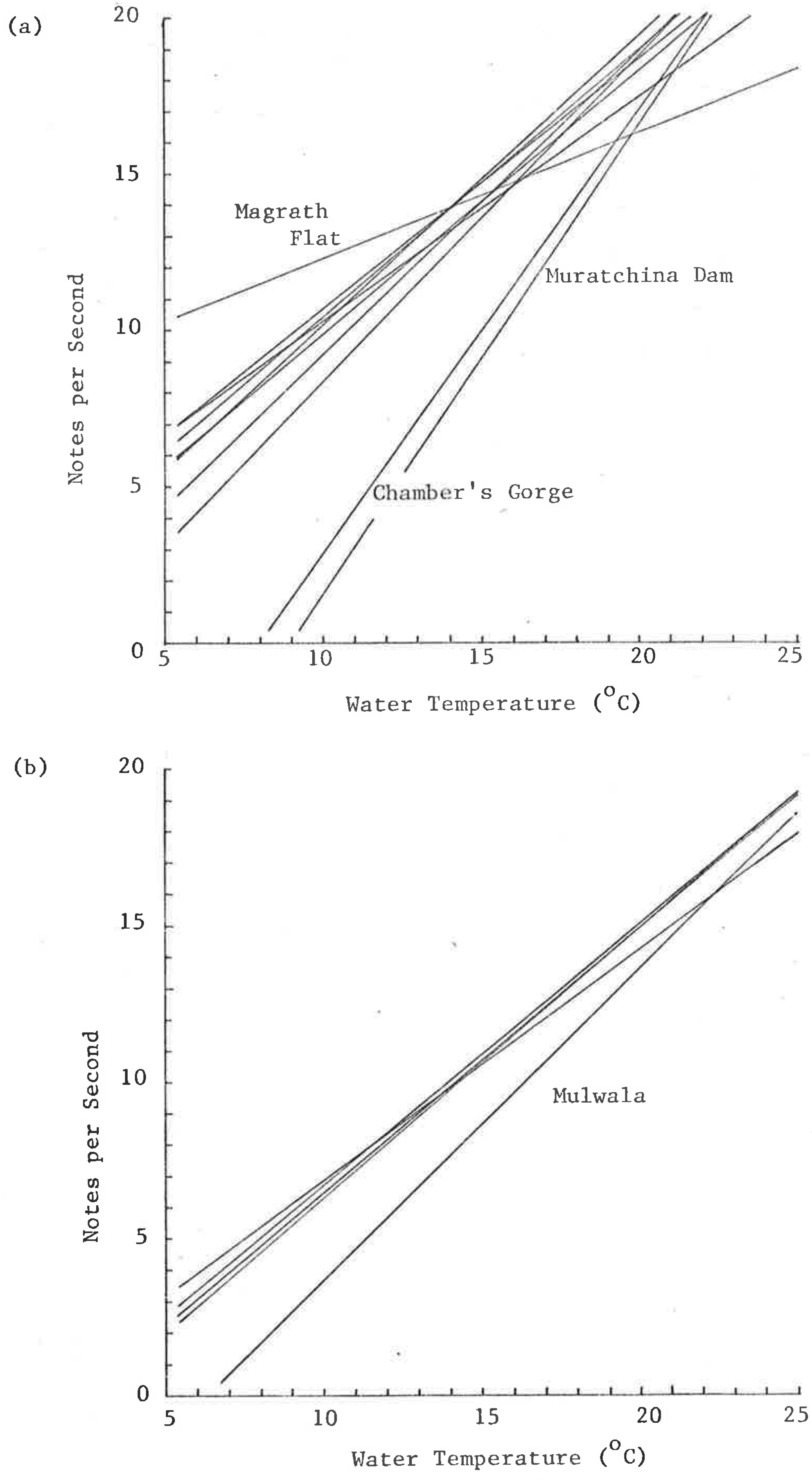


Figure 5.5 Regression lines fitted to notes per second, water temperature plots in (a) ten western and (b) five northern call race populations. Populations are as in Figures 5.3 and 5.4. In this plot only lines with markedly aberrant slopes are named.

		Variance homogeneity	Anova	Adjusted F, variances heterogeneous	Kruskal-Wallis non-parametric analysis variance
(a)	(i)	$Fmax_{5,5} = 17.56$ *	$F_{4,62} = 7.42$ ***	-----	H = 32.13 ***
	(ii)	$Fmax_{5,5} = 2.63$ n.s.	$F_{4,64} = 4.10$ **	-----	-----
(b)	(i)	$Fmax_{5,14} = 5.70$ *	$F_{4,74} = 7.13$ ***	-----	H = 20.23 ***
	(ii)	$Fmax_{5,14} = 20.62$ **	$F_{4,74} = 3.98$ **	-----	H = 9.15 n.s.
	(iii)	$Fmax_{5,14} = 23.32$ **	$F_{4,74} = 0.68$ n.s.	-----	H = 7.32 n.s.
(c)	(i)	$Chi^2_9 = 55.68$ ***	$F_{9,151} = 4.46$ ***	$F'_{9,151} = 9.61$ ***	-----
	(ii)	$Chi^2_9 = 8.72$ n.s.	$F_{9,151} = 5.57$ ***	-----	-----
	(iii)	$Chi^2_9 = 29.64$ ***	$F_{9,151} = 7.00$ ***	$F'_{9,26} = 5.68$ ***	-----

Table 5.2 Summary of statistical procedures conducted in the study of geographic variation of L.tasmaniensis call structure.

(a) Southern call race (i) Dominant frequency (ii) Note duration

(b) Northern call race (i) Dominant frequency (ii) Average notes per call (iii) Pulses per second

(c) Western call race (i) to (iii) as for (b).

Variance homogeneity tested by Bartlett's test ((c)) or the Fmax mathod ((a) and (b)). When variances were not homogeneous alternatives to anova were done; Kruskal-Wallis in (a) and (b), adjusted F ratio in (c). n.s. not significant, * p less than .05, ** p less than .01, *** p less than .001.

(a) Northern call race

(i) Comparison of slopes

Variation	d.f.	Sum of Squares	Mean Square	F
Among regression coefficients	4	0.4819	0.1205	0.2759 n.s.
Deviations from regression	69	30.1246	0.4367	

(ii) Comparison of elevations

Variation	d.f.	Sum of Squares	Mean Square	F
Among intercepts	4	34.229	8.557	20.4 ***
Deviations from pooled regression	73	30.617	0.419	

(b) Western call race

(i) Comparison of slopes

Variation	d.f.	Sum of Squares	Mean Square	F
Among regression coefficients	9	13.96	1.551	2.029 *
Deviations from regression	141	107.789	0.765	

(ii) Comparison of elevations

Variation	d.f.	Sum of Squares	Mean Square	F
Among intercepts	9	340.47	37.83	46.61 ***
Deviations from pooled regression	150	121.749	0.812	

Table 5.3 Comparison of slopes and elevations of regression lines fitted to note repetition rate, water temperature data in the northern and western call races. In (a) (i) within sample deviations from regression were homogeneous (Chi$^2_4$ = 6.59 n.s.), in (b) (i) there may be some heterogeneity (Chi$^2_9$ = 13.26 *). n.s. etc. as in Table 5.2.

with the call components individually and the call races, southern, northern and western, in turn.

5.2 Variation in the southern call race.

1) Dominant frequency.

For this call character there are significant differences between samples in both variability and mean values (Table 5.2(a)). The variance heterogeneity is largely due to the high value at Kingston relative to all other samples. The Kingston value is caused by one frog with a dominant frequency of 1005 Hz. Without this datum the standard deviation for the Kingston sample reduces from 215.0 to 78.5 whilst the mean shifts slightly from 1785 to 1834 Hz. This low value is not an artefact (cf. comments on a low dominant frequency in a frog from Morgan, section 4.4 1) a)) and I must accept it as representing the low extreme in this call race.

Causes of the significant heterogeneity in sample means are not quite as obvious but this is probably largely due to the difference between the Kingston, Petherick Road data and those from Moyston and Mt. Hope Drain (Figure 5.2). The Clunes mean falls between these two groups.

In attempting to explain these results I will consider the incidental origin hypothesis first.

Almost any adaptation might indirectly affect call structure to some extent. However, those that will be most important will effect either larynx structure or position, and therefore the form of passive elements of call structure (W.F. Martin 1971; W.F. Martin and Gans 1972; Gans 1973), or, nervous transmission rates which would result in repetition rate characteristics changing, or, finally, lung volume such that air pressures in or the amounts of air passing through the larynx alter producing changes in call durations or dominant frequencies (W.F. Martin 1971).

In conjunction with my work on calls I did not work on any physiological or morphological traits. Therefore, I cannot say whether L. tasmaniensis at Moyston possess adaptations to unique features of their environment, or, if they do, whether such adaptations effect call dominant frequency.

However, in comparison with other studies on anurans which have shown significant geographic variation in morphology or physiology, and where the selective force has been recognised (Nevo 1972, 1973; Jameson, Taylor and Mountjoy 1970; Calhoon and Jameson 1970) the environments at the five recording sites in this study are very similar. Further, there are no obvious correlations of call component values with particular features of the environment.

Moyston and Clunes are at about the same altitude, 300 m, but the three South Australian sites are all at or very close to sea level. Petherick Road, Kingston and Mount Hope Drain are all in the vegetation association described by Specht (1972) as tussock grassland. A comparable classification is not available for Victoria but at Moyston the vegetation could probably be described as open woodland. I have no plant data for Clunes.

Rainfall at the two Victorian sites is much more uniformly distributed through the year compared to South Australia where there is a marked summer drought (Figure 5.6(a)). The summer, winter temperature range is wider in Victoria (Figure 5.6(b)) at both localities and the average daily minimum is consistently lower there than at any South Australian site. In fact, climates at the three South Australian localities are more similar to each other than any is to either Moyston or Clunes. This probably also applies to many other features of the environment as well. Thus if incidental changes were important I would expect South Australia, Victoria splits, not the observed pattern.

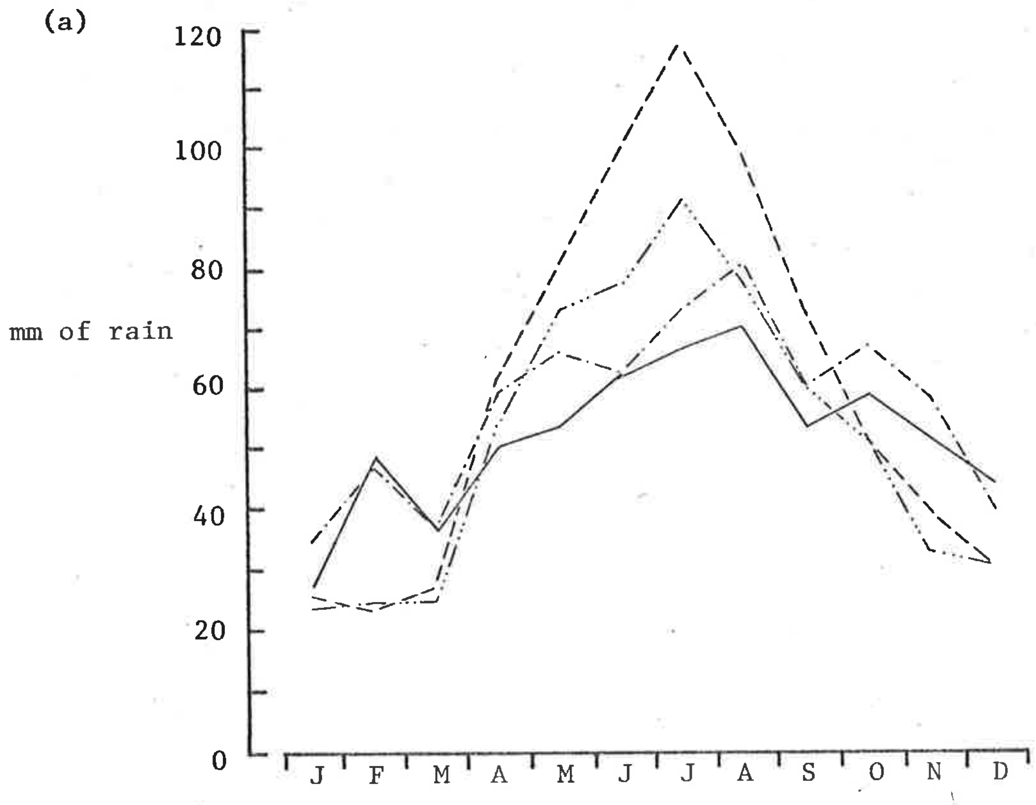


Figure 5.6 (a) Mean monthly rainfall for the standard thirty-year period 1930 to 1960 (Bureau of Meteorology 1966) for observation sites closest to southern call race recording localities.

---- Beachport (Mt. Hope Drain) —··—··— Kingston (Kingston, Petherick Road)

—·—·— Moyston ——— Clunes

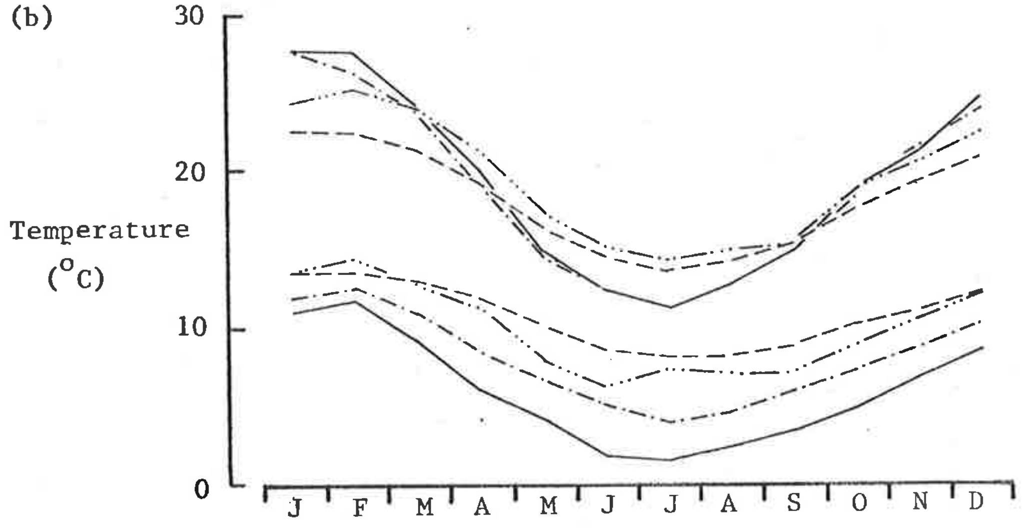


Figure 5.6 (b) as for (a) except that data are mean monthly maximum and minimum temperatures (from Bureau of Meteorology 1975).

—··—··— Policeman's Point (Petherick Road, ? Kingston) ——— Clunes

------ Robe (Mt. Hope Drain, ? Kingston) —·—·—·— Ararat (Moyston)

In considering the alternative hypothesis, i.e. that the observed differences in call structure result from adaptative change in response to signal transmission problems, similar reasoning applies. The features likely to affect sound propagation, e.g. soil type, the nature and thickness of vegetation (Morton 1975), are more similar at Petherick Road, Kingston and Mount Hope Drain than any of these is to either Clunes or Moyston.

A potentially more potent force, however, is the number and nature of anuran species calling at the same time as *L.tasmaniensis*. It is now known that frogs have an auditory system with two or more major sensitivity peaks, with one of these usually tuned closely to the male call dominant frequency (Loftus-Hills 1973; Capranica, Frishkopff and Nevo 1973; Feng, Narins and Capranica 1975; Capranica and Moffat 1975). Thus sympatric frog species with similar call dominant frequencies are likely to jam each other if they call at the same time and place.

Frog species with calling seasons overlapping that of *L.tasmaniensis* are listed in Table 5.4 for each of the five recording localities. Table 5.4 also gives call dominant frequency data for these species. Data for South Australia ~~is~~ are from my own observations but that those for Victoria is are taken from Brook (1975).

Loftus-Hills (1973) investigated the auditory system of southern *L.tasmaniensis* and found, contrary to the expectation set out above, a peak sensitivity at 1400 Hz in frogs from a population with a mean call dominant frequency of 1900 Hz. If this is a general characteristic of southern *L.tasmaniensis* it is difficult to predict which, if any, sympatric anuran species might act as sources of interference. Perhaps the two most likely possibilities are species with call dominant frequencies around either 1400 or 1800 - 2000 Hz. In either case there is no pattern of mean variation consistent with the presence of frog

Species	Locality 1.	2.	3.	4.	5.	Dominant Frequency (Hz)
Litoria aurea raniformis@	-	-	P	P	P	1200 [1. & 2.]
Litoria ewingi	P	P	P	P	P	2490 (2400 - 2550)[3.]
Litoria peroni	-	-	-	P	-	2020 [1.]
Ranidella parinsignifera	-	-	-	P	P	3400 (3200 - 3900)[2.]
Ranidella signifera	P	P	P	P	P	3240 (2750 - 3800)[2.]
Limnodynastes dumerili	P	P	P	P	P	685 (630 - 771)[4. (a)]
Limnodynastes peroni	-	-	P	-	-	998 (860 - 1150)[5.]
Limnodynastes tasmaniensis#	P	-	-	-	-	1355 (1171 - 1564)[1.]
Neobatrachus pictus	P	P	?P	-	P	1300 (1180 - 1450)[1.]

Table 5.4 Frog species which are sympatric with, and whose calling season at least partly overlaps that of, the southern call race. Localities are: 1. Petherick Road, 2. Kingston, 3. Mount Hope Drain, 4. Moyston, 5. Clunes. Symbols, - indicates the species has not been reported, P species present, ?P no data but species very likely present. Superscripts on dominant frequency refer to sources in Table 5.9.

western call race.

@ Courtice and Grigg (1975) proposed elevation of this subspecies to specific rank. However, inadequacies in their samples plus Littlejohn's (1969 b) suggestion that *L.a.raniformis* and *L.a.aurea* hybridise in eastern Victoria make this proposal suspect. I will maintain the subspecific nomenclature until the situation is clarified.

species causing interference (Table 5.4, Figure 5.2).

Neither incidental origin nor the interference hypothesis seem particularly appropriate explanations of the observed variation in southern call race dominant frequencies. I will therefore offer one other possibility.

Several investigations of frog call variation have shown that call dominant frequency and body length are negatively correlated (A.A. Martin 1972; Zweifel 1968; W.F. Blair 1955; Snyder and Jameson 1965). I do not have much data for southern L.tasmaniensis, but those that presented in Figure 5.7 suggests a similar effect in this frog. In this study head width rather than snout vent length was used as I could measure the former more reliably, particularly in live specimens. (Even so, head width and body length are highly correlated. The Product-moment correlation coefficient, is 0.906, both p less than .05, for frogs supplying data for Figure 5.7). Results of my statistical analysis of the relationship between head width and dominant frequency are given in Table 5.5.

It is fairly clear that there is some effect of head width on dominant frequency in southern L.tasmaniensis.

From this it is fair to argue that in the various populations of southern L.tasmaniensis where the I recorded calls, mean head sizes differed at the time of sampling. If the head width differences are genetically determined, and in the various populations I sampled different genes for head width were fixed, then I would have to consider reviving the incidental origin hypothesis to explain the variation in mean dominant frequencies. Alternatively though, the head width differences may represent year to year variation in morphology resulting either from differences in the nature or severity of some selective force or, from variations in the rate or form of development as a response to fluctuations in some environmental component (e.g. the weather, food availability, the

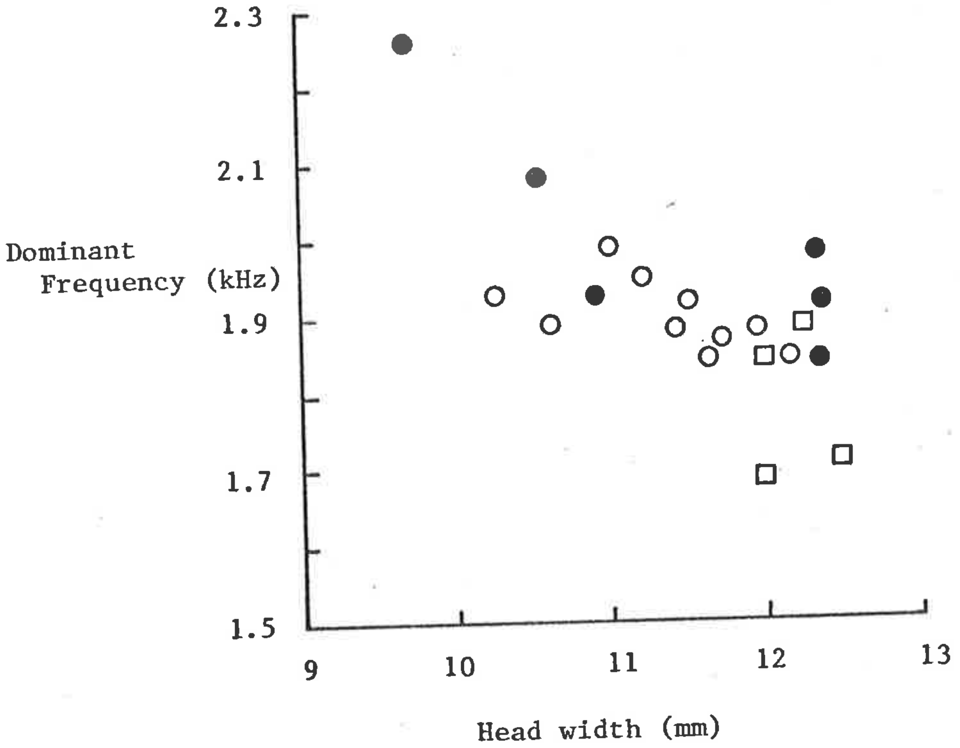


Figure 5.7 Plot of head width verses call dominant frequency for southern males from three populations; closed circle Moyston; open circle, Mt. Hope Drain; open square, Kingston.

	Product moment correlation coefficient	Kendall's rank correlation coefficient
Moyston	- 0.854 *	- 0.828 *
Mount Hope Drain	- 0.503 n.s.	- 0.440 *
All data	- 0.702 **	- 0.483 **

Table 5.5 Correlation coefficients for the relationship between head width and dominant frequency in southern call race populations. The category, All data, contains data from Moyston (6 frogs), Mt. Hope Drain (10 frogs) and Kingston (4 frogs).
n.s. not significant, * p less than .05, ** p less than .01

	Mean Note Duration	Mean Water Temperature
Clunes	11.62 ms	15.03 °C
Moyston	10.64 ms	15.50 °C
Mount Hope Drain	12.79 ms	13.05 °C
Kingston	12.26 ms	13.76 °C
Petherick Road	12.45 ms	16.17 °C

Table 5.6 Mean note durations and mean water temperatures in five southern call race populations.

presence of competitors (cf. Wilbur and Collins 1973)).

If the latter hypothesis is correct relatively large, though not unprecedented, shifts in head width would be required. Using the slope of a regression line fitted to the head width, dominant frequency data illustrated in Figure 5.7 I estimate a change in call dominant frequency of 150 Hz requires a change in head width of 1.35 mm. For a frog with an average head width of 11.5 mm (based on the twenty individuals supplying the data for Figure 5.7) this represents a variation of about 12%.

Between years variation of snout vent length in single populations of *Ranidella pseudinsignifera* and *R.insignifera* of between 4% and 7% were reported by Bull (1973). In a single population of *Hyla regilla* between years head width variation of about 10% was reported by Vogt and Jameson (1970). In this latter study size changes were correlated with the weather in the year preceding capture, that is during the period of growth and development (Vogt and Jameson 1970; Jameson, Mackey and Anderson 1973). It is interesting to note that in the relevant areas of Victoria and South Australia there was considerable variation between years in rainfall and maximum and minimum temperatures in the period 1970 to 1973 (Bureau of Meterology 1970 - 1973). This raises the possibility of correlated morphological changes in southern call race populations accounting for the observed variation of dominant frequencies.

2) Note Duration.

Within population variability of this parameter was equivalent in all samples but there are significant differences in sample means (Table 5.2(a)). Mean note duration is lowest at Moyston and highest at Mount Hope Drain and I think the difference between these two means is the major contribution to the significant F ratio (Figure 5.2).

There is even less pattern to the variation in this call component than there was with dominant frequency. Again, these data are not readily explained by either the incidental origin or communication difficulties hypotheses. However, as with dominant frequency, there is another possibility.

In the regression analyses reported in Chapter 2 there was no apparent effect of water temperature on note duration in the southern call race. In these samples the range of water temperatures was small and it is possible that some slight effect was not detected. Note duration is commonly found to depend on environmental temperature in anurans (Chapter 2, western and northern call races of L.tasmaniensis; A.A. Martin 1972; Littlejohn 1965) with a negative correlation of the two variables usually observed. Excluding Petherick Road, mean note duration in the southern call race declines as mean water temperature increases (Table 5.6).

Thus there may be a weak effect of water temperature on note duration in southern L.tasmaniensis and this would account for the observed variation in note durations.

The Petherick Road data do not fit this pattern and this may be because the sample size is relatively small, a result of past interbreeding with western L.tasmaniensis, or because the negative correlation of water temperature and note duration means is a chance result not representing reality.

I do not have any confidence in any explanation of these note duration data, but an incipient temperature effect, despite its drawbacks, seems the best alternative.

To conclude, the apparent evidence of geographic variation in dominant frequency and note duration in the southern call race may reflect variations in the time and nature of sampling rather than significant genetic differentiation. I have clearly not precluded the incidental origin or adaptation in the face of communication difficulty hypotheses. However, I can say that this analysis has not given any clues about the origin of any of the call races of L.tasmaniensis.

5.3 Variation in the northern call race.

1) Dominant Frequency.

The F_{max} text indicates significant differences in sample variability (Table 5.2(b)), and inspection of Figure 5.3 suggests this is largely due to the high variance of the Morgan sample. The various analysis of variance procedures conducted indicated significant differences between sample means (Table 5.2(b)), and again this seems due to the Morgan sample which has a much lower mean than the other four samples (Figure 5.3).

The high variance at Morgan is caused by one frog with a dominant frequency of 847 Hz. Without this datum the Morgan sample standard deviation reduces from 247.8 to 126.4, a value comparable to that in other northern populations I sampled. In section 4.4 1) a) I discussed calls of this frog and suggested that the low dominant frequency may be an artefact. If this is true then the differences in sample variance disappear.

The most obvious explanation of the low Morgan mean is introgression with the western call race. Western L.tasmaniensis generally have a lower call dominant frequency than northern (Chapter 2, 4). Morgan lies at the eastern end of a contact zone between the western and northern call races (section 4.4).

Vegetation and climate at the four South Australian sites are similar (Specht 1972; Fig. 5.8). I have no data on vegetation at Mulwala. Temperatures are similar to those at the South Australian

Figure 5.8 (a) Mean monthly maximum and minimum temperature and (b) rainfall data for observation stations closest to northern recording sites. Data from same sources as Figure 5.6.

Temperature.

– – – – – Yarrawonga (Mulwala) –·–·–·– Waikerie (Morgan)

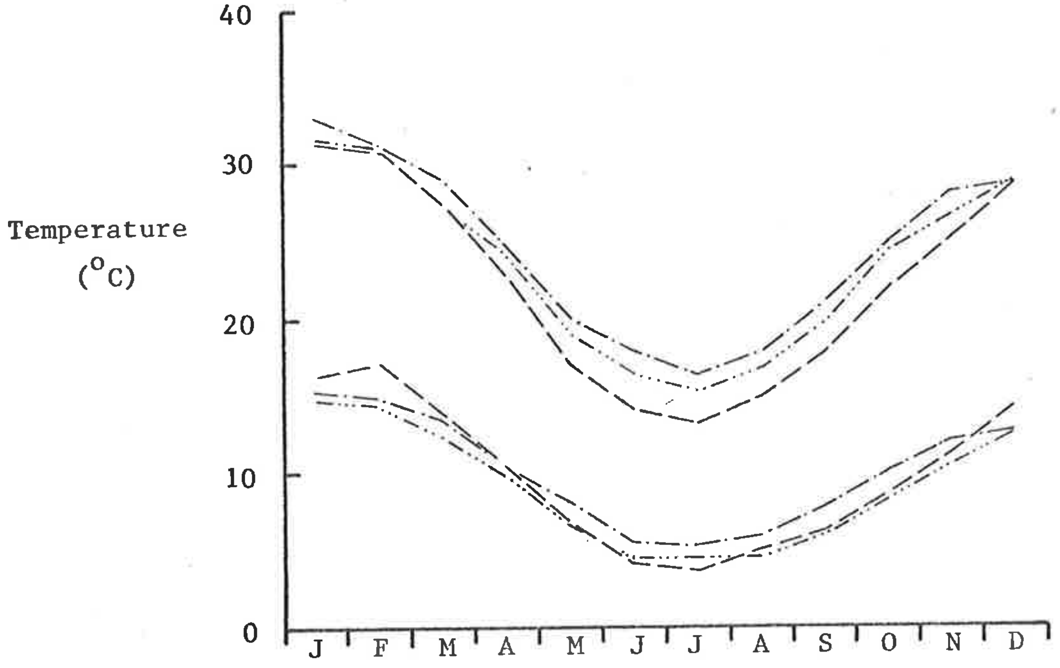
–···–···– Nildottie (Kroehn's Landing, Big Bend, ?Blanchetown)

Rainfall.

– – – – – Yarrawonga (Mulwala) –·–·–·– Morgan ———— Blanchetown

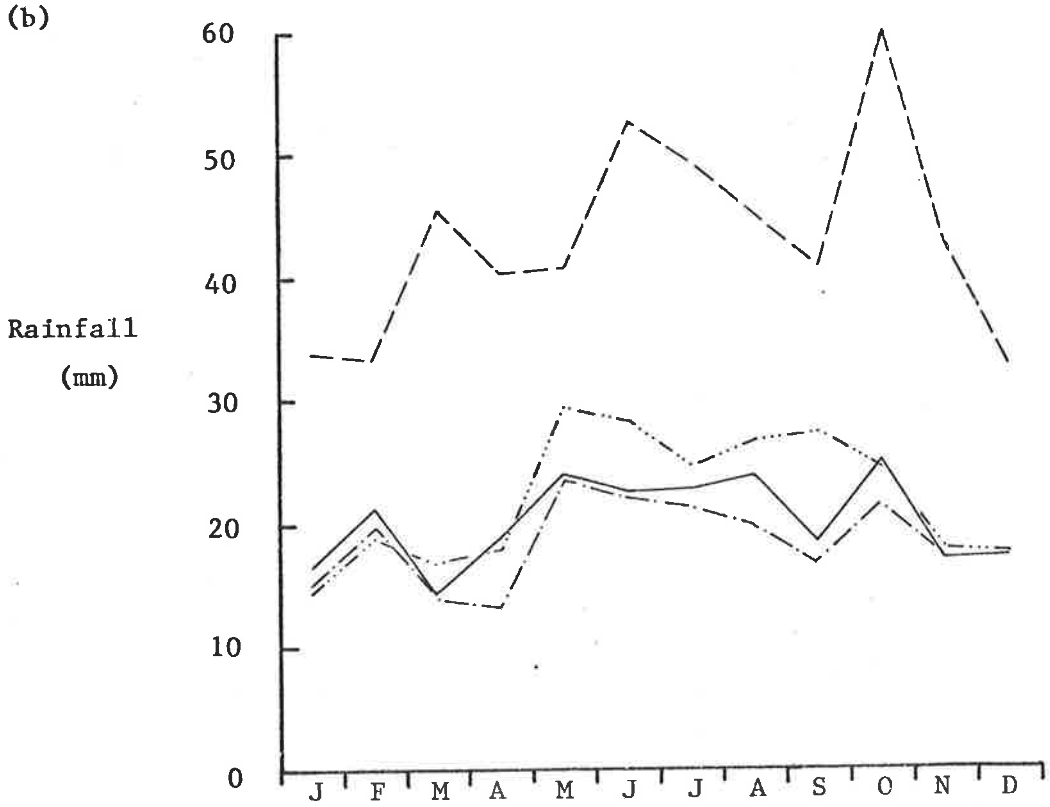
–···–···– Swan Reach (Big Bend, Kroehn's Landing)

(a)



Temperature (°C)
40
30
20
10
0
J
F
M
A
M
J
J
A
S
O
N
D

(b)



Rainfall (mm)
60
50
40
30
20
10
0
J
F
M
A
M
J
J
A
S
O
N
D

sites but rainfall is much higher (Figure 5.8). However, Morgan, Blanchetown, Big Bend and Kroehn's Landing are all subject to flooding by river Murray waters and this may reduce the significance of the higher precipitation at Mulwala.

Given the above information I would not predict major differences in morphology or physiology amongst frogs at these five recording sites and the chance of differences in dominant frequency or any other call component having an incidental origin are therefore low.

The number of sympatric anuran species varies between sites with the highest number, nine, at Mulwala (Table 5.7). There is no species occurring uniquely at Morgan which is likely to have affected dominant frequencies there. *Neobatrachus centralis* is recorded from Morgan but this is based on a single observation of a lone individual during one of many visits to this site. Apart from this I have never observed *Neobatrachus* in the river Murray valley; this species generally breeds in temporary waters.

2) Pulse repetition rate.

Although there are no differences in population means this character does exhibit significant differences in sample variance (Table 5.2(b)). The cause of the latter result is not immediately obvious as there is no one sample with an extreme sample variance (Figure 5.3). The Mulwala variance is very low; it is higher at Big Bend and Kroehn's Landing and higher again at Blanchetown and Morgan.

As I said when discussing dominant frequency there is little reason to expect differentiation between these populations. The only factor known to be different about Mulwala is the number of sympatric anuran species (Table 5.7).

Although species with a similar dominant frequency are the most likely sources of interference, sounds at other frequencies may also

Species	Locality					Dominant Frequency (Hz)
	1.	2.	3.	4.	5.	
Litoria aurea raniformis	P	P	P	P	P	1200
Litoria ewingi	-	-	-	-	P	2490
Litoria peroni	P	P	P	P	P	2020
Ranidella parinsignifera	P	P	P	P	P	3400
Ranidella signifera	P	-	P	-	P	2490 (2300 - 2600)[6.]
Ranidella sloanei	P	-	-	-	-	2600 [7.]
Limnodynastes dumerili	P	P	P	P	P	654 (582 - 765)[4.(b)]
Limnodynastes fletcheri	P	P	P	P	P	1620 [1.]
Limnodynastes interioris	P	-	-	-	-	247 (226 - 270)[4.]
Neobatrachus centralis	-	P	-	-	-	1470 (1290 - 1620)[1.]
Neobatrachus pictus	P	-	-	-	-	1300 (1180 - 1450)[1.]

Table 5.7 Frogs which are sympatric with, and whose calling season at least partly overlaps that of, the northern call race. Localities are: 1. Mulwala, 2. Morgan, 3. Blanchetown, 4. Big Bend, 5. Kroehn's Landing. Symbols as in Table 5.4. Sources of dominant frequency not repeated if already given in Table 5.4

cause problems if they are sufficiently loud (Loftus-Hills 1973). With a greater number of frog species this latter form of interference may be more severe. A decrease in pulse repetition rate variance may reflect increased selection for specificity of the signal. Pulse repetition rate is important in call discrimination in Hylid frogs (Loftus-Hills and Littlejohn 1971; Straughan 1975), and might be used as a direction cue in one Leptodactylid (Littlejohn and Watson 1974).

I cannot see any cause of the differences in pulse repetition variance between Big Bend, Kroehn's Landing and Blanchetown, Morgan.

3) Average notes per call.

This character showed significant heterogeneity of sample variances but there was no evidence of differences in population means (Table 5.2(b)). The variance in four of the five samples is roughly equal but that at Kroehn's Landing is much higher (Figure 5.3).

At Kroehn's Landing seventeen of twenty frogs had an average of four notes per call or less. The other three had values of 4.6, 5.8 and 7.0 respectively. If these last two values are excluded, the standard deviation reduces from 1.104 to 0.55; a value comparable to that in the four other northern call race populations investigated.

I have two explanations, both unproven, for the high notes per call values at Kroehn's Landing. First, they represent the effects of introgression with the western call race (section 4.4 1)). I will not consider this hypothesis further. Second, the recorded calls came from a frog involved in some sort of interaction with a conspecific.

Southern call race males occasionally give a multiple note call and elsewhere this was described as a territorial call (Littlejohn and Roberts 1975). Western call race males fight, and while so doing give a low growling call quite distinct from the normal call. I have recorded the western fight call in uninterrupted calling sequences, and in some cases it was preceded by an increase in notes per call or was contained in a series of calls which all had high note numbers. Thus, although I have no data for the northern call race, it may be a general phenomenon in *L.tasmaniensis* that interactions between calling males involve an increase in notes per call, possibly as a preliminary to more overt activities.

4) Note repetition rate.

The analysis of covariance indicated no differences in slope of the five fitted regression lines but significant heterogeneity of elevations (Table 5.3(b)). From Figure 5.5(a) it is obvious this is due to the low note repetition rate values at Mulwala.

In section 4.3 I discussed this result and suggested that it represented a residual effect of introgression with the southern call race. This still seems the most likely explanation, though introgression with western *L.tasmaniensis* is a plausible alternative.

Elsewhere (section 4.4) I have suggested that the northern call race moved down the Murray river contacting the western call race at several sites with the first contact at Morgan. If this is true, that all northern call race populations I sampled in South Australia are derived from a population which has been exposed to western genes. Note repetition rate in the western call race is higher than in the northern (Chapter 2). If western genes affecting note repetition rate were incorporated into the northern genotype on initial contact between the call races this would explain why South Australian, northern *L.tasmaniensis* had higher note repetition rates than those at Mulwala.

5) Note duration.

No trends in note duration are evident that might in any way bear on the problem of the origin of the call races of L.tasmaniensis. The lowest mean occurs at Big Bend and the widest range at Morgan (Figure 5.3). This is consistent with the idea that note duration is affected to some degree by water temperature (Chapter 2), as in my samples the highest mean water temperature occurs at Big Bend and the widest range of temperatures at Morgan.

As with the southern call race, this study of geographic variation in northern L.tasmaniensis calls has not given any real clues to the origin of any of the call races in this species. The only fact of any interest is the correlation of low pulse repetition rate variance and high number of sympatric frog species at Mulwala. However, until it is known whether pulse rate conveys information it would be unwise to make much of this correlation.

5.4 Variation in the western call race.

1) Introduction.

Ten populations were sampled and these were spread broadly through the known range of this call race. This relatively intense sampling covers a variety of physical and biotic environments and gives a greater chance of finding interpopulation differences than my studies on either the southern or northern call races.

As a preliminary to discussing each of the analysed call components in turn I will consider environmental features that may act directly on call structure (the interference hypothesis), or incidentally by selecting changes in physiology or morphology (the incidental origin hypothesis).

a) Climate.

Climates at the ten recording sites vary considerably from warm, subhumid areas with a marked summer drought in the south (Petherick Road, Magrath Flat) through warm, semi-arid regions with a moisture deficiency in all seasons (Gawler, ? Muratchina Dam, Aroona Valley) to arid warm climates in the north and east (Bower, ? Muratchina Dam, Chamber's Gorge, Finke Springs). These categorisations are based on Map 13 and associated data of Gentilli (1972). They are supported by rainfall and mean monthly maximum and minimum temperature data plotted in Figure 5.9. These data were taken from Bureau of Meteorology publications for observation stations closest to my field recording sites. Rainfall data ~~is~~ are available for nearly all sites but temperature information is more meagre and probably less reliable. For example, Leigh Creek is the closest temperature recording station to the three Flinders Ranges recording sites. However, this town is on the plain west of the ranges and may be much hotter than places at higher altitudes and in the more varied topography in the body of the ranges.

Rainfall is highest at Meningie (Magrath Flat, Petherick Road) and Roseworthy College (Gawler), lower and approximately equal at Murray Bridge, Kongolia (Black Hill), Blinman (Aroona Valley, ? Finke Springs) and Buckleboo (Muratchina Dam) and lowest at Wertaloona (Chambers Gorge)(Figure 5.9(b)).

Maximum temperatures are highest at Leigh Creek, lowest at Meningie with Kimba, Nildottie and Roseworthy College similar to each other and falling about midway between these extremes (Figure 5.9(a)). Minimum temperatures are about the same at all localities except Leigh Creek where values are higher in the spring, summer and autumn (Figure 5.9(a)).

b) Vegetation.

Not surprisingly, given the wide variations in climate, vegetation at the various recording sites was quite different. The three Flinders Ranges sites were all on creeks dominated by red gum (Eucalyptus camaldulinensis). Muratchina Dam and Bower recording sites were man made

Figure 5.9 (a) Temperature and (b) Rainfall data for observation stations closest to recording sites for the western call race. Data from same sources as listed in Figure 5.6.

Temperature.

———— Leigh Creek (?Finke Springs. ? Chamber's Gorge, ?Aroona Valley; see text for relevance of these data).

—·—·— Kimba (Muratchina Dam) —···—···— Roseworthy Agricultural College (Gawler)

- - - - - - - Meningie (Magrath Flat, Petherick Road)

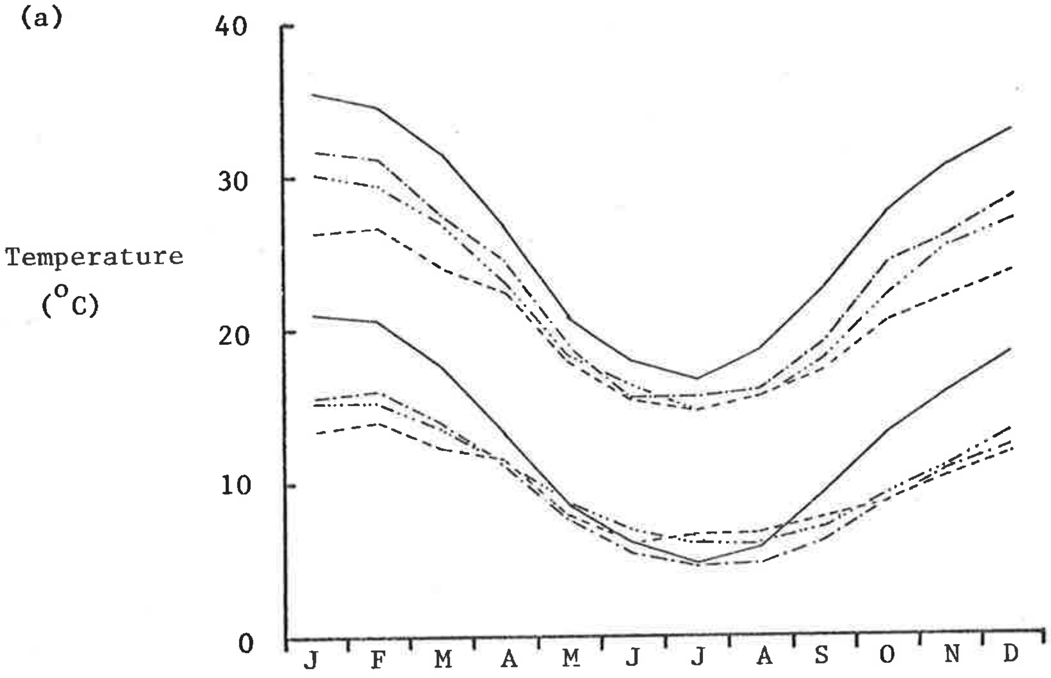
Data appropriate to Black Hill (Nildottie) and Bower (? Waikerie) have been given in Figure 5.8. Murray Bridge data not plotted but they are virtually coincident with the Roseworthy data.

Rainfall.

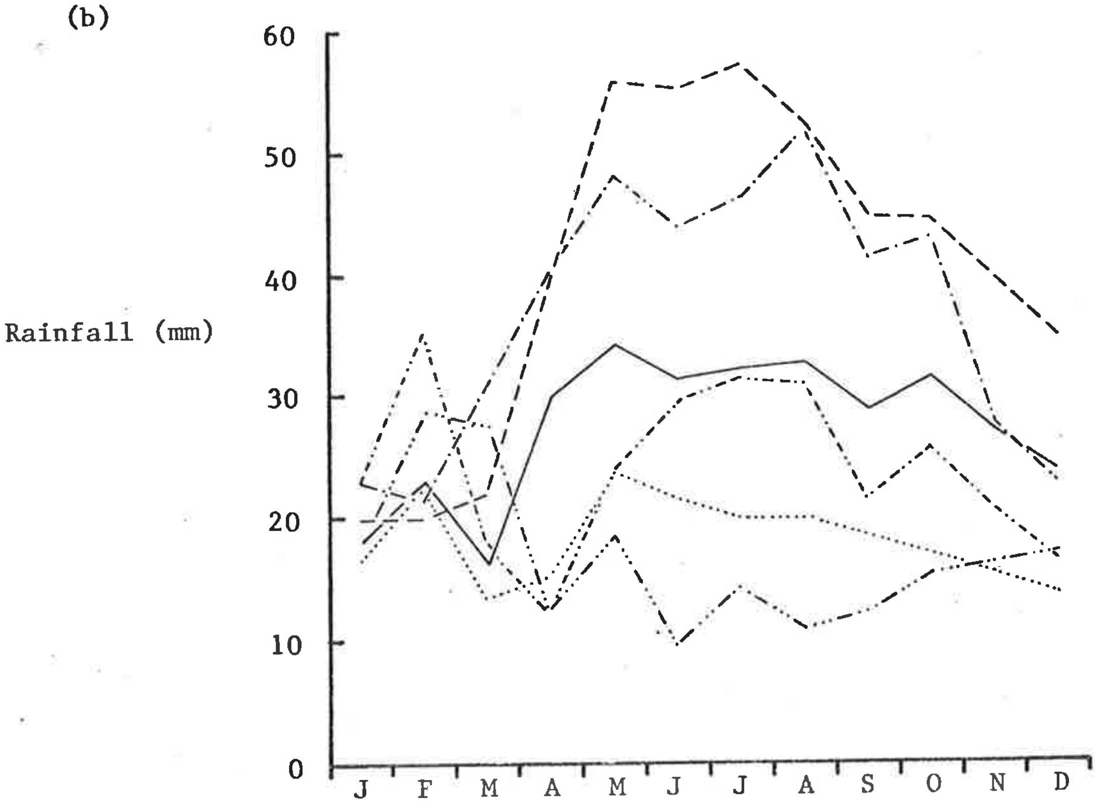
- - - - - - - Meningie (Magrath Flat) —·—·—·— Roseworthy A.C. (Gawler)
———— Murray Bridge ·············· Bower
—···—···— Wertaloona (Chamber's Gorge) - - ·- - ·· Blinman (Aroona Valley, ?Finke Springs)

To improve clarity, data for Kongolia (Black Hill) and Buckleboo (Muratchina Dam) are not plotted. Rainfall at these two stations is lower than at Murray Bridge but similarly distributed and always higher than at Bower.

(a)


40
30
20
10
0
Temperature (°C)
J
F
M
A
M
J
J
A
S
O
N
D

(b)


60
50
40
30
20
10
0
Rainfall (mm)
J
F
M
A
M
J
J
A
S
O
N
D

dams in open scrub dominated by mallee species (*Eucalyptus socialis*, *E.gracilis*; Specht 1972). Both are close to the northern edge of this vegetation type. The River Marne flows through mallee covered country but along the river there are good stands of red gum and some other plants typical of the river swamp vegetation described from the river Murray by Specht (1972). The vegetation at Murray Bridge is a mixture of the river swamp association plants and a number of introduced weeds. At Gawler, frogs were again recorded in a man-made dam in cleared country formerly covered by mallee (Specht 1972). Magrath Flat vegetation is the tussock grassland typically found in low lying swamplands in south-eastern South Australia (Specht 1972). Similarly at Petherick Road though here there are also ti trees (*Melaleuca gibbosa*) and elements of the open scrub association found on the higher land to the east (Specht 1972).

c) Sympatric frog species.

Anuran species with calling seasons at least partly overlapping that of western *L.tasmaniensis* are listed in Table 5.8. The largest number, six, occurs at Murray Bridge and the least, one, at four sites, Muratchina Dam, Aroona Valley, Chamber's Gorge and Finke Springs.

Species with call dominant frequencies close to that of western *L.tasmaniensis* (cf. section 5.2 1)) occur at all sites except Aroona Valley, Chamber's Gorge, Finke Springs and Black Hill (Table 5.8). It is conceivable that *L.tasmaniensis* at Black Hill are occasionally exposed to calls of *Neobatrachus* species, as *N.pictus* and possibly *N.centralis* almost certainly occur on the plains north and south of the Marne river. However, it is unlikely that *Neobatrachus* ever interfere with *L.tasmaniensis* calls, as in my experience both *N.pictus* and *N.centralis* usually call in temporary pools or sometimes farm dams but rarely in flowing water. At Black Hill all frogs recorded were calling in the river Marne; none were in dams or temporary swamps.

Species	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	Dominant Frequency (Hz)
Litoria aurea raniformis	-	-	P	-	-	-	-	-	-	-	1200
Litoria ewingi	P	P	P	?P	P	-	-	-	-	-	2490
Litoria peroni	-	-	P	-	-	-	-	-	-	-	2020
Litoria rubella	-	-	-	-	-	-	-	-	-	?P	no data
Ranidella riparia	-	-	-	-	-	-	-	P	P	P	2325 (2200 - 2600)[6]
Ranidella signifera	P	P	P	P	P	-	-	-	-	-	2490
Limnodynastes dumerili	P	P	P	P	P	P	-	-	-	-	654
Limnodynastes fletcheri	-	-	P	-	-	-	-	-	-	-	1620
Limnodynastes tasmaniensis ##	P	-	-	-	-	-	-	-	-	-	1966 (1832 - 2160)[1]
Neobatrachus centralis	-	-	-	-	-	P	-	-	-	-	1470
Neobatrachus pictus	P	P	-	P	-	-	P	-	-	-	1300

Table 5.8 Frogs which are sympatric with, and whose calling season partly overlaps that of, the western call race. Localities are: 1. Petherick Road, 2. Magrath Flat, 3. Murray Bridge, 4. Gawler, 5. Black Hill, 6. Bower, 7. Muratchina Dam, 8. Aroona Valley, 9. Chamber's Gorge, 10. Finke Springs. Symbols as in Table 5.4. Source of dominant frequency not repeated if already given in Table 5.4 or 5.7.

southern call race

1. J.D.Roberts, unpublished observations.
2. Loftus-Hills (1973)
3. Loftus-Hills and Littlejohn (1971)
4. Martin (1972) (a) *L.d.variegatus*
    (b) *L.d.dumerili* allopatric to *L.terrareginae*
5. Littlejohn and Martin (1965 a)
6. Littlejohn and Martin (1965 b)
7. Littlejohn (1958)

Table 5.9 Sources of dominant frequency data given in Tables 5.4, 5.7 and 5.8. Full references are given in the bibliography.

	Min.	Max.		Min.	Max.
Petherick Road	5.9	23.3	Bower	7.0	23.3
Magrath Flat	10.6	18.4	Muratchina Dam	-4.4	24.1
Murray Bridge	7.4	21.1	Aroona Valley	6.5	24.2
Gawler	5.4	23.8	Chamber's Gorge	-2.8	24.2
Black Hill	6.6	22.5	Finke Springs	4.2	24.1

Table 5.10 Maximum and minimum values of note repetition rate in various western call race populations. Method of derivation is given in the text.

This summary of relevant environmental features justifies the assertion made at the beginning of this section that the ten western call race samples came from a wide range of habitats. I will now discuss variation in each of the call components.

2) Dominant Frequency.

Statistical analysis of this variable summarised in Table 5.2(c) indicates significant differences in population means and in sample variances.

Six means (Chamber's Gorge, Muratchina Dam, Bower, Black Hill, Gawler and Petherick Road) are about equal and fall between 1400 and 1500 Hz. Means at Murray Bridge, Magrath Flat are also about equal but much lower at close to 1300 Hz while the remaining locality, Finke Springs falls between these two groups (Figure 5.4). Variances follow a different pattern with the three Flinders Ranges samples (Chamber's Gorge, Aroona Valley and Finke Springs) all having much larger sample standard deviations than the other localities (Figure 5.4).

In discussing the southern and northern call races I could generally attribute high sample variances to the presence of one or two extreme values. I cannot do this with the dominant frequency data from the Flinders Ranges. In all three samples ranges are wide relative to the more southern localities and the data are distributed through the range. Further, the ranges extend to much higher and lower values than in other western call samples. Indeed, some dominant frequencies are more characteristic of the northern call race.

Obvious explanations of these high variances are not available. In this call race there is a weak correlation of head width and dominant frequency (Figure 5.10) and the existence of some very large frogs in these populations may account for the low dominant frequencies. Increasing body size has been cited as an important adaptation to

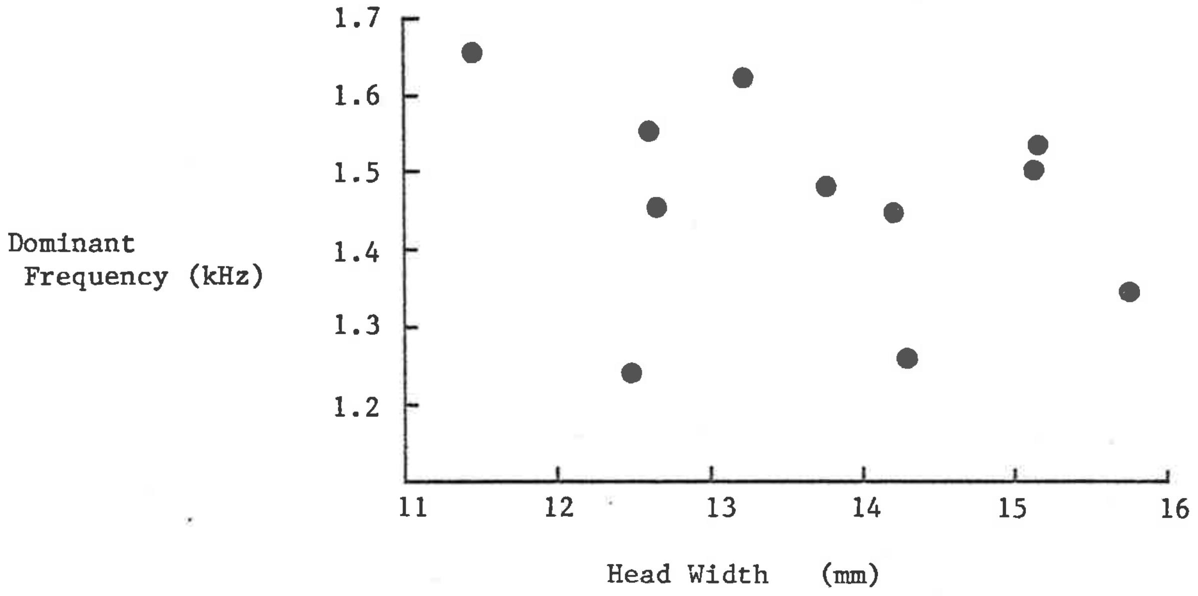


Figure 5.10 Plot of head width verses call dominant frequency for western males from various populations. Kendall's rank correlation coefficient, -0.491, p less than .05; Product moment correlation coefficient, -0.297, p greater than .05.

aridity in anurans (Nevo 1972), though it might clearly result from other effects; e.g. old age. Arguing for an incidental origin of low frequencies does not help in explaining the high frequencies also observed. Further, the environmental data summarised earlier did not definitely indicate that Aroona Valley, for example, was any drier than Bower.

At the three Flinders Ranges sites there is no frog species with a call dominant frequency close to that of western L.tasmaniensis. In fact, the sole synchronic anuran species has a much higher dominant frequency (Table 5.8). In this situation there may be no disadvantage in aberrant dominant frequencies as there is almost no risk of confusion with any other species. Thus, I would argue that the high variances in the data from Aroona Valley, Chamber's Gorge and Finke Springs result from a relaxation of a selective force imposed in other areas either by a number of sympatric frog species calling at various frequencies or by a single species with a dominant frequency very close to that of the western call race.

There is no pattern to the variation in sample means. I cannot see any correlations with any of the environmental variables discussed above (section 5.4 1)), nor is there any pattern of high or low frequencies in various years (cf. southern call race data discussed in section 5.2). I can only conclude either that some unrecognised selective agent is acting, or that some feature of my sampling routine is inadequate. For example, although sample sizes are generally large (mean size, 16.1), they may be too small to give an accurate estimation of population means. At most sites the population of calling males was much larger than the number of calling males recorded. I did not conduct any investigation to determine what an appropriate sample size might be, and as far as I am aware this has not been done with any frog call.

Without more careful investigation I cannot really say anything about the variation in mean dominant frequencies in western call race populations.

3) Pulse repetition rate.

As with dominant frequency, these data showed significant differences in population means and variances (Table 5.2(c)). Again, the largest variances occurred in the Flinders Ranges samples, but they were not particularly larger than those at Gawler, Murray Bridge and Bower (Figure 5.4). The heterogeneity of means appears to result from differences between the low means at Petherick Road, Magrath Flat and Murray Bridge and the high values at Aroona Valley and Chamber's Gorge.

In section 5.3 2) I mentioned that pulse repetition rate may be an important cue in call discrimination or localisation. Thus, as with dominant frequency, the high variance in the Flinders ranges may result from a lack of selection against aberrant pulse rates. It may be that call samples from Finke Springs, Chamber's Gorge and Aroona Valley represent the primitive condition, and that populations with low variances have experienced some form of selection. Selection may have improved distinctness of western calls from calls of a single species, e.g. either Neobatrachus pictus or N.centralis (cf. Table 5.8), or from calls of a number of species.

The quality of recordings and resultant oscillogrammes may also have contributed to the high variance of pulse repetition rate in the Flinders ranges data. All three samples were from frogs calling in running water, and in some recordings there was considerably continuous background noise from water flowing over stones. Six of the other seven western samples were from frogs calling in still water, and at the seventh (Black Hill) there was no water noise.

At Chamber's Gorge only one analysed call was affected, but the problem was worse at Finke Springs with 2 of 8 calls affected; and at

Aroona Valley 6 of 18 calls suffered some degree of interference. I am not certain that this background noise affected pulse repetition rate estimates though in some cases it seemed to impose perturbations on otherwise smooth oscillations. This may mean some pulses were measured as shorter then they really are and consequently some pulse repetition rate values are inflated.

Pulse rate means show a slight, albeit irregular, trend to higher values in more northerly populations (Figure 5.4). Thus there is a crude correlation of mean pulse repetition rate values with certain environmental features (lower rainfall and or higher temperatures, Figure 5.9). However, before incidental origin is invoked, the similarities and differences of the climate at Aroona Valley, Bower and Muratchina Dam need to be clarified (section 5.4 1)).

A more plausible alternative is that the higher means at Aroona Valley, Chamber's Gorge and Finke Springs represent adaptations allowing more efficient propagation or easier localisation of the signal in the particular local environments. As mentioned above there was often considerable water noise, and the generally stoney nature of the creeks and surrounding country (Northcote _et al_. 1968) may mean that sounds need quite different properties to be transmitted effectively (cf. Morton 1975).

4) Average notes per call.

Analyses of these data indicate that there are significant differences in population means but no sample variance heterogeneity (Table 5.2(c)).

The lowest mean was at Aroona Valley and the highest at Murray Bridge (Figure 5.4). The difference between these two means was a major contribution to the significant Anova reported in Table 5.2. The Aroona Valley mean is also probably significantly different from that in five other populations; Muratchina Dam, Bower, Black Hill, Magrath Flat and Petherick Road (Figure 5.4).

As with pulse repetition rates, low means at Aroona Valley and Chamber's Gorge may represent adaptations to the peculiarities of the local environment or southern populations of the western call race may have been selected for an increase in note number to improve call distinctness.

With this call parameter I again have no real appreciation of why population means vary as they do. However, the causes of the low means are interesting. At both Aroona Valley and Chamber's Gorge, the frequency of three note calls is much higher than in other western call race populations. At both sites one of the recorded frogs made some two note calls in the last five recorded calls. Some of this notes per call data is thus more characteristic of the northern call race (Ch. 2) as was the case with a few dominant frequency values from this area (section 5.4 2)).

5) Note repetition rate.

The analysis of covariance reported in Table 5.3(b) indicates significant differences in slope and not surprisingly therefore differences in elevation (Snedecor and Cochran 1967). Variation about the fitted regression lines was about the same at all sites (Table 5.3(b) (i)).

Inspection of Figure 5.5(b) shows the cause of the slope heterogeneity immediately. Seven of the ten lines are almost coincident, one (Magrath Flat) has a relatively low slope while two (Muratchina Dam and Chamber's Gorge) have higher than average slopes (Figure 5.5(b)).

This sort of variation is difficult to explain as it does not result in any major shift in the values of note repetition rate. If a temperature effected call component is to convey information frogs must be able to respond irrespective of the temperature of the signal producer. That is, a response must be possible at any value note repetition rate might take over the normal range of calling temperatures. Otherwise one must assume that frogs using say note repetition information are somehow temperature compensated: an unlikely event particularly if the male is calling in water and the female making an approach on land (Straughan 1975; Littlejohn and Watson 1976). Thus when looking at geographic variation in note repetition rate, the range of possible values may be the most relevant parameter to consider.

Maximum and minimum values of note repetition rate for the ten western populations sampled are given in Table 5.10. These values were obtained from the fitted regression lines for each population and assuming frogs at all sites call over the same range of water temperatures. The temperature range chosen was 6 to 25°C. This encompasses the lowest and highest water temperatures at which I have recorded western <u>L.tasmaniensis</u> calling.

The Magrath Flat limits fall within the range encountered in other areas, and the upper limits at Muratchina Dam and Chamber's Gorge coincide with those at all other localities (Table 5.10).

I am uncertain how to interpret the lower limits for Muratchina Dam and Chamber's Gorge. Clearly negative values of note repetition rate are meaningless. However, even if notes per second values as low as one occurred at Muratchina Dam or Chamber's Gorge, this would not be far outside the range found in other western populations. Thus, unless a change in the slope of the water temperature note repetition rate relationship is accompanied by a major shift in elevation notes per second values will not fall outside the normally encountered range.

Slight slope changes established by chance might persist as there would be little selection pushing the slope back to more usual values.

The most easily conceived chance event is a massive reduction in population size with the few surviving frogs having an aberrant note repetition rate water temperature relationship (the founder effect; Mayr 1963). At Chamber's Gorge, Muratchina Dam and Magrath Flat population decimation might have easily occurred: at the first two sites from drought and at the last by flooding with sea water. At Magrath Flat the fresh water swamps are only separated from the salt waters of the Coorong by a low earthern bank. This was breached in 1973, and from discussions I had with local residents it seems that this was not a unique occurrence.

There is no correlation of slope values with any of the environmental variables discussed earlier, and I think it unlikely that either incidental or adaptive origins are good explanations of the low slope at Magrath Flat and the high slopes at Muratchina Dam and Chamber's Gorge.

6) Note Duration.

The only trend in these data is a rough negative correlation of note duration and water temperature. This is not surprising given the analysis of single samples detailed in Chapter 2.

Once again this analysis of call variation has given few clues to the origin of the call races of *L.tasmaniensis*. However, it did suggest that the number or nature of sympatric anuran species and or the nature of the physical environment may have affected two call components, dominant frequency and pulse repetition rate. The latter result agrees with the interpretation I made of northern call race pulse repetition rate variances in section 5.3 2).

5.5 Conclusion.

This investigation of call structure in the *L.tasmaniensis* complex revealed little of how or why the three call races evolved. I am confident that reinforcement, at least between the extant taxa, did not contribute (Chapter 4).

Nevo (1969) felt that Littlejohn (1969) had overemphasised the role of reinforcement in the evolution of premating isolating mechanisms and argued that incidental origins might have an at least equivalent part to play. My analyses of geographic variation in L.tasmaniensis calls gave little or no evidence of incidental origins of call differences, despite the fact that in two call races populations sampled came from subtly (southern call race, section 5.2 1)), or very obviously different (western call race, section 5.4 1)), environments.

The third theory, adaptive change in differing reproductive environments, has little factual support at present but of these three ideas about the evolution of premating isolating mechanisms, this last receives best support from my data (section 5.3 2) and 5.4). However, until the significance of various call components in call function is known better, and until some of the problems associated with sampling call data (e.g. sample sizes, section 5.4 2); annual fluctuations in call component values, section 5.2 1)) are resolved, I would be foolish to suggest that this was the mechanism that produced the L.tasmaniensis complex.

From the present known distribution of the call races (Figure 2.6) and in the lack of as there is no evidence of reinforcement (Chapter 4) I think I can safely conclude that the call races evolved in allopatry. Assuming this, below, I will outline an hypothetical scheme for the evolution of the L.tasmaniensis complex.

In section 4.2 I proposed that the southern call race evolved in Tasmania and implied it diverged from a northern like call type. Equally though, the northern and southern calls may have stemmed from a common stock or the northern may have evolved from a southern call form. If adaptive differentiation of calls in differing reproductive environments was important then the last suggestion in the previous sentence makes some sense. Compared to Tasmania there is a much greater diversity of anuran species on the eastern Australian mainland (Moore 1961; Littlejohn 1967; Littlejohn and Martin 1974), and presumably therefore a more complex sound environment at many or most L.tasmaniensis breeding sites. In this situation, calls which convey more information or transmit it in a different way may be required. The greater structural complexity of northern calls, relative to southern, may meet such a requirement.

However, calls in a simple acoustic environment may also change. In south-eastern South Australia, and in Victoria, calling southern males can generally be seen easily (section 4.2 1)). No specific data are available on this point for Tasmania but the positioning of egg masses in open water (Littlejohn and Martin 1974) suggests that L.tasmaniensis males use similar calling sites there. Thus some of the acoustic cues used for male localisation in the northern call race may be replaced by visual ones in the southern.

The pattern of evolution of the western and northern call races is as difficult to determine as the southern, northern split discussed above. The close structural similarity of western and northern calls suggests that one evolved from the other. The limitation of the western call race to the Flinders, Mount Lofty ranges system and the closely adjacent plains (Figure 2.6) makes me believe that it must have evolved there. One other frog species, Ranidella riparia is endemic to this region (Tyler and Roberts 1973), but this area is not usually recognised as an important one in the evolution of the southern Australian frog fauna (Littlejohn 1967, 1971b; Moore 1961). The proto-western stock presumably had a call like that of the present northern call race. It could then be argued that the occurrence of some northern call elements in western populations at Aroona Valley, Chamber's Gorge and Finke Springs is a remnant of this base stock. This would, however, require that southerly populations of the western call race had evolved further and lost these elements (section 5.4).

The northern-like progenitor of the western call race could have entered the Flinders ranges from the north or north east by a fairly simple move from the western drainages in Queensland (e.g. Cooper's and Strzelecki creeks) or by westward moves from the Murray, Darling river system into the ridge of the Flinders range extending east toward Broken Hill (Figure 2.6). A general increase in rainfall or a series of abnormally wet years as occurred in 1973, 1974 (Bureau of Meteorology 1973, 1974) would bridge arid barriers which might generally prevent movements into the Flinders ranges from the east and north.

L.tasmaniensis occur in the predicted source areas, though I have no information on call types there. Moore (1961) reported L.tasmaniensis from Wilcannia on the Darling and from Toper Station, east of Broken Hill. I have specimens from Coongie lake, 160 km south east of Birdsville, and Nappa Merri, 225 km south east of Birdsville.

Although it is easy to dream up schemes to get a L.tasmaniensis stock to the Flinders ranges, I cannot offer any reasons why northern calls should have evolved there into what I now recognise as the western call race. One difficulty with this is that I do not know what the call of my presumed stock was like. All the northern populations that I sampled may have been affected by introgression with either the southern or western call races (section 4.3 and 5.3). The original northern call race may have been somewhat different. L.tasmaniensis calls in Queensland show some characteristics of the northern call race but note number is higher in some populations (and closer to values in the western call race), and note repetition rates are differently effected by temperature (Straughan 1966). Queensland L.tasmaniensis calls may be more like the originally evolved northern call race than any of the populations that I sampled.

This thesis has not unravelled the evoultionary history of the *L.tasmaniensis* complex, but, given the events that produced the southern, northern and western call races are over, this result is not surprising. I have not found convincing evidence supporting any of the three theories of premating isolating mechanism evolution outlined in Chapter 1. However, this reflects more on the inadequacies of this study and the study object, the *L.tasmaniensis* complex, than on the theories themselves.

6. Appendices

Appendix 1. Comparison of water and cloacal temperatures in calling males of the *L.tasmaniensis* complex.

Water and cloacal temperatures were available from seven northern, seven southern, eighteen western and five males from western, northern call race overlap zones. Twenty eight males showed either positive or negative differences between water and cloacal temperatures while in the other nine these values were identical.

Data were analysed by the Wilcoxon matched pairs signed ranks test (Siegel 1956). Results were: all data, Wilcoxon T = 176, p greater than .05. That is, the observed diffrences between water and cloacal temperatures were not significant. Water temperatures ranged from 11.1 to 24.8°C. For the western call race alone, Wilcoxon T = 46, p greater than .05.

I conclude that water temperature is a reliable indicator of cloacal temperatures in calling *L.tasmaniensis*.

Appendix 2. Comparison of data from oscillograms of the same calls with and without the Allison 2 AB passive filter.

Oscillograms of the last recorded call of twenty males from Kroehn's landing were made with and without the filter. Call components were measured as in Chapter 2 and the data analysed as matched pairs with the Wilcoxon matched pairs signed ranks test (Siegel 1956).

Results are:

(a) Dominant frequency	Wilcoxon T = 65.5	n.s.
(b) Pulses per second	Wilcoxon T = 76.0	n.s.
(c) Notes per second	Wilcoxon T = 75.0	n.s.
(d) Pulses per note	Wilcoxon T = 12.5	*
(e) Note duration	Wilcoxon T = 6.0	**

(n.s. not significant, * p less than .05, ** p less than .01)

Only two call components were affected by the filter, note duration was longer and pulses per note higher when the filter was used. Neither result is caused by a distorting effect of the filter but rather from the fact that it reduces background concealment of the beginning and end of notes.

I conclude the filter has had no important effect.

Appendix 3. Shortened citations of papers contributing data to Table 3.1.

Awbrey, F.T. (1968) Copeia 1968:420 - 422.

Blair,W.F. and M.J.Littlejohn (1960) Evolution 14:82 - 87.

Bull,C.M. (1973) Unpublished Ph.D. thesis, Department of Zoology, University of Western Australia.

Forester,H.C. (1973) Copeia 1973:60 - 67.

Gerhardt,H.C. (1973) Am. Midl. Nat. 89:81 - 88.

------------- (1974) Copeia 1974:534 - 536.

------------- (1974) Am. Midl. Nat. 91:424 - 433.

Littlejohn,M.J. (1960) Copeia 1960:370 - 371.

------------- (1961) Tex. J. Sci. 13:49 - 50.

------------- , M.J.Fouquette and C.Johnson (1960) Copeia 1960:47 - 49.

------------- and J.J.Loftus-Hills (1968) Evolution 22:659 - 663.

------------- and T.C.Michaud (1959) Tex. J. Sci. 11:86 - 92.

------------- and G.F.Watson (1974) Copeia 1974:171 - 175.

Martof,B.S. (1961) Am. Midl. Nat. 65:118 - 126.

----------- and E.F.Thompson (1964) Am. Midl. Nat. 71:198 - 209.

Menzies,J.I. (1972) Herpetologica 28:291 - 300.

Michaud,T.C. (1962) Copeia 1962:213 - 215.

Oldham,R.S. (1974) Copeia 1974:982 - 984.

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Omissions

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