A NARROW HYBRID ZONE BETWEEN TWO WESTERN AUSTRALIAN FROG SPECIES *RANIDELLA INSIGNIFERA* AND *R. PSEUDINSIGNIFERA*: THE FITNESS OF HYBRIDS

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SUMMARY

The mechanisms which maintain the stable narrow hybrid zone between *Ranidella insignifera* and *R. pseudinsignifera* are not clear. To test the hypothesis that reduced hybrid fitness is a factor preventing gene flow through the hybrid zone, a number of experimental interspecific hybrid crosses were performed. There was no indication that hybrid progeny had lower survival than those from control conspecific crosses at any stage from egg to metamorphosis. Nor was there any evidence for hybrid sterility. Alternative hypotheses to account for the narrow hybrid zone are discussed including superior hybrid fitness at an ecotone and decreased hybrid fitness as a consequence of not being able to find a mate.

1. INTRODUCTION

STABLE narrow hybrid zones between related taxa are now well known. Two theories have arisen to account for their persistence. One, originally proposed by Anderson (1948), suggests that a hybrid zone is located at an ecotone between the habitat preferred by one taxon and that preferred by the other. If intermediate habitats are found in the ecotone, then hybrids, with part of their genome adapted to each habitat, might be fitter there than either parental taxon. The theory predicts that hybrids have heterotic fitness within the ecotone, but reduced fitness in either of the habitats outside the ecotone where one or the other parental taxon is better adapted. Moore (1977) has suggested that this hybrid superiority maintains many hybrid zones between vertebrates. Hagen (1967) and Littlejohn and Watson (1973) use it to explain specific cases of narrow hybrid zones.

The second theory suggests that hybrids formed at the secondary contact of related taxa have reduced fitness as a consequence of the breakdown of the parental genomes. This would restrict gene flow across the contact beyond a hybrid zone if intertaxon mating takes place. Dobzhansky (1970) suggested that hybrid zones would be transient because reinforcement of reproductive isolating barriers might lead to assortative mating and reduced hybrid production at the contact. Loftus-Hills (1975) has pointed out that reinforcement is not commonly known in natural situations, perhaps because of the continual swamping of hybrid zone populations by parental individuals from allopatric populations outside of the influence of the selective pressure for reinforcement (Littlejohn, 1969; Watson, 1972).

The continual production of hybrids with reduced fitness has been used

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to explain the persistence of several stable hybrid zones (Watson, Loftus-Hills and Littlejohn, 1971; Hall and Selander 1973). Both models predict that there is a difference in fitness between hybrids and their parental taxa in the region of the hybrid zone. The first model suggests that hybrids will be fitter, the second that they will be less fit than either parental form. It should be possible to differentiate between these hypotheses in specific cases.

One hybrid zone in which the mechanism of maintenance remains to be explained is that between the frog species *Ranidella insignifera* and *R. pseudinsignifera* in Western Australia. These two sibling species were first detected by differences in the male mating call (Main, 1957). Their allopatric distributions abut along a long common boundary (Littlejohn, 1959), with areas of narrow contact zones containing intermediate and parental calls (Littlejohn, 1959, Bull, 1978) and abrupt changes in electrophoretic and morphological characters (Blackwell and Bull, 1978). The position and width of one such zone has remained stable for at least 19 years (Bull, 1978). These observations suggest that the stable narrow hybrid zone lies between two taxa which have several genetical differences. Either of the two hypotheses might account for the persistence of the zone. On the one hand, the zone coincides with the scarp of the low Darling Ranges (Littlejohn, 1959; Bull, 1978). *R. insignifera* is confined to the coastal plains and *R. pseudinsignifera* predominantly occupies the inland plateau. The environmental change at the base of the escarpment where the species contact might represent an ecotone where hybrids have superior fitness. On the other hand, Main (1968) reported reduced survival to hatching of eggs from crosses between the two species, and sterility of four males with aberrant call types from the hybrid zone, supporting the model of reduced hybrid success at the zone.

Critical assessment of these alternatives is difficult because data are only available from two controlled crosses between the species (Littlejohn, 1957), in both of which the hybrid progeny survived as well to hatching as the parental forms. This paper reports a more substantial investigation into the fitness of laboratory-raised hybrids between the two species, and an attempt to discriminate between the two hypotheses. If hybrids are shown to have a reduced fitness in early growth, or if hybrid adults are shown to be sterile, then there would be strong arguments in support of the reduced hybrid fitness model, and against the model of hybrid heterosis.

2. MATERIALS AND METHODS

Adult frogs were taken from allopatric populations, and from populations in the hybrid zone. Precise locations have been previously given (Bull, 1977, 1978). Crosses were made by the method of Watson (1977). Sperm suspensions were made from males of two different populations. About half of the eggs of a female from one of these populations were placed in each suspension. Thus eggs from each female were used in both an experimental interpopulation cross and a control within-population cross. The eggs were kept in finger-bowls and checked daily until hatching was completed. Those embryos which did not develop normally were removed to prevent contamination. Hatched tadpoles from some crosses were transferred to round plastic bowls (diameter 35 cm : capacity 3.5 l) and kept in a shaded area outdoors. The tadpoles browsed on the natural algal growth

on the sides of the bowls. At two week intervals the growth and survival of tadpoles was determined until all had metamorphosed. Initially, each bowl contained 20 or 30 newly hatched tadpoles. Tadpoles from inter-population crosses, and their controls from the same female, were kept in adjacent bowls.

Metamorphosed hybrids, toe-clipped for identification, were transferred to an enclosed artificial pond. Adult males from this pond were used in experimental backcrosses during the following winter. Although mature adult hybrid females were present, none was found with eggs descended into the ovisacs ready for fertilisation. (It was common to find unclashed females of the parental species in the same condition as the hybrid females.) Other backcrosses were made by using males from a natural population within the hybrid zone which were subjectively identified as hybrids by their mating call.

3. RESULTS

(i) *Survival to hatching*

(a) *F1 hybrid crosses*

The results of 54 controlled crosses between *R. insignifera* females and *R. pseudinsignifera* males from allopatric populations are summarised in table 1. Fertilisation rates were high and no significant differences were found between control and experimental batches in 40 (74 per cent) pairs. Among the remaining 14 pairs, 10 (71 per cent) showed significantly higher fertilisation rates in the experimental interspecies batches than in the controls. No significant difference was found ($T_s = 544.5$; $t_{54} = 1.70$; n.s.) between percentage fertilisation in control and experimental groups using a Wilcoxon signed rank test (Sokal and Rohlf, 1969).

In 40 (74 per cent) of the crosses there was no difference between control and experimental batches in the success of fertilised eggs reaching hatching. In 9 of the 14 crosses where differences were significant experimental interspecific crosses performed better than controls. Over all 54 crosses the Wilcoxon signed rank test showed a trend (which was not quite significant at the 5 per cent level) for higher success in interspecific batches relative to their controls ($T_s = 515.5$; $t_{54} = 1.95$; $P < 0.1$).

Table 1 also summarises 40 controlled crosses between *R. pseudinsignifera* females and *R. insignifera* males from allopatric populations. Fertilisation success was high and in 31 (78 per cent) cases there was no significant difference between control and experimental batches. In the remaining nine crosses significantly higher proportions of eggs were fertilised in the experimental interspecific batches than in the controls. The Wilcoxon signed rank test for all 40 crosses showed that experimental batches had significantly higher fertilisation success than their controls ($T_s = 225$; $P < 0.01$). (In this test a Student's t value is only derived for samples greater than 50.)

Thirty (75 per cent) pairs of crosses showed no significant difference between control and experimental batches in the proportion of fertilised eggs which hatched. Among those where there was a significant difference nine (90 per cent) had a higher proportion of the experimental batch hatching than its control. The Wilcoxon signed rank test for all 40 crosses showed significantly higher proportions hatching in the experimental batches compared to their controls ($T_s = 208$; $P < 0.005$).

TABLE 1

Summary of the results from egg to hatching in sets of laboratory crosses and their controls. In F1 crosses allopatric populations are indicated in the following manner—*R. insignifera* populations: WW—William Welshpool Road; TH—Thornlie; EB—Ellen Brook; VP—Victoria Parade. *R. pseudinsignifera* populations; PB—Pickering Brook; JS—Jacoby St.; CR—Charles Rd.; GF—Glen Forrest; WB—Wandana Bog; EG—East of Gingin T.O.

Parent population		Control crosses				Experimental crosses		
		No. of pairs of crosses	No. of eggs	Percentage of eggs fertilised	Percentage of eggs hatching	No. of eggs	Percentage of eggs fertilised	Percentage of eggs hatching
♀	♂							
<i>R. insignifera</i> ♀ × <i>R. pseudinsignifera</i> ♂								
WW	PB	12	843	84.7	80.0	733	93.5	85.3
WW	JS	10	725	88.7	83.7	600	95.3	87.9
TH	PB	5	404	89.9	95.9	505	83.4	94.1
TH	JS	14	670	87.2	95.9	553	91.7	92.1
TH	CR	7	301	85.1	87.1	300	92.7	92.1
TH	GF	2	31	83.9	88.5	29	62.1	66.7
EB	WB	1	26	50.0	92.3	15	73.3	100.0
EB	EG	3	154	90.9	77.9	176	92.6	96.9
<i>R. pseudinsignifera</i> ♀ × <i>R. insignifera</i> ♂								
JS	WW	25	1419	79.6	81.0	1175	88.9	91.0
JS	TH	6	489	87.9	96.7	474	93.0	95.9
CR	TH	1	57	89.5	94.1	62	96.8	100.0
PB	WW	3	273	86.8	69.6	194	96.9	85.1
GF	VP	1	43	95.4	95.1	54	94.4	94.1
EG	EB	2	102	92.2	97.9	206	98.1	100.0
WB	EB	2	50	98.0	98.0	101	93.1	98.9
<i>R. pseudinsignifera</i> ♀ × F1 ♂ (<i>pseudinsignifera</i> ♀ × <i>insignifera</i> ♂)								
—	—	7	257	86.0	87.4	364	71.7	69.0
<i>R. insignifera</i> ♀ × F1 ♂ (<i>pseudinsignifera</i> ♀ × <i>insignifera</i> ♂)								
—	—	4	157	88.5	88.5	236	80.9	90.6
<i>R. pseudinsignifera</i> ♀ × F1 ♂ (<i>insignifera</i> ♀ × <i>pseudinsignifera</i> ♂)								
—	—	2	33	60.6	90.0	44	65.9	86.2
<i>R. insignifera</i> ♀ × F1 ♂ (<i>insignifera</i> ♀ × <i>pseudinsignifera</i> ♂)								
—	—	2	101	98.0	98.0	105	92.4	97.9

(b) Backcrosses

The results of 15 backcrosses involving laboratory raised hybrid males and wild-caught parental females are summarised in table 1. Individual backcrosses, when compared with their controls, gave no indication that hybrid males were sterile or that there was reduced viability of their offspring. In 12 crosses fertilisation success was as high in control as in experimental batches. In the remaining three crosses the experimental batches had significantly lower fertilisation success. In 13 pairs of crosses there was no significant difference between control and experimental batches in the proportion of fertilised eggs which hatched. The control batch had a significantly higher proportion hatching in one, and the experimental batch in the other of the two remaining crosses. The Wilcoxon

signed rank test showed no significant difference between control and experimental batches for the 15 crosses for either level of fertilisation ($T_s = 37$; n.s.) or survival to hatching of fertilised eggs ($T_s = 52$; n.s.).

These data were supplemented by crossing three field-caught males with subjectively determined hybrid call types from the Wilkie-Coulston Road population, to a female from the same site. The three egg batches had an average 78.9 per cent fertilisation, and an average 88.5 per cent of fertilised eggs hatched. The control cross between this female and a male *R. pseud-insignifera* from Jacoby Street had 80 per cent fertilisation and 83.3 per cent hatching. The results gave no indication of sterility in hybrid males.

Clasped females from populations previously assessed (Blackwell and Bull, 1978; Bull, 1978) to contain high proportions of hybrid males (Helena Valley Gutter Creek, Wilkie-Coulston Road) produced viable eggs with high levels of survival to hatching (Bull, 1973), but the identity of the females could not be ascertained. Tadpoles were found in one of the raising ponds which contained only F1 hybrid adults, implying that F1 hybrid females can produce viable offspring.

(c) *Other crosses*

Females from allopatric populations were crossed to males from populations on the opposite edge of the hybrid zone. Electrophoretic data (Blackwell and Bull, 1978) and acoustic data (Bull, 1978) suggest that these latter populations consisted predominantly of one parental species with some hybrids. Thus most of the crosses would have produced F1 hybrid progeny and some backcross progeny.

Of 20 controlled crosses between *R. insignifera* females from Thornlie and from William-Welshpool Road and hybrid zone males from Hale Road and Boya, three showed significant differences in levels of fertilisation (two with experimentals exceeding controls), and three showed significant differences in survival to hatching of fertilised eggs (two with controls exceeding experimentals). Wilcoxon signed rank tests showed no significant difference between control groups and experimental groups over the whole 20 crosses for either level of fertilisation ($T_s = 87.5$; n.s.) or for survival to hatching ($T_s = 100$; n.s.).

In 24 controlled crosses between *R. pseudinsignifera* females from Charles Road, Jacoby Street and Glen Forrest and hybrid zone males from Hartfield-Lewis Road, First Dip Helena Valley Road and Gravel Pit Helena Valley Road (the latter three localities being on the *R. insignifera* edge of the hybrid zone), three showed significant differences in level of fertilisation (two with controls exceeding experimentals) and five showed significant differences in the survival to hatching of fertilised eggs (three with controls exceeding experimentals). Wilcoxon signed rank tests showed no significant difference between controls and experimentals for all 24 crosses for either level of fertilisation ($T_s = 125.5$; n.s.) or for survival to hatching of fertilised eggs ($T_s = 129$; n.s.).

(ii) *Survival from hatching to metamorphosis*

(a) *F1 hybrid crosses*

Table 2 summarises the survival until metamorphosis, of tadpole from sets of crosses between allopatric populations of the two species compared

TABLE 2

Summary of the percentages of tadpoles reaching metamorphosis in sets of laboratory crosses and their controls

No. of pairs of crosses	Control crosses		Experimental crosses	
	No. of tadpoles	Percent metamorphosing	No. of tadpoles	Percent metamorphosing
	<i>R. insignifera</i> ♀ × <i>R. pseudinsignifera</i> ♂			
11	314	46.2	290	40.7
	<i>R. pseudinsignifera</i> ♀ × <i>R. insignifera</i> ♂			
13	353	35.7	363	30.0
	<i>R. pseudinsignifera</i> ♀ × F1 ♂ (<i>pseudinsignifera</i> ♀ × <i>insignifera</i> ♂)			
5	112	28.6	108	41.7
	<i>R. insignifera</i> ♀ × F1 ♂ (<i>pseudinsignifera</i> ♀ × <i>insignifera</i> ♂)			
2	90	34.4	90	43.3
	<i>R. pseudinsignifera</i> ♀ × F1 ♂ (<i>insignifera</i> ♀ × <i>pseudinsignifera</i> ♂)			
2	18	72.2	25	60.0
	<i>R. insignifera</i> ♀ × F1 ♂ (<i>insignifera</i> ♀ × <i>pseudinsignifera</i> ♂)			
2	56	51.8	79	43.0

to their controls. Some crosses showed significant differences between control groups and experimental groups (in four cases the experimentals having higher survival, and in two cases the controls), but there was no indication that either group was consistently more successful. Wilcoxon signed rank tests showed no differences in the 11 crosses to *R. insignifera* females ($T_s = 30.5$; n.s.), in the 13 crosses to *R. pseudinsignifera* females ($T_s = 23$; n.s.) or in the pooled sample of 24 interspecies crosses ($T_s = 128$; n.s.).

(b) *Backcrosses*

Table 2 shows the survival of tadpoles to metamorphosis in 11 backcrosses to hybrid males. Individual crosses showed variable success relative to their controls. In three cases backcrosses had significantly higher survival, and in one the control did, but there was no significant trend, either among those pairs that did show significant differences, or in the whole sample (Wilcoxon signed rank test, $T_s = 33$; n.s.).

(c) *Other crosses*

Progeny of nine of the crosses between *R. insignifera* females and males from the *R. pseudinsignifera* edge of the hybrid zone were raised to metamorphosis. Two were significantly different from their controls, one having higher and the other lower survival. In 11 crosses between *R. pseudinsignifera* females and males from the *R. insignifera* edge of the hybrid zone the survival to metamorphosis was not significantly different from controls in eight cases, was greater than controls in two cases and was lower than

controls in one case. Wilcoxon signed rank tests showed no overall differences for each of the two samples ($T_s = 17$; n.s. and $T_s = 30.5$; n.s.) nor for the pooled sample of 20 crosses ($T_s = 99$; n.s.).

4. DISCUSSION

The experimental data can now be examined in the light of the hypothesis that the stable narrow hybrid zone is maintained by the reduced fitness of hybrids produced by parental individuals which have migrated into the zone and mated. The data from this study show no evidence of hybrid disadvantage related to intrinsic genetical incompatibility between the two species. Hybrid crosses were equal to or better than parental control crosses in the proportions successfully developing in all stages from egg to metamorphosis. An earlier analysis (Bull, 1977) showed no differences between the two species or their hybrids in the time to reach metamorphosis. The size and wet weight of metamorphosing hybrids was intermediate between the two parental species. Laboratory raised hybrid males, intermediate calling males from a hybrid zone population, and other males from within the hybrid zone, all were as fertile as control males and eggs fertilised by these males developed as normally as those fertilised by control males. Indirect evidence suggested hybrid females produce viable eggs. In summary, hybrids do not show reduced fitness in terms of survival and growth in laboratory conditions, nor is there any indication of infertility in hybrid adults. Thus the hypothesis that reduced hybrid fitness results from interspecific genomic incompatibility is not supported by the experimental data.

The alternative hypothesis is that hybrids have superior fitness within an ecotone at the common boundary. Clearly the laboratory is not the site to test this hypothesis. It is not necessarily supported by the demonstration of hybrid vigour from egg to hatching in laboratory containers, because the model predicts reduced hybrid fitness in all but the intermediate habitats of the ecotone. Furthermore it is not clear that there is an ecotone at the common boundary.

The hybrid zone coincides with a low escarpment, but there are no obvious major changes in macroclimate, vegetation, pond properties (Bull, 1973, 1978) or soil type (Burbridge, 1963). The scarp may act as a physical barrier reducing migration by *R. insignifera* out of the coastal plain, but there is no obvious change in habitat which would prevent *R. pseudinsignifera* from migrating on to the plain. Nevertheless the hypothesis should be tested by measuring relative survival of the two species and their hybrids at different sites across the zone.

Despite the results of this study there are two ways that reduced hybrid fitness may play a part in maintaining the hybrid zone. Firstly the fitness in laboratory containers may be quite irrelevant to the relative survival of hybrid and parental individuals in natural populations. There may be incompatibilities between the genomes of the species such that hybrids are not as well adapted to all the environmental stresses that they must face. Alternatively, hybrids may survive as well as the parental species but have lowered fitness because male hybrids with intermediate calls cannot attract mates. One case is known where calls of hybrid males attracted female frogs of the two parental species as well as the calls of the parental species (Littlejohn and Watson, 1976), but in other anuran species, females are

highly selective of the male they choose to mate with from within a chorus (Licht, 1976; Wells, 1977). Females of other species of frogs and crickets discriminate against hybrid calls when calls of parental species are offered as an alternative (Hoy and Paul, 1973; Gerhardt, 1974). This factor may reduce the reproductive success of hybrids sufficiently to prevent introgression proceeding beyond the hybrid zone. These various models still need to be tested experimentally, but this paper eliminates one simple hypothesis, that the maintenance of the stable narrow hybrid zone can be explained by a reduced hybrid fitness resulting from major genetic incompatibilities between the two species.

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