aldehyde (Baker), methyl salicylate (Rush and Hebble), *p*-hydroxybenzoic acid (Sigma), and ethyl *p*-hydroxybenzoate (Aldrich). Application of all these compounds to the vulvar area of the females caused increased ano-genital investigation of the females. This effect waned rapidly, however, and did not lead to sexual behavior or mounting. In two of six trials with propyl *p*-hydroxybenzoate (Aldrich), the males attempted to mount the female. In each trial there were two attempts to mount, but in both trials male interest waned within 10 minutes.

There have been numerous discussions regarding the chemical complexity of higher animal chemical communication systems (*5*). Specialized scent glands are common in vertebrates, but the odor profiles generated by the secretions of these glands are highly complex. Often they consist of hundreds of compounds in varying concentrations (*6*). Our own chromatographic analysis of odors produced by beagle vaginal secretions show that these too are highly complex. There can be little doubt that there is a large amount of information stored in the olfactory signatures of these secretions. The question is whether animals have a need to use all of this information in all cases.

In the case of the release of sexual behavior in the dog, it is premature to speculate. There are many other components of the vaginal odor that we have not yet identified and tested for behavioral effects. Some of these odorants may be active alone or as synergists to methyl *p*-hydroxybenzoate. The fact still remains, however, that a single synthetic compound will release sexual behavior identical to that released by an estrous female dog. It is possible that some odorants may be far more important than others as signal carriers in scent.

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Postmating Reproductive Isolation in *Pseudophryne* and the Evolutionary Significance of Hybrid Zones

Abstract. *Hybrid zones involving* Pseudophryne *(Anura: Leptodactylidae) provide direct evidence for the operation of postmating reproductive isolating mechanisms in animals in a natural situation. Significant introgression is prevented by hybrid inviability but there is no evidence for the reinforcement of premating isolating mechanisms. These parapatric hybrid zones are of unknown etiology, but are interpreted as being relatively old and persistent.*

When two distinct populations with imperfect isolating mechanisms come together, it is generally believed that they will either fuse completely or diverge to become more perfectly isolated (*1*). A third possible outcome is that some hybrid zones are relatively ancient and are at equilibrium (*2*). Factors preventing fusion or reinforcement of isolating mechanisms in such cases are seldom known but assumed to involve an interplay between selection against hybrids, and the influx of genes from adjacent homospecific populations (*3*). There is considerable evidence for postmating isolation derived from artificial hybridization (in groups where these experiments are feasible), but there are few data on their operation in nature. In the frog genus *Pseudophryne*, I found evidence of hybrid inviability in nature and identified some factors involved in long-term maintenance of narrow hybrid zones (*4*). I conclude from this study that the evolutionary significance of other similar hybrid zones now requires reassessment. Many such zones, traditionally interpreted as resulting from secondary contact following a period when the taxa were differentiating in separate refugia (*5*), may have evolved in situ by parapatric differentiation (*6*).

Pseudophryne semimarmorata, *P. bibroni*, and *P. dendyi* are small terrestrial frogs with essentially allopatric ranges in southeast Australia (Fig. 1A). Despite species-specific differences in adult coloration, the three taxa are very similar in anatomy, karyotype, size, breeding season and site, common male vocalizations, pre- and postmating behavior, reproductive rates, mating system, and pattern and rates of embryonic and larval development (*4*, *7*).

Except where unfavorable habitats prevent contact, the northern borders of *P. semimarmorata* are marked by narrow zones of parapatric hybridization (*8*) with *P. bibroni* in the west and *P. dendyi* in the east (Fig. 1A). I mapped these contacts on the basis of five diagnostic features of adult coloration and used a hybrid index to identify hybrids (*4*, *9*). Interactions between *P. semimarmorata* and *P. bibroni* (four study areas), and between *P. semimarmorata* and *P. dendyi* (three study areas) were found to be similar. A transect of the hybrid zone near Wallan, 50 km north of Melbourne is typical; the morphological transition zone is less than 9 km wide and 80 percent of the change in mean hybrid index occurs in the central 3 km (Figs. 1B and 2A). Intrasample variance in hybrid index is zero in allopatry and at the edge of the zone, and maximal in the center of the zone ($\sigma = 3.4$). The diversity of hybrid phenotypes suggests that hybridization is not restricted and that backcrossing to both parental species is occurring. Both *P. semimarmorata* and *P. bibroni* have fixed alternate alleles of heart lactate dehydrogenase (LDH) (*10*). Independent association ($P = .79$) of LDH allozymes with various parental and intermediate morphotypes suggests that there is ran-

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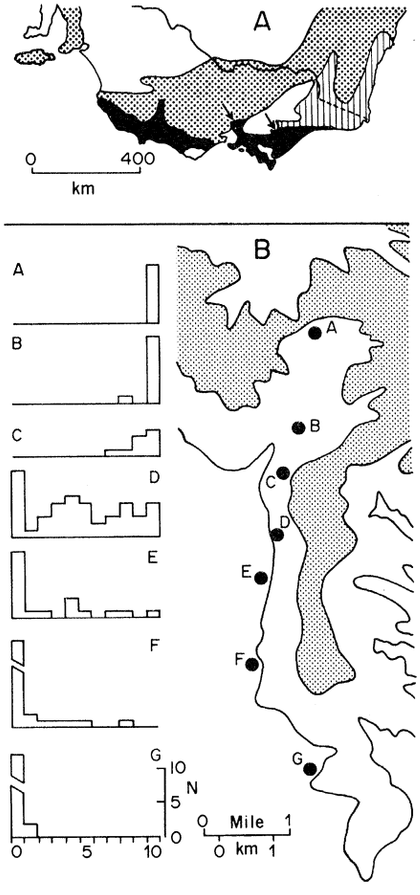
dom mating in the hybrid zone. Hybrid adults appear as healthy as homospecific adults in the field and show typical behaviors. Field observation revealed no marked changes in population density across the hybrid zone. No evidence was found for the operation of temporal, ethological, or mechanical premating isolating mechanisms (*4*). Littlejohn [in (*10*)] analyzed the common male vocalization (which he terms the mating call) of these species and concluded that there is broad overlap for each of seven call characters quantified. The hybrid zones are not clearly related to conspicuous ecotones or environmental barriers but do appear to run across a number of gradual environmental gradients. The zones are relatively stable; no changes in position or relative frequency of hybrids were detected during the period 1960 to 1974 (*4*, *10*).

Postmating isolating mechanisms are not sufficient to prevent the occurrence of healthy and fertile F_1 and backcross hybrids. No changes in the number of eggs in natural batches, the frequency of multiple matings at nest sites, or the proportion of eggs fertilized was detected across the hybrid zones. Controlled in vitro hybridization experiments confirmed that the taxa are interfertile to a considerable extent (*4*). Observation of 32 field-collected batches of eggs from the *P. semimarmorata-P. bibroni* contact near Wallan showed, however, that hybrid intracapsular mortality is two to three times as high ($P < .001$) as the maximum seen in homospecific batches (*11*; Fig. 2A). Furthermore, there is evidence (in vitro hybridization experiments, coupled with the wide range of viabilities in batches of eggs collected in hybrid zones) that various hybrid recombinations may be less viable than first generation F_1 hybrids (*4*). Similarly, with *P. semimarmorata-P. dendyi* hybrids, half the 63 batches collected within the zones showed normal (< 10 percent) mortality, but in 24 percent embryonic mortality exceeded 20 percent (Fig. 2B).

Although the histories of these narrow zones of increased variability are unknown, they were interpreted as the stable outcome of secondary contacts between taxa which diverged from one another in allopatric Pleistocene refugia (*12*). Similar hypotheses have been proposed for other hybrid zones in southeast Australia (*13*) and elsewhere (*2*, *5*). An alternative hypothesis holds that these phenomena can result from parapatric differentiation between contiguous populations along environmental gradients (*14*, *15*). Available techniques do not allow us to discriminate between the results of allopatric and parapatric speciation: both may produce the same type of hybrid zone in a few hundred generations (*14*). It follows that widely accepted views about the spatial concordance of hybrid zones between diverse species pairs in biotic suture zones (*5*) are also open to reinterpretation. The tendency of hybrid zones to run parallel to one another may have nothing to do with coalesced refugia but simply reflect a common tendency of different organisms with similar dispersal patterns to respond in a similar manner to late Cenozoic environmental gradients. Geographic concordance of such zones may also be due to the tendency of stepped clines that form within a certain critical distance of partial barriers (rivers, mountain ranges, ecotones) to move towards the barrier (*14*).

The *Pseudophryne* zones are interpreted as relatively stable phenomena in which effective reinforcement has not occurred and where introgression is limited by postmating isolating mechanisms: an evolutionary impasse. Regard-



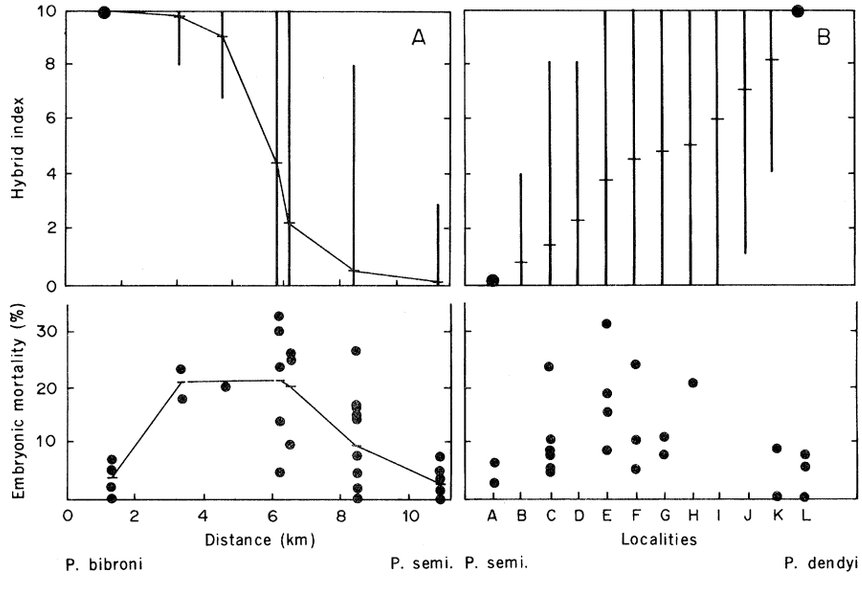


Fig. 1 (left). (A) Map of southeast Australia showing ranges of *P. semimarmorata* (solid), *P. bibroni* (dots), *P. dendyi* (lines), and the position of the Wallan (left arrow) and Tyers (right arrow) transects. (B) Morphological variation near Wallan: hybrid index diagrams illustrate change from *P. bibroni* (location A) to *P. semimarmorata* (location G) (*9*). The 306- and 355-m (shaded) contour lines are indicated. Fig. 2 (right). (A) The *P. bibroni-P. semimarmorata* hybrid zone near Wallan. Upper diagram shows mean and range of hybrid index scores of samples from localities A to G (Fig. 1B). The lower diagram shows embryonic mortality (mean values connected) in batches of eggs collected at these localities. (B) The *P. dendyi-P. semimarmorata* hybrid zone near Tyers. The 12 localities are arranged in order of mean hybridity as they are drawn from three separate road transects.

less of their mode of origin, *P. semimarmorata* now behaves as a semispecies toward both *P. bibroni* and *P. dendyi*. It is accordingly ranked as a species despite the fact that it hybridizes freely with these other taxa wherever their ranges contact. Species concepts need revision to allow for the fact that genetic isolation cannot invariably be directly equated with reproductive isolation. As others (*16*) have shown, strongly integrated and coadapted gene complexes may be effectively protected against introgression even in the apparent absence of premating reproductive isolation.

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11. Observation of saprovoric fungal infestations in 50 batches of eggs collected away from the hybrid zones permitted the determination of natural embryonic mortality patterns. From 98 to 100 percent of the ova were fertilized and commenced development. Embryonic mortality was generally < 5 percent (range 0 to 11 percent) and most failures occurred during gastrulation.
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The *Salmonella* Mutagenicity Assay: Recommendations

A number of recommendations have recently been made (*1*) concerning the use of the *Salmonella* plate assay for determining chemical mutagenicity. This assay was described in detail by Ames and his co-workers in 1975 (*2*), and since that time, many variations of the procedure have been incorporated into the test as it is used in individual laboratories. In ongoing efforts to identify mutagenic chemicals that may pose a hazard to human health, the diversity of existing protocols presents several questions. Among them are: (i) What are the most likely sources of variation in results among laboratories? (ii) What protocol is currently best suited for routine testing? (iii) What constitutes an adequate test? The recommendations made therefore provide basic guidelines for conducting the assay and constitute minimum criteria by which reports of mutagenicity testing in the *Salmonella* plate assay can be evaluated.

Bacterial strains. For routine testing, strains TA1535, TA1537, TA1538, TA98, and TA100 should be used. The recommendation that TA1538 might be deleted (*2*) was not considered advisable since this strain may show greater sensitivity than TA98 with some chemicals. The use of other strains is considered optional.

Preparation of suspensions of tester strains. The growth of bacterial strains in overnight nutrient broth cultures has been found to vary considerably depending on the nutritional quality of the medium. There seem to be marked differences not only between media from different sources but also among batches from a single source. Overnight cultures which have just reached a density of 1 to 2×10^9 viable cells per milliliter are considered most desirable for mutagen testing. Instead of using washed cells, investigators should take inocula directly from nutrient broth cultures. A fresh cell suspension should be used for each day's experiments; use of cultures kept overnight in a refrigerator should be avoided. Cell suspensions should be maintained through the day at ice-bath temperature since storage at room temperature may result in loss of viability or mutagen sensitivity.

Checking tester strain genotypes. The procedures outlined by Ames *et al.* (*2*) are satisfactory with regard to confirming histidine requirement, deep-rough character, and ultraviolet sensitivity of tester strains. However, tests for the presence of the R factor conferring ampicillin resistance in strains TA100 and TA98 can be conducted more conveniently by using commercially available filter-paper disks containing 10 μg of ampicillin. Ampicillin-containing disks are placed in the center of petri dishes overlaid with each of the tester strains. Zones of inhibition should be observed with strains TA1535, TA1538, and TA1537, but not with TA98 or TA100. For the three sensitive strains, the diameter of the zone of inhibition has been found to be reproducible and characteristic within a given laboratory, although some differences have been found between laboratories.

Because this procedure will not determine what fraction of the culture has lost the R factor, it is important to check stock cultures periodically to ensure that close to 100 percent of the bacteria contain the R factor. This can be done by replica plating (preferably with 100 or more colonies) onto ampicillin-free plates and plates containing 25 μg of ampicillin per milliliter of medium.

Preparation of S9. For routine screen-

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