eight patients, behavioral disturbances were so severe that fluphenazine administration was begun after 14 medication-free days. There was no relationship between the number of medication-free days and either the initial concentration of plasma HVA or baseline psychosis ratings. Fluphenazine was administered orally at 10 mg/day and was increased to a final daily dose on the basis of individual clinical state by the end of the first week. This dose (mean ± standard error of the mean, 36 ± 6 mg/day) was maintained whenever possible throughout the remaining 4 weeks of the study. In each patient, benztropine was added during the first week (dose range, 0.5 to 2.0 mg) to reduce extrapyramidal side effects.

15. W. E. Bunney, Jr., and D. A. Hamburg, *Arch. Gen. Psychiatry* **9**, 153 (1962). Scores of 10 and above correspond to severe psychotic symptomatology. In addition to global ratings of psychosis, physicians performed the Brief Psychiatric Rating Scale (BPRS) weekly. We have used the withdrawal-retardation subscale of the BPRS to assess general motor activity [J. E. Overall and D. R. Gorham, *Psychol. Rep.* **10**, 799 (1962)].
16. Blood was collected as often as three times per week (Monday, Wednesday, and Friday); clinical limitations occasionally precluded a blood collection (*18*).
17. W. H. Chang, M. Sheinin, R. S. Burns, M. Linnoila, *Acta Pharmacol. Toxicol.* **53**, 275 (1983). All samples from each patient were assayed on the same day and within the same run. Interassay and intrassay variability were 6.3 and 2.2 percent, respectively.
18. A maximum of 18 possible blood collections were possible: three pretreatment samples followed by three samples for each of five treatment weeks. For these analyses, if a patient missed an individual blood collection, the corresponding clinical rating for that day was excluded from group means for that time point. In all cases, group means for plasma HVA and psychosis ratings during drug treatment were less than at baseline; thus, changes in HVA and psychosis ratings represent a reduction in plasma HVA and clinical improvement, respectively. Means and standard errors of individual patients' variation coefficients for any of the 6 weeks of study ranged from 5.44 ± 1.13 percent to 11.2 ± 3.0 percent; for all weeks, 7.2 ± 0.83 percent. For the correlative analysis we used conservative degrees of freedom based on two-thirds of the number to minimize the effects of repeated inclusion of individuals [M. H. Quenouille, *Associated Measurements* (Academic Press, New York, 1952); p. 170].
19. K. S. Kendler, R. C. Mohs, K. L. Davis, *Psychiatr. Res. Rep.* **8**, 215 (1983).
20. N. C. Andreasen and S. A. Olsen, *Arch. Gen. Psychiatry* **39**, 789 (1982); N. C. Andreasen, S. A. Olsen, J. W. Dennert, O. A. Scott, *Am. J. Psychiatry* **139**, 297 (1982).
21. J. F. Casey, I. F. Bennet, C. J. Lindley, *Arch. Gen. Psychiatry* **2**, 210 (1960); National Institute of Mental Health Psychopharmacology Service Center Collaborative Study, *ibid.* **10**, 246 (1964); D. R. Gorham and A. D. Pokorny, *Dis. Nerv. Syst.* **25**, 77 (1964).
22. All controls were studied as outpatients; dietary restrictions were identical to those of the schizophrenic patients.
23. N. G. Bacopoulas, S. E. Hattox, R. H. Roth, *Eur. J. Pharmacol.* **56**, 225 (1979).
24. N. G. Bacopoulas, D. E. Redmond, J. Balla, R. H. Roth, *J. Pharmacol. Exp. Ther.* **212**, 1 (1980).
25. R. O'Keefe, D. F. Sharman, M. Vogt, *Br. J. Pharmacol.* **38**, 287 (1970); H. Asper *et al.*, *Eur. J. Pharmacol.* **22**, 287 (1973); J. A. Nielsen, N. J. Duda, K. E. Moore, *Life Sci.* **31**, 1495 (1982).
26. P. Lerner, P. Nose, E. K. Gordon, W. Lovenberg, *Science* **197**, 181 (1977).
27. M. B. Bowers, *Psychopharmacologia* **28**, 309 (1973); G. Sedvall *et al.*, *J. Psychiatr. Res.* **11**, 75 (1974).
28. R. M. Post and F. K. Goodwin, *Science* **190**, 488 (1975).
29. J. Gerbach, K. Thorsen, R. Fog, *Psychopharmacologia* **40**, 341 (1975); B. Wode-Helgodt, B. Fyro, B. Gullberg, G. Sedvall, *Acta Psychiatr. Scand.* **56**, 129 (1977); M. B. Bowers, *Biol. Psychiatry* **13**, 375 (1978); ——— and G. R. Heninger *Psychiatr. Res. Rep.* **4**, 285 (1981).
30. D. E. Sternberg, D. P. van Kammen, P. Lerner, W. E. Bunney, *Science* **216**, 1423 (1982).

31. We thank J. Boronow, P. Ninan, A. Doran, and O. Wolkowitz for their help in this study; the nursing staff of the 4-East clinical research unit of the NIH Clinical Center who provided clinical care and behavioral rating data; and T. Miller, M. Thomas, and M. Stipetic for their laboratory support. A. Hobbs provided editorial assistance in the preparation of the manuscript.

9 March 1984; accepted 15 June 1984

Albumin and Australian Frogs: Molecular Data a Challenge to Speciation Model

Abstract. *Vertebrate speciation in the southwest of Australia has long been viewed as resulting from multiple invasions of eastern source stocks during the Pleistocene. Microcomplement fixation studies of serum albumin evolution in frogs of the genus* Heleioporus *provide the first hard data on age and phylogenetic relationships among species in this genus and lead to rejection of the multiple invasion model in favor of speciation occurring in Western Australia. The albumin molecular clock was used to estimate that the species divergences in this genus occurred between 4 million to 12 million years ago in the late Tertiary (Pliocene-Miocene), rather than in the Quaternary (the last 2 million years).*

Ideas on the biogeography of southern Australia have been influenced by allopatric speciation models that were based on isolation by deserts or marine barriers. In Western Australia the absence of the major geographic barriers presumed essential for allopatric speciation led Main *et al.* (*1*) to propose a model of repeated invasions of source stocks from eastern Australia to account for the high species diversity in several genera of frogs in Western Australia. This "multiple invasion hypothesis," which White (*2*) recently described as "ingenious, even if completely imaginary," has been widely adopted to explain species diversity in many vertebrate and invertebrate taxa (*3*).

For the past quarter of a century this multiple invasion hypothesis has enjoyed widespread popularity, in part because of its general applicability to so many taxonomic groups, but also because previously (*4*) there have not been new data available to test objectively the validity of this model.

Initially, the hypothesis suggested that there were two source stocks per frog genus in eastern Australia and that frog stocks were dispersed from east to west across the continent during Pleistocene glaciations. During interglacials, gene pools were disrupted, and the eastern stock and its western cognate populations diverged. Main *et al.* (*1*) recognized up to three migration and differentiation cycles. The model was based on information on the structure of male advertisement calls, the results of artificial hybridization studies, information on morphology, and knowledge of the breeding ecology of frogs in three genera: *Heleioporus*, *Neobatrachus*, and *Crinia*. Although criticized (*2, 5*), the model was never tested directly (*4*). An adequate test requires (i) independent evaluation of the phylogenetic relationships of the species under consideration, and (ii) an estimate of the timing of lineage divergence events. Microcomplement fixation studies of the evolution of serum albumin can provide these important data (*6*).

Albumin was obtained from serum preserved in phenoxyethanol (*7*) for all six species of *Heleioporus*. Established procedures (*8*) were used for the purifi-

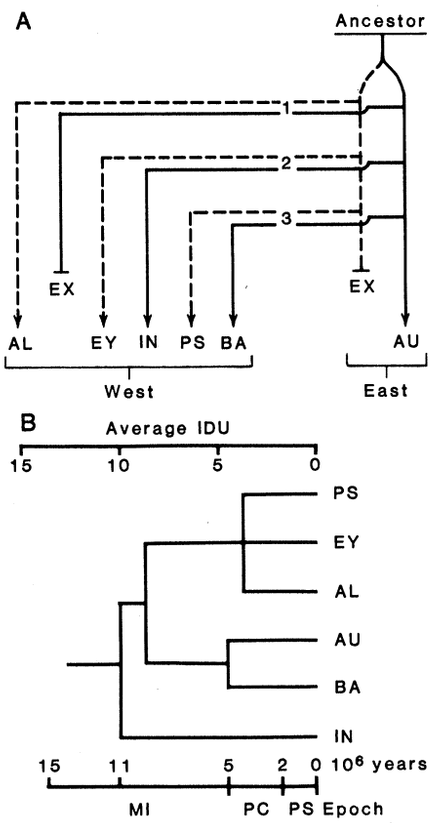

Fig. 1. (A) Predicted relationships in the genus *Heleioporus*; the dashed lines represent descendants of an arid adapted form, solid lines descendants of a related mesic adapted form [after Lee (*10*)]. Numbers 1, 2, and 3 refer to migrations across Australia, with 1 the oldest, 3 most recent. EX, extinct; AU, *H. australiacus*; PS, *H. psammophilus*; EY, *H. eyrei*; AL, *H. albopunctatus*; BA, *H. barycragus*; IN, *H. inornatus*. (B) Albumin relationships among *Heleioporus*. On the basis of MC'F analysis, the estimated immunological distance between any two species is the sum of the horizontal linkages. The vertical distances between species have no evolutionary significance. PS, Pleistocene; PC, Pliocene; and MI, Miocene epochs.

cation of albumins, the preparation of antiserums, and the comparison of albumin by means of microcomplement fixation. Data are reported as immunological distance units (IDU's) where one IDU is equivalent to approximately one amino acid difference between the albumins compared, and roughly 10 IDU's accumulate between lineages every 5.5 million to 6.0 million years of independence (*9*).

The western species, *H. eyrei*, *H. albopunctatus*, and *H. psammophilus* form a cluster distinct from the other *Heleioporus* species as predicted by Lee (*10*) (Fig. 1A). The most simple interpretation of speciation events in this subgroup is that a trifurcation occurred in this lineage during the Pliocene (Fig. 1B). Microcomplement fixation is not suited to discriminating temporally close divergences (Table 1) (*11*), and thus we cannot preclude the possibility that the *H. eyrei* subgroup forms a graded series as Main predicted (*1*). *Heleioporus inornatus* has an albumin that is very distinct from that of all other species. Moreover, *H. inornatus* is as distinct from the *H. eyrei* subgroup as it is from the remaining species, emerging 11 million to 12 million years ago in the mid-Miocene (Fig. 1B). As predicted, *H. barycragus* and *H. australiacus* are each other's closest relatives, and their divergence is estimated to have occurred about 5 million years ago, considerably earlier than the postulated Pleistocene scenario. There is no evidence of a multiple invasion pattern of which *H. inornatus*, *H. barycragus*, and *H. australiacus* were a part. We see no need to invoke a hypothetical extinct ancestor for the *H. eyrei* subgroup as it is an average of only 14 IDU's from the *H. australiacus–H. barycragus* lineage; it is in fact closer than *H. inornatus*.

The multiple invasion model of Main *et al.* (*1*) was highly regarded in that it represented a common pattern recognizable in several frog genera and other faunal elements. Further, it was difficult for biologists to envisage in situ speciation in Western Australia in the absence of major topographic barriers that might cause vicariance events. Our data on *Heleioporus*, as well as studies on *Litoria* (*12*) and on *Crinia* (*4*), and current studies of *Neobatrachus* and other leptodactylids, demonstrate that speciation within the southwest of Western Australia is not only feasible but that some of these events have occurred quite recently.

Chromosome studies on frogs in the genus *Neobatrachus*, and redefinition of species in this genus and their ranges in eastern Australia (*13, 14*) have destroyed the patterns of relationships postulated by Main *et al.* (*1*) for this group. Mahony's work (*13*) indicates that major karyotype alterations, including tetraploidy and translocations, have contributed to speciation events in *Neobatrachus* rather than migration and differentiation cycles. Electrophoretic analyses of relationships in the genus *Crinia* (*4*) have also resulted in the rejection of the multiple invasion model in favor of in situ speciation events.

Table 1. Albumin IDU's measured by microcomplement fixation tests with antiserums to *Heleioporus australiacus*, *H. barycragus*, and *H. eyrei* (*21*). Albumin IDU's are estimates of the number of amino acid differences in the two albumins compared and are subject to a variation of ±2 units (*11*). The standard deviation of reciprocal measurements for these three antiserums is 12.4 percent, average values from more extensive studies range from 10 to 15 percent (*8, 9, 12*).

Frog	IDU's measured with antiserum to		
	H. australiacus	*H. barycragus*	*H. eyrei*
H. australiacus	0	9	20
H. barycragus	9	0	17
H. eyrei	20	11	0
H. albopunctatus	25	15	8
H. psammophilus	19	11	6
H. inornatus	17	25	20

To account for the occurrence of *H. australiacus* in eastern Australia, we postulate a single transcontinental migration. The occurrence of several congeneric species pairs in eastern and western Australian frogs (*15*) indicates that there were opportunities for frog dispersal across the continent in the past. Ages of divergence of species pairs in the genera *Litoria* (*12*), *Crinia* (*4*), and *Heleioporus* indicate the possibility of faunal exchange across southern Australia until the late Pliocene but not during the Pleistocene. This is in accord with palynological and paleoclimatic data that suggest a relatively wet Pliocene (*17, 18*). The Pleistocene had dry glacial periods and interglacials no wetter than the present (*17, 19*). The Nullarbor Plain or its maritime extensions, exposed during periods of lowered sea level in the Pleistocene, were effective barriers to frog dispersal throughout this time. Climatic fluctuations during the Pleistocene and later Tertiary may have caused complex patterns of isolation related to vegetation and substrate changes sufficient to cause speciation events in genera such as *Heleioporus* (*20*).

Our data on albumin evolution in *Heleioporus*, the karyotype data for the genus *Neobatrachus* (*13*), and the electrophoretic data for the genus *Crinia* (*4*) collectively point to a comprehensive rejection of the multiple invasion model as applied to frogs. As this model formed the basis of speciation scenarios in many taxa, it is clear that reevaluations are needed. Because studies of albumin evolution provide both phyletic and time perspectives on the speciation process, they offer a powerful method for testing hypotheses about historical biogeography.

LINDA R. MAXSON
J. DALE ROBERTS*
Department of Genetics and Development, University of Illinois, Urbana 61801

References and Notes

1. A. R. Main, A. K. Lee, M. J. Littlejohn, *Evolution* **12**, 224 (1958); A. R. Main, *Adv. Ecol. Res.* **5**, 37 (1968).
2. M. J. D. White, *Modes of Speciaton* (Freeman, San Francisco, 1978).
3. Frogs: see (*15*); reptiles: D. R. Horton, *J. Herpetol.* **6**, 101; birds: A. Krash, *Monogr. Biol.* **41**, 1585 (1981); invertebrates: I. M. MacKerras, in *The Insects of Australia* (Melbourne Univ. Press, Melbourne, 1970), pp. 187–203; B. Y. Main, *Monogr. Biol.* **41**, 809 (1981); J. A. L. Watson, *ibid.*, p. 1139.
4. Since we submitted this report, data rejecting the model as applied to frogs in the genus *Crinia* (W. R. Barendse, *Evolution*, in press).
5. W. R. Heyer and D. S. Liem, *Smithson. Contrib. Zool.* **233**, 1 (1976).
6. L. R. Maxson, *Herpetologica* **37**, 96 (1981).
7. M. Nakanishi, A. C. Wilson, R. A. Nolan, G. C. Gorman, G. S. Bailey. *Science* **163**, 681 (1969).
8. L. R. Maxson, R. Highton, D. B. Wake, *Copeia* **1979**, 502 (1979).
9. L. R. Maxson and A. C. Wilson, *Syst. Zool.* **24**, 1 (1975); A. C. Wilson, S. S. Carlson, T. J. White, *Annu. Rev. Biochem.* **46**, 573 (1977); S. S. Carlson, A. C. Wilson, R. D. Maxson, *Science* **200**, 1183 (1978).
10. A. K. Lee, *Aust. J. Zool.* **15**, 367 (1967).
11. L. R. Maxson and R. D. Maxson, *Evolution* **33**, 1057 (1979).
12. L. R. Maxson, M. J. Tyler, R. D. Maxson, *Aust. J. Zool.* **30**, 643 (1982).
13. M. J. Mahony and E. S. Robinson, *Chromosoma* **81**, 199 (1980).
14. J. D. Roberts, *Trans. R. Soc. S. Aust.* **102**, 97 (1978).
15. M. J. Littlejohn, *Monogr. Biol.* **41**, 1303 (1981).
16. C. H. Daugherty and L. R. Maxson, *Herpetologica* **38**, 341 (1982).
17. R. W. Galloway and E. M. Kemp, *Monogr. Biol.* **41**, 51 (1981).
18. A. N. Blint, *Aust. J. Bot.* **29**, 277 (1981).
19. J. M. Bowler, G. S. Hope, J. N. Jennings, G. Singh, D. Walker, *Quat. Res.* **6**, 359 (1976).
20. D. Hopper, *Annu. Rev. Ecol. Syst.* **10**, 399 (1979).
21. *Heleioporus australiacus* from Watagan State Forest, New South Wales; *H. barycragus* from Darlington, Western Australia (voucher specimen deposited in the Western Australian Museum, WAM R73915); *H. eyrei* from Perth (WAM R73919); *H. albopunctatus* from Toodyay (WAM R73916); *H. psammophilus* from Midlands; *H. inornatus* from Toodyay.
22. We thank R. D. Maxson, W. R. Heyer, D. L. Nanney, and G. S. Whitt for helpful comments. This work was supported in part by grant DEB 82-01587 from the National Science Foundation (L.R.M.) and by funds from the Department of Zoology, University of Western Australia (J.D.R.).
* Permanent address: Department of Zoology, University of Australia, Nedlands 6009, Australia.

5 December 1983; accepted 29 May 1984