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Range Expansion and Its Genetic Consequences in Populations of the Giant Toad, *Bufo marinus*

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INTRODUCTION

During the past few thousand years there have been major global climatic changes. These have resulted in regional shifts in biotic composition and in alterations of the ranges of many species. These events are sufficiently recent that any effects they had on the populations involved may still be evident. More recently still, other major changes in the distributions of species have occurred as a result of human activities. Many species have been introduced to regions outside their natural range and the distributions of others have been altered through habitat modification. Range alterations may be general and frequent occurrences and their potential effects need to be considered in studying natural populations.

A range expansion may have two kinds of evolutionarily significant effect. First, it may introduce populations to new environments. This will impose new selection pressures on the populations and cause them to evolve in ways they would not otherwise have done. Second, during the process of colonization, populations may experience demographic conditions, particularly reduction and subsequent growth in population number, that lead to evolutionary genetic change. As a result, populations

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formed by a range expansion initially may not be adapted well to their new environments and may not be at equilibrium with respect to the processes of mutation, genetic drift, and gene flow.

These effects can be investigated through the study of species with well-known recent histories of range expansion. In this chapter I shall review one such case, that of the Giant Toad, *Bufo marinus*. *Bufo marinus* is native to southern North America, Central America, and northern South America; it has been introduced elsewhere, mainly as a biological control agent. As a result it now occurs extensively throughout the Caribbean and Pacific regions. I shall review the analysis of its spread, particularly in Australia, and discuss how this history of spread has provided a framework for investigating evolutionary genetic questions.

THE SPREAD OF *BUFO MARINUS*

The Introductions

The natural range of *Bufo marinus* extends from northwest Mexico to southeast Peru (Zug and Zug, 1979; Easteal, 1986*a*). Listings of records of *B. marinus* from its natural range are in Easteal *et al.* (1981*a*,*b*). The species is most abundant in human-modified habitat (Zug and Zug, 1979; Zug *et al.*, 1975). It occurs only rarely in forested areas and it is especially rare in rainforests (Zug *et al.*, 1975; Duellman, 1961). Lescure (1975) failed to find it in Amerindian villages isolated in the Amazon rainforest, and both he and Zug and Zug (1979) proposed that rainforest acts as a barrier to the dispersal of the species. The species range has thus undoubtedly been extended with the expansion of human settlement and the clearing of forests in some regions within the Americas.

A far greater expansion occurred when the species was introduced to the Caribbean and Pacific regions as a biological control agent between the early 18th century and the 1940s. I have described the history of these introductions in detail elsewhere (Easteal, 1981).

With the exception of introductions to the west coast of Florida from Colombia (King and Krakauer, 1966) and to the Indian Ocean island of Mauritius from Trinidad (Commonwealth Institute of Biological Control, unpublished documents), the source of all the introductions was the Guianas. The first introductions were from French Guiana to Martinique and from there to Barbados (both before 1844) and from Guiana to Barbados (1833) and to Bermuda (at least 24 individuals in 1855). From Barbados they were taken to Puerto Rico (12 individuals at Mayaguez in 1920)

and to Jamaica (1944). A further introduction was made to Puerto Rico from Jamaica (40 individuals at Rio Piedras in 1923–1924). From Puerto Rico successful introductions were made to St. Croix (1934) and Cuba (1946). In the Caribbean region the species was also introduced to Grand Cayman Island, Hispaniola, the Virgin Islands, Antigua, Nevis, Montserrat, Guadeloupe, Dominica, St. Lucia, St. Kitts, and Grenada. None of the sources of these introductions is known. Listings of records of *B. marinus* in the Caribbean are in Easteal *et al.* (1981*c*).

In 1932, 148 individuals were transported to Hawaii (Oahu) from Puerto Rico. From there they were taken in large numbers to the other major Hawaiian Islands (Kauai, Molokai, Maui, and Hawaii) between 1933 and 1935. From Oahu, Hawaii they were also introduced to Viti Levu, Fiji (57 individuals in 1935) and thence to other Fijian islands (including Vanua Levu, Ovelau, Taveuni, Rambi, and Kadavu), to the Solomon Islands (Baniki, Buka, Guadelcanal, Gavutu, Malaita, and Vanikova, in 1940) and to Tuvalu (150 individuals in 1939). Introductions from Oahu were also made to Taiwan (1935) and thence to the Japanese island of Minami-Daitujima, to the Philippines (27 individuals in 1934 to Luzon and thence to Guimaras, Marinoluque, Mindanao, Mindoro, Negros, and Panay), to Guam (less than 39 individuals in 1939), to Papua New Guinea [New Britain in 1937 and thereafter to the main island and many smaller islands (Zug *et al.*, 1975)], and to Australia (101 individuals at Gordonvale in 1935 and from there in large numbers to the Ingham, Giru/Ayr, Mackay, and Bundaberg regions, all in Queensland, between 1936 and 1937). In the Pacific region the species was also introduced to American Samoa, Palau, the Caroline Islands (Yap, Truk, and Ponape), and the Northern Marianas (Sapian, Tinian, Rota); however, the sources of these introductions are not known. Listings of records and reports of *B. marinus* in the Pacific and in Australia are in Easteal *et al.* (1971*d*) and Floyd *et al.* (1981), respectively.

During the course of the species spread, introductions were made involving both small and large numbers of individuals. Both of these are of interest. The former allow the effects of population size reductions or bottlenecks to be investigated; the latter allow the study of the gradual divergence, following isolation, of populations that initially were genetically the same.

Population bottlenecks or founder events can result in changes in allele frequencies due to genetic drift and to the loss of genetic variants and of genetic variability. They may also cause the reorganization of polygenic systems that can lead to morphological change and reproductive isolation (Mayr, 1954; Carson and Templeton, 1984).

Testing the theoretical predictions about these effects with the *B.*

marinus populations presents some difficulties. First, the numbers of individuals involved in the very early introductions from French Guiana to Martinique and thence to Barbados and from Guiana to Barbados are not known, and these early introduced populations were the source of almost all the subsequent introductions.

Second, even where the exact number of introduced individuals is known, this may not be the same as the effective number introduced. The effective number will be smaller than the actual number if the individuals selected for introduction were close relatives, which they might have been if they were collected within a small area, as occurred, for example, with the introduction from Barbados to Puerto Rico. In that case all of the 12 individuals introduced were found feeding under a single beehive (Wolcott, 1934). The effective number of introduced individuals would also have been reduced if there were unequal numbers of males and females involved in the introduction or if all the introduced individuals did not breed successfully.

Some reduction in effective introduction number is likely for most of the introductions for one or more of the above reasons. An exception is the introduction from Oahu, Hawaii to Australia. The individuals involved in this introduction were collected from both Waipio and Honolulu (Mungomery, 1935, 1936). There were equal numbers of males and females, and because of the care taken following introduction, it is likely that all of the introduced individuals bred successfully at least once (Easteal, 1985*a*).

Despite these limitations to quantitative theoretical testing, there are some predictions that can be compared qualitatively with the available genetic data from introduced *B. marinus* populations.

One interesting feature of the introductions is that many of them were followed by a rapid increase in population numbers, followed, after a few years, by an equally dramatic population decline. This is known to have occurred in Barbados, Puerto Rico, Bermuda, Hawaii, the Philippines, and parts of Australia (Easteal, 1981; Freeland *et al.*, 1986). The cause of the population declines is not known. In Australia it appears not to be due to parasites or to food or water shortages (Freeland *et al.*, 1986).

The Australian Range Expansion

The first attempt to document the spread of *B. marinus* from its initial release sites in Australia was made by Covacevich and Archer (1975). They used the results of a questionnaire survey to plot the approximate distribution in 1974. Sabath *et al.* (1981) used data on dates of first sighting

obtained from the same survey to determine the species' rate of spread up to 1974. Easteal *et al.* (1985) extended this analysis up to 1980, and van Beurden and Grigg (1980) described the distribution and rate of spread of an isolated population in the Byron Bay area of northern New South Wales. Easteal and Floyd (1986) analyzed the detailed pattern of spread in particular regions and provided an overall analysis of the data (Fig. 1).

Sabath *et al.* (1981) and Easteal *et al.* (1985) found a strong linear relationship between the log of area occupied and time. L. L. Cavalli-Sforza (see Parsons, 1983) found a strong linear relationship between the square root of area occupied and time. Table II shows that there is also a strong linear relationship between time and distance moved within the different regions analyzed by Easteal and Floyd (1986). These relationships are similar to that found by Ammerman and Cavalli-Sforza (1971, 1984) for the spread of farming through Europe during the Neolithic, and Cavalli-Sforza and Feldman (1981) point out that this kind of relationship appears to be a general feature of the geographical expansion of biological populations. These authors concluded that such a relation implies that expansion is continuous and occurs at a uniform rate. However, as examination of Tables I and II makes clear, such a conclusion is erroneous. In the Australian *B. marinus* populations the relationship exists in all the regions studied despite highly significant heterogeneity in the rate of spread among 5-year intervals in all but two of the regions. In one region, Ayr, for example, there was no apparant expansion during five of the nine 5-year intervals between initial release and 1981. During the most recent 5-year intervals, spread occurred at an average rate of 19.9 km/year and between 1960 and 1964 spread occurred at an average rate of 53.7 km/year. Despite this obvious heterogeneity (significant at the 0.0001 level) the regression coefficient between time and distance moved (0.945) is also highly significant ($p < 0.0001$).

The reason for this apparent anomaly is that the observed relationships are between time and successive cumulative totals of area occupied or distance moved. Since any one of these totals is made up largely of the total preceding it in time, a strong correlation between the totals and time is almost inevitable whatever the pattern of expansion, unless it involves periodic contractions. A significant correlation between total distance moved and time does not imply a continuous spread of uniform rate. The detailed analysis (Easteal and Floyd, 1986) of the pattern of spread of *B. marinus* in Australia shows that it was both highly variable and that it involved numerous discontinuities.

Variations in the rate of spread within regions can largely be explained by variation in topography. Expansion occurred more slowly in

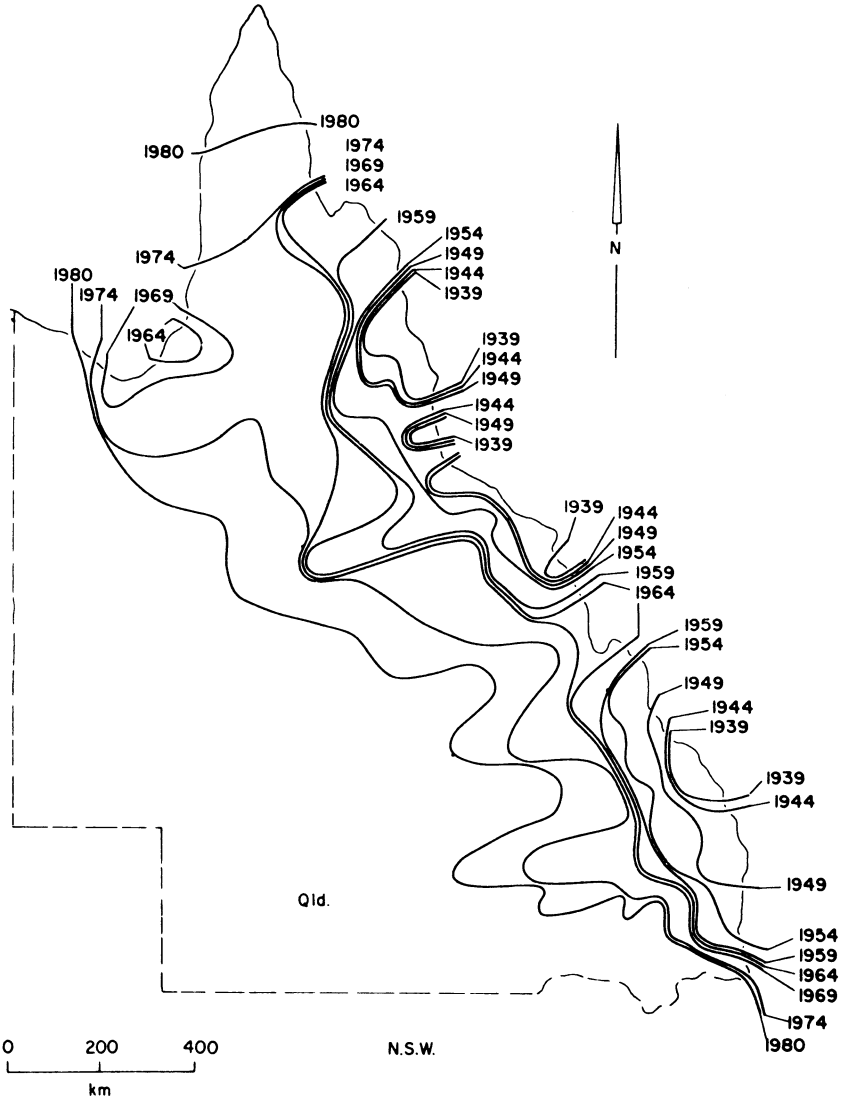

Fig. 1. Pattern of colonization of Queensland by *Bufo marinus*, showing the areas occupied at each 5-year interval from 1939 to 1980.

TABLE I. Overall Mean Annual Area Occupied during Successive Time Intervals by *Bufo marinus* in Australia and Mean Annual Distances Moved in Different Regions[a]

Time interval	Mean annual area occupied, km^2	Mean annual distance moved,[b] km							
		N	G	A	M	B	MB (N)	MB (S)	BB
1937–1939			15.0	0	6.9	12.2			
1940–1944	4,600		8.6	19.7	2.7	2.0			
1945–1949	3,560		0	0	0	10.6			
1950–1954	12,880		26.1	0	0	14.5	1.4	1.3	
1955–1959	12,880		4.3	8.6	14.4	11.4	1.9	0.8	
1960–1964	11,000	17.1	0	53.7	0	3.4	2.4	4.6	2.6
1965–1969	8,800	8.9	1.6	0	0	3.5	4.3	3.7	2.5
1970–1974	56,600	20.1	29.7	0	31.2	16.0	4.0	4.6	2.4
1975–1980	33,500		42.5	29.9	31.2	12.1			
G	106,949	12.2	430.4	811.2	437.6	68.9	6	14.1	0
p	<0.0001	<0.005	<0.0001	<0.0001	<0.0001	<0.0001	>0.05	<0.01	

[a] The results of tests for uniformity among time intervals (*G*) are shown.
[b] Regions: N, Normanton; G, Gordonvale; A, Ayr; M, Mackay; B, Bundaberg; MB, Moreton Bay; BB, Byron Bay.

TABLE II. Cumulative Totals of Overall Area Occupied and of Distance Moved in Different Regions by *Bufo marinus* in Australia[a]

Time since introduction, years	Total area occupied, km²	Total distance moved,[b] km							
		N	G	A	M	B	MB (N)	MB (S)	BB
2	32,800		45	0	20.7	36.6			
7	55,800		88	98.5	34.2	46.6			
12	73,600		88	98.5	34.2	99.6			
17	138,000		218.5	98.5	34.2	172.1	8.4	7.8	
22	202,000		240	141.5	106.2	229.1	17.9	11.8	
27	257,000	68.4	240	410	106.2	246.1	29.9	34.8	
32	301,000	112.9	248	410	106.2	263.6	51.4	53.3	23.4
37	584,000	213.4	396.5	410	262.2	343.6	71.4	76.3	35.9
43	785,000		651.5	589	449.4	416.2			47.9
r	0.924	0.976	0.911	0.945	0.851	0.989	0.986	0.892	0.998
F	40.92	20.11	34.31	58.32	18.44	303.19	105.02	11.74	241.80
p	<0.001	>0.05	<0.001	<0.0001	<0.01	<0.0001	<0.01	<0.05	<0.05

[a] *r*, Coefficient of regression of either total area occupied or total distance moved with time since introduction.
[b] Regions: N, Normanton; G, Gordonvale; A, Ayr; M, Mackay; B, Bundaberg; MB, Moreton Bay; BB, Byron Bay.

mountainous regions than on the coastal and inland plains. Some of the variation undoubtedly resulted from occasional long-distance transportation to new areas by humans. This is known to have occurred at Byron Bay in northern New South wales (van Beurden and Grigg, 1980), at Normanton on the Gulf of Carpentaria (Sabath *et al.* 1981), and at several localities on Cape York Peninsula (Easteal *et al.*, 1985; Easteal and Floyd, 1986).

In the Moreton Bay region in southeast Queensland, where the pattern of spread was analyzed in detail, there were numerous discontinuities (Easteal and Floyd, 1986), suggesting that discontinuities may also have been numerous in other areas where such detailed analysis was not possible.

Discontinuities are of potential significance with respect to the genetics of the expanding populations, as they provide a means by which effective population size could have been reduced during colonization if the establishment of new, isolated populations involved relatively small numbers of individuals.

Over the entire Australian range there is variation in expansion rate that correlates with latitude (Table III). The rate is greater in the tropical north than in the temperate south. Some of this variation may be due to there being more unrecognized discontinuities in the pattern of spread in the north, since fewer data were obtained from there. However, many amphibians are known to be more active and to disperse more when temperature and humidity are high. The greater rate of colonization in the north probably reflects a real difference in continuous dispersal rate.

TABLE III. Mean Annual Rates of Linear Spread of *Bufo marinus* Populations in Different Australian Regions

Region	Latitude (S)		Mean annual spread rate, km/year
	Mean	Range	
Normanton	17°	15°–19°	15.1
Gordonvale	18°30′	17°–20°	14.2
Ayr	20°45′	19°30′–22°	12.1
Mackay	22°	21°–23°	9.7
Bundaberg	25°	23°–27°	8.6
Moreton Bay (N)	27°15′	27°–27°30′	3.0
Moreton Bay (S)	27°45′	27°30′–28°	3.0
Byron Bay	28°45′	28°30′–29°	2.5

GENETIC VARIATION IN THE INTRODUCED POPULATIONS

The Data

The genetic data obtained from the introduced *B. marinus* populations consist of genotypes at 21 enzyme loci determined by electrophoretic separation of tissue homogenates in starch gels or cellulose acetate strips followed by histochemical staining. Genotypes were obtained for a total of 4700 toads collected from 64 localities in Hawaii (ten localities) and Australia (54 localities). Isozyme nomenclature and methods of collection, tissue preparation, electrophoresis, and staining have been described elsewhere (Easteal, 1982, 1985*a,b,* 1986*b*). Eleven of the loci studied were monomorphic in all sampled populations. The remaining ten loci were polymorphic in the majority of populations. The names and geographical positions of the sample localities are in Table IV and Fig. 2, respectively.

Genetic Variability

The levels of genetic variability in the populations were estimated as average heterozygosity H. The H values for the ten Hawaiian populations and for 12 Australian populations are shown in Table V. In Hawaii the values range from 0.131 ± 0.044 at Hawaii Kai to 0.189 ± 0.050 on Kauai. The mean value for the populations is 0.159. In the Australian populations H values range from 0.091 ± 0.034 at Burleigh Heads to 0.169 ± 0.049 at Woodstock, with a mean value of 0.136. The mean value for Australia is lower than that for Hawaii, but not significantly so. There is thus no detectable loss of genetic variability associated with the introduction of 101 individuals from Hawaii to Australia.

The H values in these populations are at the high end of the range of values for populations of other animal species and especially of other vertebrate species (Nevo, 1978). Populations of other *Bufo* species also have relatively high H values. One has $H = 0.12$ in *B. americanus* (Guttman, 1975), $H = 0.16$ in *B. arenarnum* (Mathews, 1975), and $H = 0.13$ in *B. viridis* (Dessauer *et al.,* 1975; Nevo *et al.,* 1975). The high level of genetic variability in *B. marinus* may therefore reflect some feature of its biology that it shares with other members of its genus.

Data on genetic variability are not available for native populations of *B. marinus,* so that evaluation of the effects of population size reductions associated with the introductions cannot be made. However, the fact that the H values in the Hawaiian and Australian populations are so

TABLE IV. List of Localities from Which *B. marinus* Populations Were Sampled for Isozyme Analysis[a]

Hawaii
1. Kauai
2. Molokai
3. Maui
4. Hawaii
5. Oahu, Aiea
6. Oahu, Manoa
7. Oahu, Waikiki
8. Oahu, Aina Haina
9. Oahu, Hawaii Kai
10. Oahu, Kahalu'u

Australia
11. Coen
12. Cooktown
13. Gordonvale
14. Normanton
15. Ingham
16. Woodstock
17. Bucasia
18. Emu Park
19. Bundaberg
20. Lennox Head

Townsville, Australia
52. Magnetic Island
53. Pallarenda
54. Garbutt
55. Bohlevale
56. Hermit Park
57. Anderson Park
58. James Cook University
59. Birigaba
60. Alligator Creek
61. Upper Ross River
62. Ross River Dam
63. Giru

Moreton Bay, Australia
21. Caboolture
22. Deception Bay
23. North Pine Dam
24. Sandgate Lagoon
25. Nudgee
26. Virginia
27. Mt. Nebo
28. Keperra
29. Enoggera
30. Wynnum
31. Mt. Cootha
32. Dunwich
33. Kenmore
34. Mansfield
35. Longpocket
36. Jindalee
37. Griffith University
38. Rocklea
39. Capalaba
40. Redland Bay
41. Pallara
42. Forestdale
43. Bundamba Lagoon
44. Reedy Lagoon
45. Jacobs Well
46. Upper Coomera
47. Albert River
48. Helensvale
49. Southport
50. Beaudesert
51. Burleigh Heads

[a] The numbers correspond to those in Fig. 2.

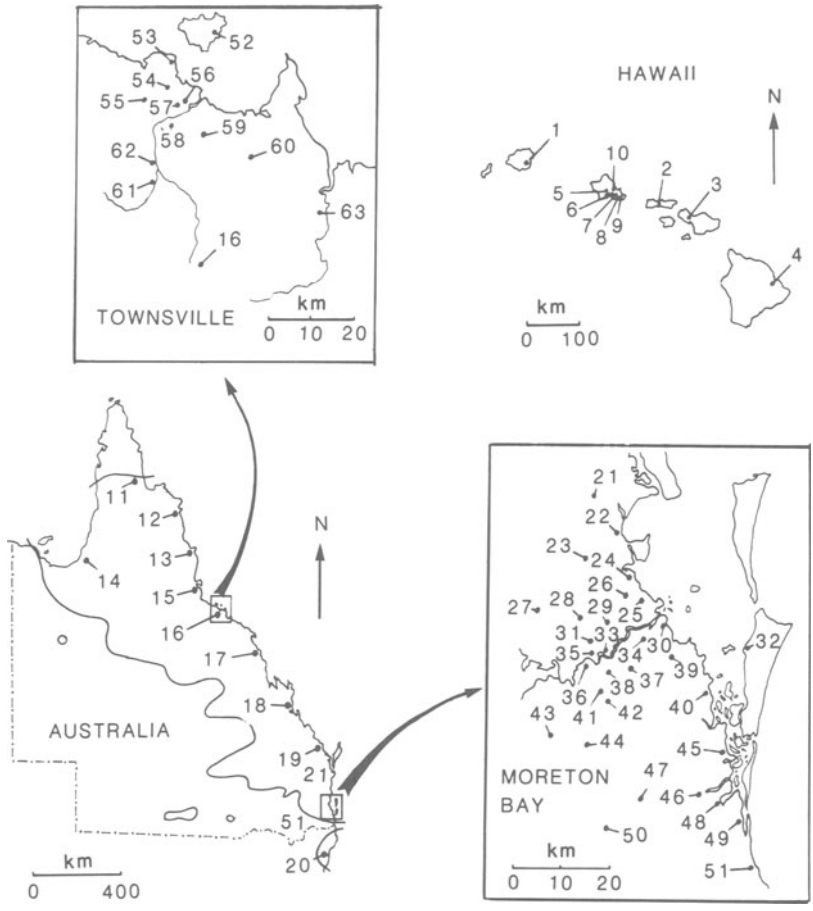

Fig. 2. Positions of localities from which *Bufo marinus* samples were collected for genetic analysis. Locality names are in Table IV.

high suggests that they have not been substantially reduced during the course of the introductions that gave rise to them.

An interesting feature of the data is that all but one of the ten polymorphic loci examined have only two detectable alleles; the average number of alleles is 2.3. This is low when compared to other species. For the animal species listed by Nevo (1978), which have H values of 0.15 or more, the average number of alleles per polymorphic locus is 3.9, with a range of 2.6–6.5. The number for the Hawaiian and Australian *B. marinus*

TABLE V. Average Heterozygosity *H* in Populations 1–51 of Table IV

Locality	*H*	*SE*	Locality	*H*	*SE*
Hawaii			Moreton Bay, Australia		
Kauai	0.189	0.050	Caboolture	0.148	0.045
Molokai	0.173	0.050	Deception Bay	0.152	0.043
Maui	0.172	0.048	North Pine Dam	0.157	0.042
Hawaii	0.142	0.043	Sandgate Lagoon	0.155	0.043
Oahu			Nudgee	0.144	0.043
Aiea	0.163	0.047	Virginia	0.151	0.045
Manoa	0.152	0.047	Mt. Nebo	0.163	0.044
Waikiki	0.162	0.045	Keperra	0.122	0.038
Aina Haina	0.142	0.044	Enoggera	0.135	0.041
Hawaii Kai	0.131	0.044	Wynnum	0.103	0.036
Kahalu'u	0.162	0.046	Mt. Cootha	0.143	0.046
			Dunwich	0.119	0.042
Australia			Kenmore	0.130	0.040
Coen	0.119	0.039	Mansfield	0.122	0.042
Cooktown	0.120	0.038	Longpocket	0.129	0.042
Gordonvale	0.169	0.048	Jindalee	0.137	0.043
Normanton	0.114	0.037	Griffith University	0.129	0.041
Ingham	0.152	0.041	Rocklea	0.131	0.041
Woodstock	0.169	0.049	Capalaba	0.102	0.039
Bucasia	0.133	0.045	Redland Bay	0.117	0.036
Emu Park	0.118	0.036	Pallara	0.110	0.037
Bundaberg	0.152	0.042	Forestdale	0.110	0.040
Lennox Head	0.145	0.045	Bundamba Lagoon	0.100	0.035
			Reedy Lagoon	0.108	0.037
			Jacobs Well	0.125	0.039
			Upper Coomera	0.117	0.037
			Albert River	0.129	0.043
			Helensvale	0.132	0.039
			Southport	0.141	0.044
			Beaudesert	0.135	0.045
			Burleigh Heads	0.091	0.034

populations is below the range for populations of other highly variable species.

The low number of alleles in the *B. marinus* populations suggests that some alleles have been lost during the course of the introductions. The suggestion of a loss of alleles but little loss of genetic variability is consistent with the theoretical predictions for the effects of population bottlenecks (Chakraborty and Nei, 1977; Maruyama and Feurst, 1985).

Effective Population Size

In both Hawaii and Australia the agencies responsible for the introduction of *B. marinus* instigated programs of toad breeding and distribution that resulted in many tens of thousands of the progeny of the introduced individuals being taken in both cases from the initial release sites, on Oahu in Hawaii and at Gordonvale in Australia, to four other areas. These were the islands of Kauai, Maui, Molokai, and Hawaii in Hawaii and the sugar-cane-growing areas of Ingham, Ayr/Giru, Mackay, and Bundaberg in Australia.

Because of the large numbers of individuals involved in these secondary releases, it can be assumed that the allele frequencies in the resulting populations were approximately the same at the time of population establishment. In both cases the five initial populations have probably remained isolated from each other since their establishment—the Hawaiian populations because they are on separate islands and the Australian populations because of the large distances separating them. Estimates of the rates of dispersal and gene flow (Easteal and Floyd, 1986; Easteal, 1986*b*) indicate that the exchange of genes among the five Australian populations is unlikely in the short time that they have been in existence.

The rate of allele frequency divergence among the populations since their establishment was estimated as the standardized variance of allele frequencies F_{st}. Mean F_{st} values for the ten polymorphic loci in the two sets of populations were 0.056 for Hawaii and 0.063 for Austalia.

Assuming approximate selective neutrality of the enzyme variants and a 1-year generation time (Easteal and Floyd, 1986), one can estimate the average effective size N_e of the populations, using the relationship $N_e = t/2\ [\ln(1\text{-}F_{st})]$ derived from Wright (1931), to be 390 (with 95% confidence limits of 119 and 812) for the Hawaiian populations and 346 (with 95% confidence limits of 104 and 719) for the Australian populations (Easteal 1985a). The N_e is the effective size of local populations, loosely equivalent to Wright's (1931) genetic neighborhood. The validity of its estimation depends on the assumption of selective neutrality.

The isolation and nonhierarchical relationship of the populations allows testing for evidence of the action of natural selection by comparison of the observed and expected variances of F_{st} values among loci, using the method proposed by Lewontin and Krakauer (1973). There is no significant difference between observed and expected variances in either set of populations and thus no evidence that natural selection has acted on these enzyme variants. The Lewontin–Krakauer test is insensitive to the effects of weak selection acting over short periods of time. This negative result therefore cannot be taken as a conclusive demonstration that nat-

ural selection has not acted on these variants. However, if there were undetected effects of natural selection, these would have been slight and would not have substantially affected the estimates of N_e.

The similarity of the estimates of N_e for Hawaii and Australia further suggests they have not been substantially affected by the action of natural selection. It is unlikely that selection would have acted to produce two similar but incorrect estimates, particularly since there is no correlation between the F_{st} values at the different loci in the two sets of populations.

The implication of the N_e values with respect to the genetic structure of *B. marinus* populations is that they are small enough that appreciable differentiation will exist among equilibrium populations as a result of genetic drift and mutation alone (Wright, 1943).

Dispersal, Gene Flow, and Neighborhood Size

Dispersal rate was estimated from the rate of continuous spread during colonization to be approximately 5.5 km/generation in the north of the Australian range and approximately 2.5 km/generation in the south of the range (Easteal and Floyd, 1986). The rate of gene flow was estimated from measurement of the degree of admixture in introgressing populations in the Townsville area to be approximately 2 km/year (Easteal, 1986*b*). The parameters being estimated in these two studies are approximately equivalent and the similarity of the two estimates suggests that they are reasonably accurate.

These estimates of dispersal and gene flow were used in combination with data on sex-ratio disparity and estimates of population density and of the degree of offspring number variance to estimate the size of a genetic neighborhood, which is approximately equivalent to the effective population size determined from allele frequency variances. The neighborhood size estimates were found to be several orders of magnitude greater than those for effective population size.

There are two possible explanations of this discrepency. Either the effective population size estimates are incorrect because the allele frequency variances were substantially affected by natural selection, or the neighborhood size estimates are inaccurate because of inaccuracies in the values of the parameters on which the estimates are based.

The latter is the more likely. The reasons for not thinking that the allele frequencies were substantially affected by natural selection have already been discussed. The parameters used to determine neighborhood size, by their nature and because of the way in which they are estimated, will tend to be overestimated, possibly to a large extent (Easteal and

Floyd, 1986). The procedures used in neighborhood size estimation are for the most part not different from those used in similar investigations of other species. If the above conclusion is correct, it implies that the use of ecological parameters to determine neighborhood size may result in a substantially misleading picture of the genetic structure of populations.

The Genetic Effects of Range Expansion

The discontinuous pattern of the *B. marinus'* range expansion in Australia provides a means by which genetic changes could have occurred during the colonizing process as a result of inbreeding and reduced effective population size if the establishment of new, isolated populations involved small numbers of individuals.

The possibility that this occurred was investigated by analysis of isozyme variation in samples collected from 40 populations located throughout *B. marinus'* Australian range. These included the five populations at the sites of initial release. Thirty-two of these populations are in the Moreton Bay region in southeast Queensland. The remaining ten are widely spread throughout the Australian range (Table IV, Fig. 2).

The F_{st} values among the populations outside the initial release sites ($F_{st} = 0.131 \pm 0.026$ for the Moreton Bay populations and $F_{st} = 0.241 \pm 0.057$ for the widely dispersed populations) are greater than those within the initial release sites (0.063 ± 0.008). This implies that allele frequency changes occurred during the colonizing process. These changes may have been caused by genetic drift or by natural selection resulting from the different environmental conditions experienced by the populations as they colonized new areas.

There are a number of reasons for thinking that the variation resulted substantially from genetic drift. First, a large proportion of the allele frequency variances among the derived populations is accounted for by large frequency differences occurring at a few loci between a comparatively small number of the populations (Easteal, 1985*b*). Furthermore, many of the populations exhibiting these large differences are in close proximity to each other and experience similar climatic and presumably other environmental conditions. Many of them are also of very recent origin.

Thus, for example, in the Moreton Bay region the frequency of the *Iddh* F allele ranges from a high of 0.93 at Redland Bay to a low of 0.10 at Burleigh Heads, and the frequency of the *Est* 100 allele ranges from a high of 0.80 at North Pine Dam to a low of 0.08 at Upper Coomera. These populations are separated from each other by less than 40 km and had been in existence for less than 25 years when they were sampled. The

Burleigh Heads population had been in existence for only 7 years when it was sampled. On the larger scale, there are frequency differences between the Cooktown and Coen populations of 0.45 for the *Est* 100 allele and the *Mpi* F allele. These populations are separated by less than 300 km and the Coen population had been in existence for only 5 years when it was sampled.

Extremely large selection coefficients would be required to explain these differences. There are, however, no obvious environmental differences between the localities that could account for large selection coefficients. Furthermore, these differences between populations in close proximity to each other are far greater than any existing between the initial release site populations, although the latter occur in both Hawaii and Australia, and in Australia they span more than 1000 km and occur in both tropical and subtropical regions.

The role of genetic drift in producing the allele frequency variation is also suggested by the absence in some peripheral, derived populations of several alleles that are rare in the initial release site populations (Easteal, 1985*b*). The loss of these alleles indicates that genetic drift did occur as the populations from which they were lost were formed through range expansion.

Further evidence for the role of genetic drift in generating the variation is provided by an analysis of the geographical districution of the variation in relation to the pattern of population establishment in the Moreton Bay region (Easteal, 1985*b*). A number of populations were established in isolation within the region. Most of the allele frequency variation exists between groups of populations derived from different isolates. There is little variation within such groups of populations. This suggests that the genetic variation arose as a result of allele frequency changes occurring during the formation of the isolates.

Analysis of the pattern of two-locus linkage disequilibrium also suggests the role of genetic drift. Estimates of two-locus linkage disequilibrium were made from the sample genotype frequencies between alleles at all polymorphic loci in populations 1–51 using Burrow's composite estimator Δ (Cockerham and Weir, 1977). Significance of interlocus associations was determined by χ^2 test following a Z transformation (Fisher, 1932) of the correlation coefficients R derived from Δ (Weir, 1979).

There were a total of 138 significant interlocus associations (Table VI). This is more than can be expected to occur by chance alone. Of particular interest is the association between *Iddh* and *Mpi* alleles, which occurs in 18 separate populations. In Hawaii, populations from all five of the Islands have the *Iddh*:*Mpi* association. In all cases the association is in the same direction (Δ is positive). On Oahu, only one (Aiea) of the

TABLE VI. Significant Nonrandom Associations between Alleles at Different Loci in Populations 1–51 of Table IV[a]

Alleles and population	Δ	R	χ^2
Adk_2:Adk_3			
Aiea	+0.038	+0.247	3.947*
Redland Bay	+0.029	+0.413	8.475*
Bundamba Lagoon	+0.036	+0.295	5.644*
Helensvale	+0.047	+0.295	5.368*
Southport	+0.041	+0.296	4.872*
Adk_2:Est			
Kauai	+0.044	+0.228	4.137*
Aina Haina	+0.047	+0.321	4.777*
Adk_2:G3pdh			
Reedy Lagoon	+0.016	+0.293	5.750*
Upper Coomera	+0.012	+0.312	6.443*
Adk_2:Hbdh			
Aiea	+0.051	+0.304	6.202*
Adk_2:Sod			
Virginia	+0.017	+0.406	8.331**
Albert River	+0.020	+0.335	4.002*
Adk_2:Mdh_2			
Aina Haina	−0.017	−0.390	7.448**
Adk_2:Mpi			
Deception Bay	+0.029	−0.264	4.380*
Sandgate Lagoon	+0.030	+0.320	4.963*
Redland Bay	−0.044	−0.403	8.009**
Adk_2:Iddh			
Aina Haina	−0.043	−0.307	4.419*
Mauai	+0.048	+0.252	5.106*
Coen	−0.023	−0.336	8.898**
Normanton	−0.017	−0.230	4.228*
Rocklea	−0.035	−0.344	6.940**
Adk_3:Est			
Bucasia	+0.024	+0.234	4.815*
Lennon Head	+0.043	+0.208	3.998*
Deception Bay	+0.078	+0.327	6.782**
Keperra	+0.061	+0.314	6.354*
Kenmore	−0.052	−0.321	4.646*
Jindalee	+0.084	+0.306	4.104*
Griffith University	+0.074	+0.273	3.851*
Jacobs Well	−0.064	−0.366	6.187*
Adk_3:G3pdh			
Bucasia	−0.025	−0.216	4.182*
Keperra	+0.026	+0.356	8.587**
Beaudesert	+0.017	+0.460	12.363***
Adk_3:Hbdh			
Waikiki	+0.044	+0.271	4.309*
Hawaii	−0.008	−0.396	9.317**
Deception Bay	−0.041	−0.255	4.019*
Burleigh Heads	−0.027	−0.368	7.902**

TABLE VI. (*Continued*)

Alleles and population	Δ	*R*	χ^2
Adk_3:Sod			
Southport	−0.060	−0.356	7.195**
Adk_3:Mdh_2			
Manoa	−0.012	−0.265	4.851*
Hawaii	−0.017	−0.443	10.210**
Adk_3:Mpi			
Helensvale	+0.071	+0.383	9.295**
Adk_3:Iddh			
Cooktown	+0.060	+0.283	5.497*
Lennox Head	+0.036	+0.208	3.905*
Mt. Cootha	−0.073	−0.349	6.757**
Longpocket	−0.068	−0.329	6.407*
Jindalee	−0.074	−0.338	5.080*
Capalaba	−0.042	−0.307	5.029*
Est:G3pdh			
Waikiki	+0.073	+0.281	4.588*
Bucasia	−0.089	−0.332	9.506**
Mt. Nebo	−0.051	−0.263	4.131*
G3pdh:Mdh_1			
Burleigh Heads	−0.021	−0.313	5.454*
G3pdh:Mdh_2			
Deception Bay	−0.016	−0.340	5.638*
G3pdh:Mpi			
Mt. Nebo	−0.057	−0.264	4.101*
Albert River	−0.018	−0.289	3.990*
Burleigh Heads	−0.024	−0.537	19.328***
G3pdh:Iddh			
Caboolture	−0.071	−0.280	4.060*
Keperra	−0.018	−0.268	4.684*
Mansfield	+0.023	+0.337	6.404*
Jindalee	−0.027	−0.299	3.910*
G3pdh:Mpi			
North Pine Dam	−0.058	−0.312	5.298*
Hbdh:Sod			
Kauai	−0.054	−0.371	6.374*
Southport	−0.027	−0.344	7.698**
Burleigh Heads	−0.015	−0.317	5.502*
Hbdh:Mdh_1			
Gordonvale	+0.063	+0.395	8.220**
Bundamba Lagoon	+0.029	+0.279	4.699*
Southport	+0.030	+0.305	5.649*
Hbdh:Mdh_2			
Hawaii	+0.023	+0.465	16.995***
Dunwich	+0.011	+0.380	5.910*

(*continued*)

TABLE VI. (*Continued*)

Alleles and population	Δ	R	χ^2
Hbdh:Iddh			
Manoa	+0.021	+0.317	4.630*
Virginia	+0.036	+0.432	8.773**
Jindalee	−0.024	−0.254	4.317*
Upper Coomera	−0.013	−0.284	4.351*
Burleigh Heads			
Sod:Mdh$_1$			
Redland Bay	−0.054	−0.342	5.716*
Sod:Mdh$_2$			
Aiea	+0.057	+0.308	5.066*
Waikiki			
Est:Hbdh			
Waikiki	+0.085	+0.308	5.488*
Redland Bay	+0.038	+0.322	4.894*
Est:Sod			
Bucasia	+0.067	+0.279	3.935*
Deception Bay	−0.068	−0.266	4.440*
Pallara	+0.036	+0.272	3.965*
Est:Mdh$_1$			
Hawaii Kai	+0.100	+0.379	8.129**
Lennox Head	−0.055	−0.231	5.083*
Deception Bay	+0.086	+0.377	9.425**
North Pine Dam	+0.067	+0.427	11.846***
Burleigh Heads	+0.661	+0.417	10.077**
Est:Mdh$_2$			
Gordonvale	+0.057	+0.345	6.598*
Lennox Head	+0.007	+0.209	4.192*
Virginia	−0.048	−0.377	6.910**
Mt. Cootha	−0.072	−0.316	5.260*
Jindalee	+0.047	+0.293	4.176*
Est:Mpi			
Virginia	−0.078	−0.345	7.104**
Est:Iddh			
Pallara	+0.032	+0.324	5.751*
Bundamba Lagoon	−0.024	−0.268	4.511*
G3pdh:Hbdh			
Coen	+0.040	0.236	4.591*
Normanton	+0.029	0.216	3.848*
Bucasia	−0.031	−0.245	4.835*
Sandgate Lagoon	−0.039	−0.255	3.880*
Mt. Nebo	−0.078	−0.319	6.123*
North Pine Dam	−0.069	−0.325	6.495*
G3pdh:Sod			
Aina Haina	−0.040	−0.341	5.287*
Bucasia	−0.085	−0.318	4.982*
Albert River	−0.025	+0.387	7.341**

TABLE VI. (*Continued*)

Alleles and population	Δ	*R*	χ^2
Sod:Mpi			
Emu Park	+0.074	+0.546	5.640*
Mt. Cootha	+0.081	+0.286	4.077*
Mansfield	−0.072	−0.354	6.861**
Upper Coomera	−0.065	−0.348	9.348**
Sod:Iddh			
Manoa	+0.092	+0.368	5.817*
North Pine Dam	+0.050	+0.256	3.897*
Beaudesert	−0.077	−0.435	11.057***
Mdh_1:Mdh_2			
Dunwich	−0.030	−0.359	5.211*
Mdh_1:*Mpi*			
Maui	−0.056	−0.236	4.215*
Woodstock	−0.066	−0.267	4.810*
Jindalee	+0.079	+0.322	5.113*
Mdh_1:*Iddh*			
Molokai	−0.059	−0.231	4.324*
Maui	−0.067	−0.286	6.558*
Normanton	−0.053	−0.395	13.762***
Beaudesert	−0.056	−0.283	4.222*
Mdh_2:*Iddh*			
Hawaii	+0.037	+0.299	6.103*
Kenmore	−0.047	−0.309	5.110*
Longpocket	−0.034	−0.327	7.831**
Mpi:Iddh			
Kauai	+0.049	+0.264	4.531*
Aiea	+0.070	+0.394	14.264***
Molokai	+0.080	+0.334	9.403**
Maui	+0.134	+0.512	24.031***
Hawaii	+0.080	+0.300	5.959*
Ingham	−0.064	−0.382	12.009***
Caboulture	−0.147	−0.559	19.494***
Deception Bay	−0.067	−0.281	4.748*
Virginia	+0.063	+0.300	5.085*
Enogerra	+0.064	+0.485	14.322***
Dunwich	−0.067	−0.471	9.658**
Pallandra	−0.025	−0.280	4.207*
Forestdale	−0.023	−0.335	6.188*
Upper Coomera	−0.097	−0.440	14.499***
Albert River	−0.135	−0.603	21.935***
Helensvale	−0.100	−0.679	38.279***
Southport	−0.116	−0.602	29.066***
Burleigh Heads	−0.041	−0.435	11.068***

[a] $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

six sampled populations has the association. This pattern is consistent with the linkage disequilibrium being established by inbreeding during the establishment of the populations.

The introduction of *B. marinus* to Hawaii involved two releases, one at the Manoa Arboretum at the upper end of Manoa Valley, the other at the Hawaiian Sugar Planters Association substation at Waipio to the west of Honolulu. The Aiea population is close to Waipio and is probably derived from it, as are the populations in the other islands. The remaining populations on Oahu are probably derived from the Manoa release, with the possible exception of the Kahalu'u population. The populations are all closer to Manoa than to Waipio, there are no records of toads being introduced to Honolulu from Waipio, and it is recorded that toads from the Manoa release spread down the Manoa valley into the Honolulu suburbs, where all of the populations except those at Aiea and Kahulu'u are located (Easteal, 1981).

Thus, in all of the populations derived from the Waipio release there is significant linkage disequilibrium with the same sign between *Iddh* and *Mpi* alleles, but in none of the populations derived from the Manoa release is there any linkage disequilibrium between these loci. This suggests that the linkage disequilibrium results from a population bottleneck occurring at the time of the Waipio release.

The Australian populations were established by 101 individuals collected both from Waipio and from suburban Honolulu (i.e., derived from Manoa), more than half coming from the latter (Easteal, 1985*a*). Since the Australian populations are only partially derived from the Waipio release, these would not be expected to reflect any linkage disequilibrium established in the Waipio population. In only one of the initial release populations in Australia (Ingham) is there significant linkage disequilibrium between *Iddh* and *Mpi* alleles, and in this case Δ is negative.

All of the remaining cases of *Iddh*:*Mpi* linkage disequilibrium are in Moreton Bay region populations. In all but two of these cases, Δ is negative. Of particular interest are the strong interlocus associations observed in the Upper Coomera, Albert River, Helensvale, Southport, and Burleigh Heads populations. These populations are all derived from a single isolated release in the Southport area (Easteal, 1985*b*; Easteal and Floyd, 1986). It seems likely that the linkage disequilibrium in these populations was established during a population bottleneck at the time of the formation of the Southport isolate.

Other explanations for the observed linkage disequilibrium are possible. However, the pattern of its occurrence in relation to the known history of population establishment strongly suggests that it arose as a

result of population bottlenecks occurring during the founding of the populations.

The single-locus genotype frequencies also show some interesting features that may be the result of demographic events occurring during the range expansion. Wright's inbreeding coefficient (F_i) was calculated from the genotype frequencies at the ten polymorphic loci in populations 1–51 (Table VII). A positive value of F_i indicates a deficiency of heterozygotes and a negative value indicates an excess of heterozygotes. The genotype frequencies were χ^2-tested for goodness of fit to Hardy–Weinberg expectations.

Of the 471 such tests, 41 showed significant deviations from Hardy–Weinberg expectations (22 at the 0.05 level, 16 at the 0.01 level, and 3 at the 0.001 level). This is more than can be expected to occur by chance alone. There are 35 cases of significant heterozygote deficiencies and 6 cases of significant heterozygote excess. The heterozygote deficiencies are not randomly distributed with respect to locus or region. Five of the ten Hawaiian populations have a deficiency of heterozygotes at the *Est* locus, and 10 of the 30 Moreton Bay populations have a heterozygote deficiency at the *Sod* locus.

These heterozygote deficiencies may be due to assortative mating, population mixing (the Wahlund effect), selection against heterozygotes, or the presence of null alleles. The *Est* and *Sod* deficiencies are of particular interest because of their relatively frequent occurrence and because they occur predominantly in particular regions.

There is no obvious reason why toads should mate assortatively with respect to alleles at either the *Est* or *Sod* loci, or why they should do so on a regional basis.

The Wahlund effect results from the inclusion, in samples, of individuals from separate subpopulations that have different allele frequencies, either because of the way they are sampled or because of natural subpopulation mixing. The degree of the resultant deficiency of heterozygotes is proportional to the variance in allele frequency among subpopulations. The degree of allele frequency variance varies among loci in the *B. marinus* populations, so that if there were a Wahlund effect it would only be manifest as significant heterozygote deficiencies at some loci. However, neither the *Est* locus in Hawaii nor the *Sod* locus in Moreton Bay show the most allele frequency variance in these regions. Furthermore, if there were Wahlund effects, although it is not expected that all loci would exhibit heterozygote deficiencies, it is expected that there would be a tendency for loci to have positive F_i values. In none of the localities at which heterozygote deficiencies were observed is there an overwhelming predominance of positive F_i values except at Griffith Uni-

TABLE VII. Values of F_i and Results of χ^2 Tests for Goodness of Fit of Observed Genotype Numbers to Hardy–Weinberg Expectations in Populations 1–51 of Table IV[a]

Locality	*Adk*$_2$	*Adk*$_3$	*Est*	*G3pdh*	*Hbdh*	*Iddh*	*Mdh*$_1$	*Mdh*$_2$	*Mpi*	*Sod*
Hawaii										
Hawaii	−0.018	+0.418**	+0.303**	+0.162	−0.039	+0.026	−0.068	−0.083	+0.239*	−0.143
Maui	−0.035	−0.025	+0.024	−0.050	—	+0.182	−0.109	−0.067	+0.039	+0.063
Molokai	+0.091	−0.102	+0.236*	+0.182	+0.211	+0.131	+0.112	−0.060	−0.157	+0.167
Kauai	−0.162	−0.187	+0.252*	−0.017	−0.083	−0.089	+0.153	+0.032	+0.138	+0.145
Oahu (Aiea)	+0.193	−0.050	+0.052	+0.281	+0.283*	+0.122	+0.044	+0.194	+0.011	+0.063
Oahu										
Kahalu'u	−0.060	−0.016	+0.329*	+0.294	−0.103	−0.028	+0.083	+0.067	−0.090	+0.283
Hawaii Kai	−0.055	+0.005	+0.071	+0.256	—	+0.044	+0.139	—	+0.036	+0.050
Manoa	−0.022	−0.131	+0.111	+0.283*	−0.030	−0.026	−0.082	+0.353*	+0.297*	+0.196
Waikiki	+0.250	+0.089	+0.358**	+0.182	+0.330*	+0.017	+0.108	−0.045	+0.131	−0.006
Aina Haina	−0.067	−0.133	+0.020	−0.055	+0.107	0.000	+0.093	+0.021	−0.073	+0.270
Australia										
Gordonvale	+0.175	−0.056	−0.009	−0.040	+0.186	+0.078	+0.206	−0.007	−0.069	+0.021
Ingham	+0.234	+0.105	−0.274*	+0.077	−0.038	−0.070	+0.049	−0.049	+0.156	+0.114
Woodstock	+0.110	−0.001	+0.050	+0.133	−0.019	+0.146	+0.248	−0.019	−0.073	+0.315*
Bucasia	−0.050	−0.011	+0.111	+0.243*	−0.076	−0.093	+0.108	—	+0.031	+0.138
Bundaberg	+0.121	−0.125	+0.333**	+0.225	+0.080	−0.045	+0.059	+0.125	+0.028	+0.013
Coen	+0.052	−0.044	+0.036	+0.194	+0.130	+0.172	+0.154	—	−0.272**	−0.009
Cooktown	+0.094	−0.027	−0.135	+0.009	+0.406*	+0.148	+0.736***	—	−0.272*	−0.154
Normanton	+0.030	+0.384	+0.038	+0.141	−0.019	+0.121	−0.061	—	−0.167	+0.541*
Emu Park	+0.266	+0.210	−0.004	+0.096	−0.025	−0.126	+0.053	—	−0.045	−0.161
Lennox Head	+0.126	−0.116	−0.036	−0.020	+0.010	+0.096	+0.203	−0.005	+0.006	+0.223
Moreton Bay, Australia										
Caboolture	—	−0.007	−0.301	+0.221	+0.064	−0.007	−0.100	−0.019	+0.229	−0.039
Mt. Nebo	−0.012	−0.018	−0.161	+0.379**	+0.017	−0.347**	+0.050	−0.109	−0.025	+0.477*

North Pine Dam	−0.121	+0.021	+0.152	+0.148	+0.271	−0.231	−0.002	−0.034	−0.283	+0.084
Deception Bay	−0.068	+0.097	+0.165	+0.152	−0.070	+0.079	+0.038	−0.011	−0.061	−0.049
Sandgate Lagoon	−0.055	−0.023	−0.172	+0.048	+0.030	−0.235	−0.071	−0.043	−0.144	−0.022
Virginia	−0.011	+0.160	+0.189	0.000	−0.021	−0.122	−0.064	−0.100	−0.153	+0.030
Keperra	−0.008	+0.148	+0.082	−0.040	−0.016	−0.048	−0.226	+0.062	+0.004	+0.328**
Enogerra	—	−0.202	+0.422**	−0.079	−0.009	−0.172	−0.257	+0.067	−0.271	+0.316*
Mt. Cootha	—	+0.009	+0.243	−0.019	—	+0.201	+0.236	+0.205	+0.128	− +0.167
Kenmore	−0.023	+0.026	−0.179	−0.023	−0.023	−0.126	−0.044	−0.031	−0.057	+0.333*
Longpocket	—	−0.156	−0.016	−0.071	−0.013	−0.120	−0.076	−0.059	+0.126	+0.333*
Jindalee	—	+0.166	+0.165	−0.060	−0.048	−0.107	+0.113	+0.120	−0.021	+0.253
Griffith University	+0.063	+0.185	+0.350*	−0.050	—	+0.035	+0.350*	—	+0.272	+0.203
Rocklea	−0.065	−0.112	−0.073	+0.026	—	−0.043	−0.050	−0.018	+0.169	+0.458**
Nudgee	−0.026	+0.085	+0.237	+0.112	+0.063	+0.113	−0.050	−0.019	−0.164	+0.293
Wynnum	−0.038	+0.036	+0.089	−0.019	—	+0.023	+0.148	—	−0.136	−0.027
Dunwich	—	+0.184	+0.063	—	−0.039	−0.174	+0.226	−0.026	−0.194	+0.517**
Mansfield	−0.028	−0.184	+0.034	−0.058	—	−0.146	−0.021	—	−0.083	+0.110
Capalaba	−0.010	−0.032	+0.197	−0.010	−0.051	+0.150	−0.025	—	−0.040	+0.283
Pallara	−0.128	−0.019	−0.125	−0.011	+0.655*	−0.236	−0.133	—	−0.091	+0.088
Forestdale	−0.080	+0.012	+0.148	−0.009	−0.019	−0.149	−0.003	—	−0.059	+0.427
Redland Bay	−0.080	−0.099	+0.229	−0.067	+0.140	+0.366**	−0.085	—	+0.109	−0.900
Bundamba Lagoon	−0.073	+0.152	−0.054	−0.023	−0.056	−0.082	−0.138	—	−0.068	+0.056
Reedy Lagoon	−0.065	−0.212	+0.036	−0.065	−0.031	−0.098	+0.101	—	−0.068	+0.258
Jacobs Well	−0.072	−0.150	−0.005	−0.030	−0.104	−0.050	−0.198	—	−0.105	+0.014
Albert River	−0.028	+0.137	−0.239	−0.022	−0.032	+0.123	−0.099	—	−0.093	+0.415*
Upper Coomera	−0.048	+0.015	+0.094	−0.035	−0.056	+0.216	+0.100	—	+0.103	+0.413**
Helensvale	+0.053	+0.014	−0.051	−0.089	+0.518**	−0.160	−0.318*	—	+0.090	+0.474**
Southport	−0.143	−0.129	−0.127	−0.050	−0.049	−0.180	−0.102	—	−0.130	+0.252
Burleigh Heads	−0.014	−0.009	−0.113	−0.108	−0.028	−0.113	+0.083	—	+0.038	+0.190
Beaudesert	−0.019	+0.117	−0.155	−0.010	−0.019	−0.032	+0.109	—	−0.324*	+0.148

[a] $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

versity. Griffith University is unusual in that it was the site to which collections were brought for analysis. Some population mixing there due to escapes from collections made elsewhere is quite possible.

Disruptive natural selection coefficients would have to be large to account for the degree of heterozygote deficiency observed in some populations. The problem with a disruptive natural selection explanation is to account for the occurrence of alleles with major underdominant effects at intermediate frequencies in the populations. There are two ways in which this could have occurred during the colonizing process. First, the variants may originally have existed at low frequencies and attained intermediate frequencies due to genetic drift during the founding of new populations. This explanation, however, is not consistent with the data. The heterozygote deficiencies exist in populations with quite different allele frequencies and other populations exist with similar allele frequencies that show no heterozygote deficiency. Second, the underdominance may be conditional on the genomic or external environment, both of which may have changed during colonization. Alleles that initially were not underdominant may have become underdominant as populations experienced bottlenecks and became established in new areas. The latter explanation would account for the regional pattern of the heterozygote deficiencies.

For the deficiencies to be explained by the presence of null alleles would require that the null alleles occur at moderately high frequencies, given the extent of the deficiencies, despite their probably being deleterious in homozygous form. As in the case of underdominant alleles, increases in null allele frequencies could have occurred by genetic drift during the founding of new populations.

Geographical Patterns of Variation

In the previous section evidence was presented for the predominant role of genetic drift in producing the allele frequency variation in the Australian populations of *B. marinus*. The genetic drift occurred during the range expansion of *B. marinus* and the populations provide an opportunity to investigate the kinds of geographical pattern of allele frequency variation that can be produced in this way.

Spatial autocorrelation analysis (Sokal and Oden, 1978) of the geographical variation showed that on both a large scale (populations 11–21, 51) and a small scale (populations 21–51) there are nonrandom patterns of variation at almost all loci (Easteal, 1985*b*). It also showed that there

was heterogeneity of pattern among loci. At some loci, allele frequencies varied clinally, while others did not.

Sokal and Wartenberg (1983) showed by computer simulation that for an isolation-by-distance model of population structure, variation arising from the interaction of gene flow and genetic drift will tend to assume a clinal pattern, and that all loci will tend to form similar patterns. They suggested therefore that heterogeneity of pattern among loci would provide evidence of the action of natural selection. The data from the *B. marinus* populations indicates that this is not always the case. Heterogeneity of pattern can result from genetic drift occurring during a range expansion. On the large scale this conclusion was confirmed by computer simulations (Easteal, 1988) in which the course of establishment of the introduced populations was modeled and no natural selection was assumed to occur. The results of a similar analysis of the small-scale variation are presented in Table VIII. In this work the populations were assumed to be related hierarchically in a way consistent with the course of their establishment. They were further assumed to have experienced bottlenecks during their establishment of a magnitude that would have generated the observed amount of variation. The simulations were conducted using a modification of the POPGEN program written by G. D. Schnell, J. K. Brown, and C. C. Vaughn. The populations being simulated were assumed to have the same spatial locations as the *B. marinus* populations 21–51. The allele frequencies resulting from the simulations were analyzed for association with latitude as evidence of clinal pattern.

Ten simulations were run, and in all ten, heterogeneity of pattern was found; some loci showed clinal patterns, while others showed none. These results, together with those of the large-scale simulations, show that heterogeneity of pattern among loci is expected as a result of genetic drift occurring during a range expansion. Such heterogeneity does not provide evidence of the action of natural selection unless the possibility of a recent range expansion can be ruled out.

As an extension of the use of geographical patterns of variation to test for the action of natural selection, Barbujani (1985) proposed that gene flow and genetic drift alone could not produce an association among loci between the occurrence of a nonrandom or clinal pattern and amount of variation. He has also shown (G. Barbujani, personal communication) that this is generally true for a stepping-stone model of population structure.

The computer simulations of the *B. marinus* populations provide an evaluation of the validity of Barbujani's proposal with respect to populations experiencing a range expansion. On the large scale (Easteal, 1988), an association between allele frequency variation and clinal pattern, eval-

TABLE VIII. Values of F_{st}, Values of F for Regression of Allele Frequency with Latitude, and Mann–Whitney U Values for Association between F_{st} and Latitudinal Cline for Populations 22–52 of Table IV and for Computer Simulations

	Values for given allele (1–11)										
	1	2	3	4	5	6	7	8	9	10	11
Bufo marinus											
F_{st}	0.050	0.029	0.287	0.223	0.111	0.191	0.236	0.099	0.085	0.075	0.061
F_{st} rank	10	11	1	3	5	4	2	6	7	8	9
F	0.42	2.93	36.18***	21.10***	14.88***	4.66	6.58*	1.03	6.68*	3.67	13.66**
			F_{st} = 0.131				U = 3*				
Simulation 1											
F_{st}	0.054	0.155	0.051	0.057	0.089	0	0.060	0.094	0.062	0.050	0.509
F_{st} rank	8	2	9	7	4	—	6	3	5	10	1
F	1.12	7.68*	0.42	4.04	0.02	—	2.68	6.84*	1.80	0.04	15.05**
			F_{st} = 0.118				U = 0**				
Simulation 2											
F_{st}	0.090	0.033	0.062	0.116	0.114	0.064	0.240	0.152	0.124	0.101	0.079
F_{st} rank	7	11	10	4	5	9	1	2	3	6	8
F	0.50	0.28	0.20	6.17*	0.35	6.02*	9.62**	0.01	3.20	5.21*	12.10**
			F_{st} = 0.107				U = 13				
Simluation 3											
F_{st}	0.094	0.062	0.077	0.148	0.248	0.044	0.134	0.156	0.199	0.175	0.151
F_{st} rank	8	10	9	6	1	11	7	4	2	3	5
F	1.56	4.24	0.69	0.95	4.13	19.77**	1.69	6.00*	4.53	2.23	2.54
			F_{st} = 0.135				U = 12				
Simulation 4											
F_{st}	0.029	0.120	0.156	0.121	0.284	0.022	0.228	0.211	0.027	0.101	0.172
F_{st} rank	9	7	5	6	1	11	2	3	10	8	4
F	0.28	0.08	1.50	23.57***	8.08*	3.12	7.35	5.07*	0.68	1.00	0.54
			F_{st} = 0.151				U = 2*				

Simulation 5											
F_{st}	0.114	0.174	0.083	0.401	0.131	0.104	0.184	0.067	0.236	0.089	0.074
F_{st} rank	5	4	9	1	7	6	3	11	2	8	10
F	4.54	7.17*	1.51	6.97*	4.47	1.36	2.51	0.11	3.41	5.81*	1.55
			$F_{st} = 0.151$				$U = 7$				
Simulation 6											
F_{st}	0.353	0.076	0.189	0.135	0.148	0	0.096	0.055	0.063	0.761	0.159
F_{st} rank	2	8	3	6	5	—	7	10	9	1	4
F	2.06	2.02	6.66*	13.44**	2.99	—	10.48**	0.62	6.90*	1.90	5.30*
			$F_{st} = 0.204$				$U = 14$				
Simulation 7											
F_{st}	0.137	0.211	0.161	0.227	0.249	0	0.121	0.101	0.237	0.085	0.045
F_{st} rank	6	4	5	3	1	—	7	8	2	9	10
F	0.00	3.83	0.53	0.01	5.43*	—	14.62**	0.19	0.00	1.32	1.43
			$F_{st} = 0.157$				$U = 5$				
Simulation 8											
F_{st}	0.148	0.499	0.268	0.183	0.110	0.131	0.191	0.064	0.085	0.038	0.301
F_{st} rank	6	1	3	5	8	7	4	10	9	11	2
F	1.75	12.12**	13.93**	1.83	1.30	0.00	0.22	8.46*	0.96	0.29	2.59
			$F_{st} = 0.183$				$U = 8$				
Simulation 9											
F_{st}	0.096	0.048	0.707	0.158	0.174	0.129	0.064	0.083	0.082	0.398	0.310
F_{st} rank	7	11	1	5	4	6	10	8	9	2	3
F	0.56	4.96*	6.55*	1.69	1.63	5.35*	1.07	0.29	1.09	3.75	13.78**
			$F_{st} = 0.204$				$U = 11$				
Simulation 10											
F_{st}	0.344	0	0.280	0.084	0.238	0	0.063	0.062	0.071	0.103	0.082
F_{st} rank	1	—	2	5	3	—	8	9	7	4	6
F	10.56**	—	16.78***	10.77**	9.50**	—	0.22	1.67	0.06	1.45	1.38
			$F_{st} = 0.147$				$U = 1$*				

[a] *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

uated by a Mann–Whitney U test, was observed in six of the ten simulations. Three of the ten Moreton Bay simulations show a similar association (Table VIII). These results show that when a recent range expansion has occurred, a resulting association caused by genetic drift between clinal pattern and amount of variation is quite possible and cannot be taken as evidence of the action of natural selection.

In the case of the large-scale variation, it is easy to understand why this would be the case. With the exception of the Emu Park (18) population, the populations from the center of the distribution are all from initial release sites. The peripheral populations to the north and south are all derived from these by range expansion. Variation resulting from the range expansion will be due to changes occurring at either end of the sample transect. When a change occurs in the same direction in both the north and the south, no cline will result. However, when changes occur in opposite directions at the two extremities, or when a change occurs at one extremity but not the other, a cline will form. Thus, in at least some cases, loci showing allele frequency variation as a result of colonization will also show clinal patterns of variation.

The pattern of colonization of the Moreton Bay region is more complex (Easteal and Floyd, 1986) and it is not immediately apparent that any association would be expected to result from genetic drift during this colonization. The Moreton Bay simulations show that associations can arise even with this more complex pattern of colonization.

The association discussed by Barbujani may also arise in the absence of natural selection as the result of the introgression of previously disjunct populations. Populations in a zone of introgression contain alleles derived from the ancestal populations approximately in inverse proportion to their distance from the ancestral populations. If the allele frequencies of a locus differ substantially between the ancestral populations, then a cline will be formed across the zone of introgression. If there is no allele frequency difference between the ancestral populations, then there will be uniformity of allele frequency variation across the introgression zone. The accuracy with which the degree of admixture in a mixed population is reflected by the population's allele frequency depends on the extent of allele frequency difference between the ancestral populations (Cavalli-Sforza and Bodmer, 1971). For this reason smooth clinal patterns of variation are likely to occur only at loci at which there are large allele frequency differences between ancestral populations. Thus, in zones of introgression associations are expected between the amount of variation at a locus and the occurrence of a clinal pattern of variation.

An empirical demonstration of this is provided by the *B. marinus* populations in the Townsville region (53–63). Populations were estab-

TABLE IX. Values of F_{st}, Values of F for Regression of Allele Frequency with Distance from Townsville, and Mann–Whitney U Values for Association between F_{st} and Clinal Pattern of Variation along Two Zones of Introgression in the Townsville Area Populations (52–63 of Table IV)[a]

	Townsville–Giru			Townsville–Woodstock		
Allele	F_{st}	F_{st} rank	F	F_{st}	F_{st} rank	F
Adk$_3$	0.009	8	0.679	—	—	—
Est$_{100}$	0.125	3	11.8	0.198	1	144**
Est$_{170}$	0.069	5	3.13	0.073	3	441**
G3pdh	0.017	7	3.49	0.009	6	2.01
Iddh	0.165	2	51.3*	0.133	2	46.4*
Mdh$_1$	0.041	6	0.263	0.020	5	3.87
Mpi	0.094	4	3.09	0.006	7	0.363
Sod	0.184	1	5.30	0.035	4	129**
	$U = 1$			$U = 0$*		

[a] $*p < 0.05$; $**p < 0.01$.

lished in the vicinity of Woodstock and in Townsville city in isolation from the nearest initial release site at Giru. The populations have since merged along two zones of introgression between Townsville and Woodstock and between Townsville and Giru (Easteal, 1986*b*). Allele frequencies were determined in populations in Townsville and at Giru and Woodstock and at two localities along each of the introgression zones (populations 59–62). A clinal pattern was assumed if there was a significant association between allele frequency and distance from Townsville.

Along the Townsville–Woodstock transect there are four loci showing clinal pattern and a significant association between clinal pattern and degree of allele frequency variance F_{st} (Table IX). Along the Townsville–Giru transect only one locus (*Iddh*) shows a clinal pattern of variation. Although there is no significant association between clinal pattern and F_{st} value along this transect, this may be due to the small number of clines. There is an indication of an effect in that the three most variable alleles have the three largest F values in the analysis of variance (Table IX).

Barbujani's principle does not provide the basis for a test for natural selection in populations that have undergone a recent range expansion or are in a zone of introgression. His own application of the principle to testing for natural selection (Barbujani and Milani, 1986) was to human populations in Europe and the Near East. The histories of these populations are not known; however, they may have been formed by range expansions or population introgressions or some combination of the two,

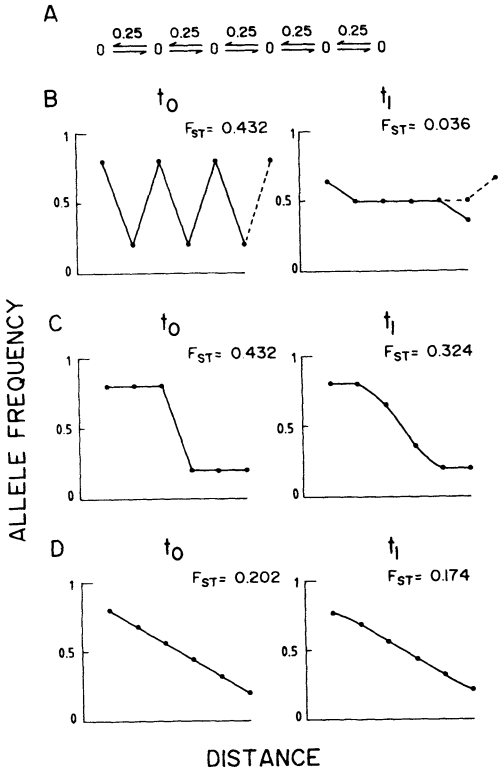

Fig. 3. Changes in the patterns of allele frequency variation existing at time t_0 after a single generation (t_1) with 25% gene flow for (A) a linear stepping model of population structure, (B) a "saw-tooth" pattern, (C) a step cline, and (D) a gradual cline.

and it is quite possible that the significant association between clinal pattern and degree of allele frequency variance in these populations does not result from the action of natural selection.

It is of interest to know the persistence of the effects discussed above. This depends on the extent of genetic drift and gene flow among populations occurring after a range expansion or introgression is complete. This question can be investigated using a linear stepping-stone model of population structure (Kimura and Weiss, 1964) for populations established with different patterns of allele frequency variation (Fig. 3).

As already discussed, in the case of introgression, patterns will tend to clines or uniformity from the outset. In the case of a range expansion the variation produced will tend to be spatially autocorrelated, since the frequency of an allele at one stage of an expansion is dependent on its frequency at the previous stage. However, the direction of change of frequency at any stage is independent of its direction at the previous stage. The result is that there may be, but usually there will not initially be,

monotonic change in frequency along the path of the expansion, and a variety of patterns may result.

At one extreme, the direction of frequency change may fluctuate and the extent of change remain constant over time periods. The result would be a "sawtooth" pattern of variation (Fig. 3B). At the other extreme, the direction of change may remain the same, resulting in a clinal pattern of variation (Figs. 3C and 3D). Loci showing these two extreme types of patterns may initially show the same amount of overall variation. However, the effect of gene flow is dramatically different in the two cases.

In the case of the sawtooth pattern, a population will exchange genes with its two neighbors, both of which have allele frequencies that differ in the same direction. The effect of one generation of 25% gene flow (or 25 generations of 1% gene flow) is to eliminate most of the allele frequency variance and the pattern of variation (Fig. 3B). The only remaining departure from uniformity is due to edge effects.

In contrast to this, in a clinal pattern of variation a population will exchange genes with two neighbors that have allele frequencies that differ in opposite directions, one being higher and the other lower. The result is that the effects of the immigrant genes cancel each other out. There is a small reduction in the amount of variation and the overall pattern is little affected. There are edge effects and a smoothing of the step cline (Figs. 3C and 3D).

The effect of gene flow is to smooth out irregularities or changes in direction of the pattern of variation. Except when local effective population size is very small and the extent of genetic drift is large compared to the amount of gene flow, allele frequencies will tend to become either variable and clinal in pattern or uniform.

The effect of gene flow following a range expansion or introgression will be to accentuate the association between clinal pattern and amount of variation.

CONCLUSION

This study of the introduced populations of *B. marinus* demonstrates some of the ways in which a geographical expansion by a species that has been well documented can be used to address evolutionary and population genetic problems that are otherwise difficult to investigate. There are many species whose ranges either have recently been or presently are being altered by human activities. These range alterations represent natural experiments. As such they are potentially far more informative

than surveys of stable populations whose demographic histories are usually not known. There is great potential for further investigations of this kind.

The study also shows some of the ways in which a range expansion can affect the patterns of genetic variation within and among populations, and how the possible occurrence of a recent range expansion may affect the interpretation of such variation. This is particularly relevant to the study of two of the most extensively investigated species, *Drosophila melanogaster* and *Homo sapiens*. Both of these species are known to have experienced recent, large-scale range expansions.

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REFERENCES

Ammerman, A. J., and Cavalli-Sforza, L. L., 1971, Measuring the rate of spread of early farming in Europe, *Man* **6**:674–688.

Ammerman, A. J., and Cavalli-Sforza, L. L., 1984, *The Neolithic Transition and the Genetics of Populations in Europe*, Princeton University Press, Princeton, New Jersey.

Barbujani, G., 1985, A two step test for the heterogeneity of *Fst* values at different loci, *Hum. Hered.* **35**:292–295.

Barbujani, G., and Milani, F., 1986, Diversity of some gene frequencies in European and Asian populations. Effects of longitude, *J. Hum. Evol.* **15**:61–69.

Carson, H. L., and Templeton, A. R., 1984, Genetic revolutions in relation to speciation phenomena: The founding of new populations, *Annu. Rev. Ecol. Syst.* **15**:97–131.

Cavalli-Sforza, L. L., and Bodmer, W. F., 1971, *The Genetics of Human Populations*, Freeman, San Francisco.

Cavalli-Sforza, L. L., and Feldman, M. W., 1981, *Cultural Transmission and Evolution*, Princeton University Press, Princeton, New Jersey.

Chakraborty, R., and Nei, M., 1977, Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model, *Evolution* **31**:347–356.

Cockerham, C. C., and Weir, B. S., 1977, Digenic descent measures for finite populations, *Genet. Res. Camb.* **30**:121–147.

Covacevich, J., and Archer, M., 1975, The distribution of the Cane Toad, *Bufo marinus,* in Australia and its effects on indigenous vertebrates, *Mem. Queensl. Mus.* **17**:305–310.

Dessauer, H. C., Nevo, E., and Chuang, K. C., 1975, High genetic variability in an ecologically variable vertebrate, *Bufo viridis, Biochem. Genet.* **13**:651–661.

Duellman, W. E., 1961, The amphibians and reptiles of Michoacan Mexico, *Univ. Kans. Publ. Mus. Nat. Hist.* **15**:1–148.

Easteal, S., 1981, The history of introductions of *Bufo marinus* (Amphibia: Anura); A natural experiment in evolution, *Biol. J. Linn. Soc.* **16**:93–113.

Easteal, S., 1982, The genetics of introduced populations of *Bufo marinus:* A natural experiment in evolution, Ph.D. dissertation, Griffith University, Brisbane, Australia.

Easteal, S., 1985*a*, The ecological genetics of introduced populations of the Giant Toad *Bufo marinus*. II. Effective population size, *Genetics* **110**:107–122.

Easteal, S., 1985*b*, The ecological genetics of introduced populations of the Giant Toad, *Bufo marinus*. III Geographical patterns of variation, *Evolution* **39**:1065–1075.

Easteal, S., 1986*a*, *Bufo marinus, Cat. Am. Amphib. Reptiles* **395**:1–4.

Easteal, S., 1986*b*, The ecological genetics of introduced populations of the Giant Toad, *Bufo marinus. IV*. Gene flow estimated from admixture in Australian populations, *Heredity* **56**:145–156.

Easteal, S., 1988, The effects of range expansion on geographical patterns of variation: a computer simulation of the colonization of Australia by *Bufo marinus*, *Biol. J. Linn. Soc.* (in press).

Easteal, S., and Floyd, R. B., 1986, The ecological genetics of introduced populations of the Giant Toad, *Bufo marinus* (Amphibia: Anura): Dispersal and neighbourhood size. *Biol. J. Linn. Soc.* **27**:17–45.

Easteal, S., Floyd, R. B., and Sabath, M. D., 1981*a*, The distribution records of the Marine Toad, *Bufo marinus*. V South America, AES Working Paper 2/81, Griffith University, Brisbane, Australia.

Easteal, S., Floyd, R. B., and Sabath, M. D., 1981*b*, The distribution records of the Marine Toad, *Bufo marinus*. IV. Central America, AES Working Paper 8/81, Griffith University, Brisbane, Australia.

Easteal, S., Floyd, R. B., and Sabath, M. D., 1981*c*, The distribution records of the Marine Toad, *Bufo marinus*. III. The Caribbean. AES Working Paper 14/84, Griffith University, Brisbane, Australia.

Easteal, S., Floyd, R. B., and Sabath, M. D., 1981*d*, The distribution records of the Marine Toad, *Bufo marinus*. II. The Pacific. AES Working Paper 4/81, Griffith University, Brisbane, Australia.

Easteal, S., van Beurden, E. K., Floyd, R. B., and Sabath, M. D., 1985, Continuing geographical spread of *Bufo marinus* in Australia: Range expansion between 1974 and 1980, *J. Herpetol.* **19**:185–188.

Fisher, R. A., 1932, *Statistical Methods for Research Workers,* Oliver and Boyd, London.

Floyd, R. B., Boughton, W. C., Easteal, S., Sabath, M. D., and van Beurden, E. K., 1981, Distribution records of the Marine Toad (*Bufo marinus*). I. Australia, 2nd ed., AES Working Paper 3/81, Griffith University, Brisbane, Australia.

Freeland, W. J., Delvinquier, B. L. J., and Bonnin, B. 1986, Decline of Cane Toad, *Bufo marinus,* populations: Status of urban toads, *Aust. Wildl. Res.* **13**:597–601.

Guttman, S. I., 1975, Genetic variation in the genus *Bufo*. II. Isozymes in northern allopatric populations of the American toad, *Bufo americanus,* in: *Izozymes, Vol. IV, Genetics and Evolution* (C. L. Markert, ed.), pp. 679–698, Academic Press, New York.

Kimura, M., and Weiss, G. H., 1964, The stepping-stone model of population structure and the decrease of genetic correlation with distance, *Genetics* **49**:561–576.

King, W., and Krakauer, T., 1966, The exotic herpetofauna of Southern Florida, *Q. J. Fla. Acad. Sci.* **29**:144–154.

Lescure, J., 1975, Observations écologiques sur les amphibiens dans l'amazonie du nord-ouest: Leur place dans l'environment humain, in: *Culture sur Brulis et Evolution du Milieu Forestier en Amazonie du Nord Oest* (P. Centivres, J. Gasche, and A. Lourteig, eds.), pp. 65–69, Société Suisse d'Ethnologie, Actes du Colloque de l'Institut d'Ethnologie de Neuchâtel, Basel, Switzerland.

Lewontin, R. C., and Krakauer, J., 1973, Distribution of gene frequency as a test of the theory of selective neutrality of polymorphisms, *Genetics* **74**:174–195.

Maruyama, T., and Feurst, P. A., 1985, Population bottlenecks and nonequilibrium models in population genetics. III. Genic homozygosity in populations which experience periodic bottlenecks, *Genetics* **111**:691–703.

Mathews, J. C., 1975, Biochemical polymorphism in populations of the Argentine toad, *Bufo arenarum, Copeia* **1975**:454–465.

Mayr, E., 1954, Change of genetic environment and evolution, in: *Evolution as a Process* (J. S. Huxley, A. C. Hardy, and E. B. Ford, eds.), pp. 157–180, Allen and Unwin, London.

Mungomery, R. W., 1935, The giant American toad (*Bufo marinus*), *Cane Growers' Q. Bull.* **3**:21–27.

Mungomery, R. W., 1936, A survey of the feeding habits of the Giant Toad (*Bufo marinus* L.) and notes on its progress since introduction into Queensland, *Proc. Int. Soc. Sugar Cane Technol.* **1936**:63–74.

Nevo, E., 1978, Genetic variation in natural populations: Pattern and theory, *Theor. Popul. Biol.* **13**:121–177.

Nevo, E., Dessaur, H. C., and Chuang, K. C., 1975, Genetic variation as a test of natural selection, *Proc. Natl. Acad. Sci. USA* **72**:2145–2149.

Parsons, P. A., 1983, *The Evolutionary Biology of Colonizing Species*, Cambridge University Press, Cambridge.

Sabath, M. D., Boughton, W. C., and Easteal, S., 1981, Expansion of the range of the introduced toad *Bufo marinus* in Australia from 1935 to 1974, *Copeia* **1981**:676–680.

Sokal, R. R., and Oden, N. L., 1978, Spatial autocorrelation in biology. 1. Methodology, *Biol. J. Linn. Soc.* **10**:199–228.

Sokal, R. R., and Wartenberg, D. E., 1983, A test of spatial autocorrelation analysis using an isolation-by-distance model, *Genetics* **105**:219–237.

Van Beurden, E. K., and Grigg, G. C., 1980, An isolated and expanding population of the introduced toad *Bufo marinus* in New South Wales, Australia, *Aust. Wildl. Res.* **7**:305–310.

Weir, B. S., 1979, Inferences about linkage disequilibrium, *Biometrics* **35**:235–254.

Wolcott, G. W., 1934, The present status of white grub parasites in Puerto Rico, *J. Agric. Univ. Puerto Rico* **18**:436–441.

Wright, S., 1931, Evolution in mendelian populations, *Genetics* **16**:97–159.

Wright, S., 1943, Isolation by distance, *Genetics* **28**:114–138.

Zug, G. R., Lindgren, E., and Pippet, J. R., 1975, Distribution and ecology of the Marine Toad, *Bufo marinus*, in Papua New Guinea, *Pac. Sci.* **29**:31–50.

Zug, G. R., and Zug, P. B., 1979, The Marine Toad, *Bufo marinus:* A natural history resume of native populations, *Smithson. Contrib. Zool.* **284**:1–58.