J. Zool., Lond. (1995) **235**, 9–31

Rapid chromosome evolution in Jamaican frogs of the genus *Eleutherodactylus* (Leptodactylidae)

J. P. BOGART

College of Biological Science, Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

AND S. BLAIR HEDGES

Department of Biology, 208 Mueller Laboratory, Pennsylvania State University, University Park, PA 16802, USA

(*Accepted 19 November 1993*)

(With 5 plates and 10 figures in the text)

Chromosomes from all 17 species of native Jamaican *Eleutherodactylus* as well as introduced *E. johnstonei* were subjected to computer-assisted analyses. Diploid chromosome numbers of 24, 26, 28, 30 and 32 were found and no two species had identical karyotypes. Karyotypic data were superimposed on a phylogeny derived from allozyme and immunological data in order to assess karyotypic changes that occurred in lineages of Jamaican *Eleutherodactylus*. Chromosome number changes have occurred at least nine times on the island and have involved both fission and fusion mutational events. C-bands and the sites of secondary constrictions varied and provide very little phylogenetic information. In most instances, karyotypically determined interspecific evolutionary relationships corresponded with the molecular data. The combination of karyological analyses and molecular data clarified lineages which involved convergent chromosome numbers or extremely divergent karyotypes. Karyotypic changes in Jamaican *Eleutherodactylus* are best explained by chromosome fission, fusion, translocations and inversions which arose in isolated demes and have been fixed through inbreeding and genetic drift. Rates of karyotypic evolution among Jamaican *Eleutherodactylus* are much faster than previous published rates for frogs. Karyotypic evolution appears to be dictated by behavioural factors and effective population sizes irrespective of taxonomic groupings.

Contents

© 1995 The Zoological Society of London

Introduction

Eleutherodactylus, with more than 500 described species (Duellman, 1993), is the largest vertebrate genus. These terrestrial-breeding frogs range from Argentina to Texas and have speciated extensively in the Antilles where they may be the only amphibians on some islands and the dominant amphibian genus on most islands (Schwartz & Henderson, 1991). Most genera of anurans are reported to be karyologically conservative (Bogart, 1972; Morescalachi, 1973; Bogart & Tandy, 1981) but *Eleutherodactylus* demonstrates considerable variation in chromosome numbers and chromosome morphology (Bogart, 1970, 1973, 1981*a*, 1991; DeWeese, 1976; Savage & DeWeese, 1981; King, 1990). Chromosome analyses of Cuban and Puerto Rican species of *Eleutherodactylus* (Bogart, 1981*a*) demonstrated distinct chromosomal groupings which were based on chromosome number and morphology. For the most part, the chromosomally derived species groupings on these two islands conformed to morphologically derived groups (Schwartz, 1969, 1976; Lynch, 1976). However, based on karyotype, Bogart (1981*a*) suggested a few changes in species group affiliations and found *Sminthillus limbatus* to be a morphologically specialized member of a West Indian group of *Eleutherodactylus* (the genus *Sminthillus* was recently synonymized with *Eleutherodactylus*; Hedges, 1989*a*).

Eleutherodactylus is an obvious exception to the generalization that frogs have a very slow rate of chromosomal evolution. When all the number variations in *Eleutherodactylus* are considered, this genus would certainly predate the earliest known frog fossil (Triassic) if the chromosome number in frogs only changes once in 70 million years (Wilson, Sarich & Maxson, 1974). Based on the criteria used by Wilson *et al.* (1974) and Bush *et al.* (1977) to calculate rate of chromosomal evolution (number of chromosomes and number of chromosome arms), there are other documented exceptions in frogs, mostly known since 1974. Chromosome number variation and telocentric chromosomes are found in various genera included in several families (Dendrobatidae, Hylidae, Leptodactylidae, Ranidae) (Bogart, 1972, 1973, 1981*a*; Blommers-Schlösser, 1978; Bogart & Tandy, 1981; King, 1990; Kuramoto, 1990).

The present study is a continuation of chromosome analyses of eleutherodactyline anurans and is part of a much larger study which attempts to outline the evolutionary history and zoogeography of the Antillean herpetofauna using molecular techniques (Hedges, Hass & Maxson, 1992). Starch-gel electrophoresis (Hedges, 1989*b*) and immunology (Hass & Hedges, 1991), using frogs from the same populations as those from which chromosomes were obtained, provide an opportunity to compare the rates of karyotypic evolution and allozyme and immunological data in *Eleutherodactylus*.

Materials and methods

Frogs were collected on Jamaica over a period of 3 years and were either carried to the University of Maryland, where they were processed for chromosomes, or shipped onward from Maryland to Guelph for processing. Chromosome methodology was as described by Bogart (1981*a*) but additional chromosomal information was obtained from squashing intestinal epithelium, which was prepared and fixed in the field following the procedure of Kezer & Sessions (1979).

The C-banding protocol was a modification of Sumner's (1972) method as outlined by Kezer & Sessions (1979) and Sessions (1982). The best results were obtained if the cover slip was removed within 1 day of the squash, the slides baked for 48 h at 60 °C, and the BaOH treatment elevated to 50 °C for 5 min. Chromosomes were analysed using CHROMPAC, which is a computer package similar to that described

by Green *et al.* (1980) but modified for the IBM PC and embellished with a plotting program which utilizes a Hewlett Packard 7470A plotter.

Metaphase spreads were observed, photographed, and the chromosomes were counted. Only those spreads that had well separated chromosomes in the same stage of contraction were considered suitable for intraspecific and interspecific karyotypic comparisons because overlapped or stretched chromosomes provided standard deviations which were unrealistically high. Idiograms were plotted from the averaged results of measuring all arms of homologous chromosomes from 1–6 chromosome spreads.

Karyotype evolution in the Jamaican *Eleutherodactylus* was examined by comparing interspecific similarities and differences in chromosome number, secondary constriction sites, C-bands, arm length ratios, and chromosome size. Karyotypes of Jamaican *Eleutherodactylus* were also compared with previously analysed Cuban and Puerto Rican *Eleutherodactylus* karyotypes (Bogart, 1981*a*) in an attempt to determine inter-island relationships. The rate of chromosomal evolution in Jamaican *Eleutherodactylus* was estimated by comparing the chromosome number changes believed to have occurred with the estimated time of arrival of the first *Eleutherodactylus* on Jamaica. This timing was based on biochemical and immunological data (Hedges, 1989*b*) and the emergence of the island in the late Oligocene/early Miocene, which is the earliest possible time for the arrival of terrestrial fauna on Jamaica.

The specimens used in the present study are catalogued in the U.S. National Museum of Natural History (USNM) collection and include (F = females, M = males, J = juveniles): *E. alticola*, USNM 266342-46 (5M); *E. andrewsi*, USNM 266347-51 (IF, 4M); *E. cavernicola*, USNM 266353-4 (2M); *E. cundalli*, USNM 266360-1 (1F, 1M); *E. fuscus*, USNM 266381-2 (2M); *E. gossei*, USNM 266388-90 (3M); *E. glaucoreius*, USNM 266370-1, USNM 266374-5 (4M); *E. grabhami*, USNM 266396-400 (3F, 2M); *E. griphus*, USNM 266406-11 (4F, 1M, 1J); *E. jamaicensis*, USNM 266412-3 (2M); *E. johnstonei*, USNM 266415-20 (3F, 3M); *E. junori*, USNM 269239 (1M); *E. luteolus*, USNM 266421-5 (3M, 2J); *E. nubicola*, USNM 266431-5 (3F, 2M); *E. orcutti*, USNM 266436-45 (10M); *E. pantoni*, USNM 269249-53 (5M); *E. pentasyringos*, USNM 266456-60 (5M); *E. planirostris*, USNM 266465 (1M); and *E. sisyphodemus*, USNM 266468 (1M).

Results

Five diploid chromosome numbers (24, 26, 28, 30 and 32) were found among the 17 endemic Jamaican species. *Eleutherodactylus planirostris* is considered to be a Cuban species which has been introduced widely through the Antilles and into Florida (Goin, 1947). Jamaican populations of this species have 32-chromosome karyotypes which are similar to the Cuban populations analysed earlier (Bogart, 1981*a*) but the quality of the chromosome spreads obtained for Jamaican *E. planirostris* were not sufficient to make a detailed comparison with the Cuban and Florida members of this species, and it was therefore not considered further in the analysis of the Jamaican species. However, Hass & Hedges (1991) found that the Cuban and Jamaican populations of *E. planirostris* are nearly identical in albumin immunological distance. *Eleutherodactylus johnstonei* is a Lesser Antillean species that was introduced to Jamaica in 1890 from Barbados (Lynn & Dent, 1943). It is included with the analyses of Jamaican species because it has not been previously subjected to detailed chromosomal analysis. Chromosome analyses (Table I) are derived from averaged digitized measurements and these data were used to construct idiograms.

24, 26 and 28-chromosome species
(Plate I, Figs 1, 2)

The two 26-chromosome species, *E. gossei* and *E. pantoni*, have several similar chromosome pairs (1–3, 6, 7, 9, 12) but the secondary constriction site is on different chromosomes and they do

TABLE I

Haploid karyotypic analyses of Jamaican species of ***Eleutherodactylus*** *derived from computer assisted measurements and calculations. The normalized length (% Length) is the length that each chromosome pair represents in relation to the total genome length (TL in μm). Ratio is the centromeric ratio derived from dividing the long arm by the short arm. Type refers to the classification derived from the centromeric ratio: metacentric chromosomes (m) have centromeric ratios of 1·00 to 1·69; submetacentric chromosomes (sm) have ratios of 1·70 to 2·99; subtelocentric chromosomes (st) have ratios of 3·00 to 6·99; and telocentric chromosomes (t) have ratios 7·00 to infinity (∞). The species are presented in alphabetical order*

	Chromosome Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E. alticola (TL = 129·2)																
% Length	11·09	10·14	9·57	8·81	7·96	6·83	6·29	6·06	5·76	5·42	4·83	4·36	3·95	3·44	3·18	2·33
Ratio	∞	∞	19·00	∞	∞	∞	1·50	∞	1·33	19·00	19·00	19·00	∞	∞	∞	∞
Type	t	t	t	t	t	t	m	t	m	t	t	t	t	t	t	t
E. andrewsi (TL = 120·3)																
% Length	11·17	10·02	8·61	8·07	7·93	7·72	6·99	5·78	4·94	4·77	4·47	4·41	4·24	4·10	3·61	3·15
Ratio	∞	∞	∞	∞	∞	∞	∞	∞	1·10	∞	∞	∞	1·04	∞	∞	∞
Type	t	t	t	t	t	t	t	t	m	t	t	t	m	t	t	t
E. cavernicola (TL = 112·8)																
% Length	12·17	10·29	9·83	8·70	8·06	6·90	6·28	6·13	5·61	5·46	4·81	4·45	3·94	3·89	3·44	
Ratio	2·57	13·29	24·00	∞	∞	∞	∞	1·78	1·56	∞	∞	∞	11·50	∞	∞	
Type	sm	t	t	t	t	t	t	sm	m	t	t	t	t	t	t	
E. cundalli (TL = 115·3)																
% Length	12·89	9·58	8·97	7·65	7·33	6·79	6·78	6·71	6·51	5·74	5·18	4·53	4·22	3·90	3·25	
Ratio	3·50	∞	∞	∞	1·69	∞	1·20	∞	∞	15·48	∞	19·38	∞	∞	∞	
Type	st	t	t	t	m	t	m	t	t	t	t	t	t	t	t	
E. fuscus (TL = 136·0)																
% Length	15·24	10·70	9·94	8·52	7·70	7·60	6·81	6·58	5·39	5·04	4·65	4·27	4·01	3·53		
Ratio	1·17	∞	∞	∞	∞	1·78	∞	1·50	∞	1·44	∞	∞	1·22	∞		
Type	m	t	t	t	t	sm	t	m	t	m	t	t	m	t		
E. glaucoreius (TL = 134·4)																
% Length	11·85	11·04	8·99	8·74	8·22	7·15	6·80	5·69	5·57	5·21	4·91	4·40	4·12	3·69	3·65	
Ratio	∞	4·00	24·00	∞	∞	∞	24·00	1·78	24·00	1·13	19·00	19·00	15·67	19·00	∞	
Type	t	st	t	t	t	t	t	sm	t	m	t	t	t	t	t	
T. gossei (TL = 156·0)																
% Length	18·47	16·98	11·23	8·74	7·09	6·67	5·83	5·06	5·05	4·17	4·11	3·36	3·25			
Ratio	1·22	1·17	∞	∞	15·67	2·03	2·03	1·17	19·00	∞	4·00	10·11	∞			
Type	m	m	t	t	t	sm	sm	m	t	t	st	t	t			
E. grabhami (TL = 108·8)																
% Length	12·00	11·93	9·92	8·88	8·69	7·92	7·74	5·19	5·18	4·29	4·14	3·81	3·76	3·29	3·25	
Ratio	1·56	10·11	8·09	4·26	5·25	6·14	3·55	2·85	1·27	3·00	1·50	4·26	2·23	3·00	1·44	
Type	m	t	t	st	st	st	st	sm	m	st	m	st	sm	st	m	

E. griphus (TL = 111·3)																
% Length	11·67	10·33	9·63	8·68	7·51	7·33	6·78	5·62	4·86	4·59	4·34	4·17	3·94	3·84	3·57	3·14
Ratio	∞	24·00	∞	∞	∞	∞	∞	∞	∞	1·33	24·00	∞	1·38	∞	∞	∞
Type	t	t	t	t	t	t	t	t	t	m	t	t	m	t	t	t
E. jamaicensis (TL = 138·2)																
% Length	12·04	10·90	9·91	9·14	8·12	7·86	7·79	7·23	4·77	4·26	4·13	3·89	3·47	3·46	3·09	
Ratio	7·33	4·26	10·11	11·50	∞	6·69	1·08	7·33	1·17	19·00	1·38	24·00	6·69	∞	15·67	
Type	t	st	t	t	t	st	m	t	m	t	m	t	st	t	t	
E. johnstonei (TL = 172·5)																
% Length	11·22	9·67	9·52	8·95	7·97	7·95	7·42	6·46	6·40	5·39	5·30	4·97	4·63	4·14		
Ratio	1·18	13·89	∞	1·21	7·93	26·38	∞	10·51	∞	1·52	11·28	∞	1·07	27·51		
Type	m	t	t	m	t	t	t	t	t	m	t	t	m	t		
E. junori (TL = 110·0)																
% Length	18·77	16·41	13·66	10·91	8·75	6·40	5·35	4·29	4·27	3·95	3·51	3·49				
Ratio	1·17	1·13	1·27	∞	49·00	2·13	2·13	2·45	∞	10·11	10·11	3·76				
Type	m	m	m	t	t	sm	sm	sm	t	t	t	st				
E. luteolus (TL = 101·6)																
% Length	12·21	10·94	9·19	8·75	8·24	7·98	7·76	7·33	5·63	4·99	4·54	4·33	4·13	3·98		
Ratio	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞	1·78	∞		
Type	t	t	t	t	t	t	t	t	t	t	t	t	sm	t		
E. nubicola (TL = 96·9)																
% Length	10·31	9·47	8·50	8·21	7·81	7·33	6·46	6·41	5·46	5·44	4·88	4·47	4·11	3·96	3·75	3·48
Ratio	∞	∞	∞	∞	∞	∞	∞	1·13	1·13	∞	∞	19·00	∞	∞	∞	∞
Type	t	t	t	t	t	t	t	m	m	t	t	t	t	t	t	t
E. orcutti (TL = 111·2)																
% Length	10·44	9·27	8·73	8·40	7·92	7·68	7·27	5·94	5·80	5·29	4·92	4·34	4·12	3·94	3·42	2·52
Ratio	∞	∞	∞	∞	24·00	∞	∞	∞	1·78	∞	24·00	19·00	1·13	∞	∞	∞
Type	t	t	t	t	t	t	t	t	sm	t	t	t	m	t	t	t
E. pantoni (TL 121·3)																
% Length	18·19	15·01	11·21	10·96	9·31	6·33	6·02	5·45	4·47	3·99	3·40	3·36	2·32			
Ratio	1·08	1·13	15·67	3·76	9·00	1·94	2·57	15·67	19·00	∞	∞	9·00	15·67			
Type	m	m	t	st	t	sm	sm	t	t	t	t	t	t			
E. pentasyringos (TL = 110·1)																
% Length	18·86	11·70	10·36	8·52	7·75	6·67	6·57	6·35	5·02	4·57	3·94	3·75	3·41	2·54		
Ratio	1·17	3·35	∞	∞	∞	1·94	2·45	∞	∞	∞	∞	10·11	∞	∞		
Type	m	st	t	t	t	sm	sm	t	t	t	t	t	t	t		
E. sisyphodemus (TL 111·9)																
% Length	11·67	10·83	9·42	8·60	8·50	8·06	6·43	5·23	4·52	4·48	4·23	3·86	3·78	3·71	3·50	3·20
Ratio	13·05	10·74	∞	7·73	6·82	20·60	∞	1·46	∞	3·70	∞	3·60	26·86	1·62	1·72	5·04
Type	t	t	t	t	st	t	t	m	t	st	t	st	t	m	sm	st

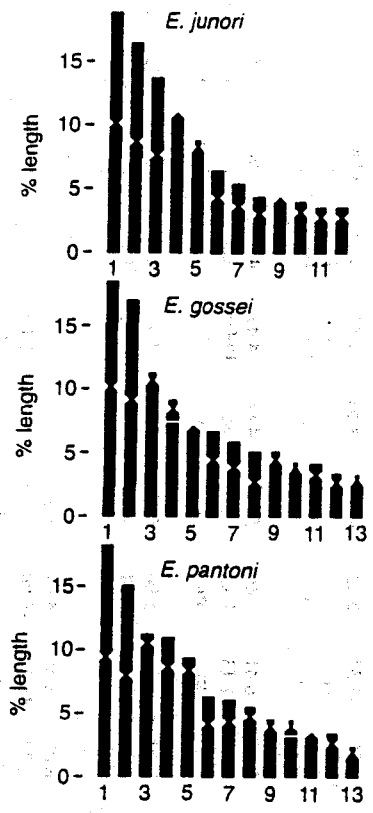


FIG. 1. Idiograms of 24 and 26-chromosome species of Jamaican *Eleutherodactylus*. Each bar represents the averaged measurements of each arm from both homologous chromosomes. The length of the bar represents the percentage length of the total genome length for the particular chromosome. The position of the centromere is indicated by a constriction which is determined by dividing the long arms by the short arms. Positions of secondary constriction are indicated by gaps in the bar. The data used to plot the idiograms are provided in Table I.

not have the same number of telocentric chromosomes. This suggests that the karyotypes of *E. gossei* and *E. pantoni* have 26 chromosomes by convergence and have been derived through the fusion of different telocentric chromosomes in a 28-chromosome ancestral karyotype (Fig. 3). None of the 28-chromosome species (Fig. 2) has two pairs of large metacentric chromosomes which would be expected in an ancestral lineage, assuming that these chromosomes were not involved in fission events. The large metacentric chromosome (1) in *E. pentasyringos* is most similar to the largest metacentric chromosome in the 26-chromosome species, while chromosome 1 in *E. fuscus* is most similar to chromosome 2 in 26-chromosome *E. pantoni*. *Eleutherodactylus luteolus*, with 13 telocentric pairs and only one submetacentric chromosome pair (13) which bears a secondary constriction, is very different from all Jamaican species. Most of the chromosomes in *E. johnstonei* are proportionally larger than found in the native Jamaican species. No distinctly similar chromosomes were found that could relate the karyotype of *E. johnstonei* to any Jamaican species.

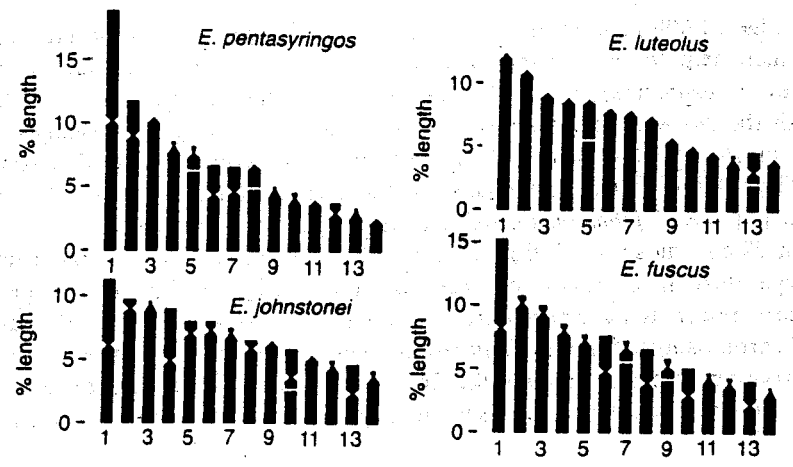


FIG. 2. Idiograms of 28-chromosome species of Jamaican *Eleutherodactylus* derived from the data in Table I.

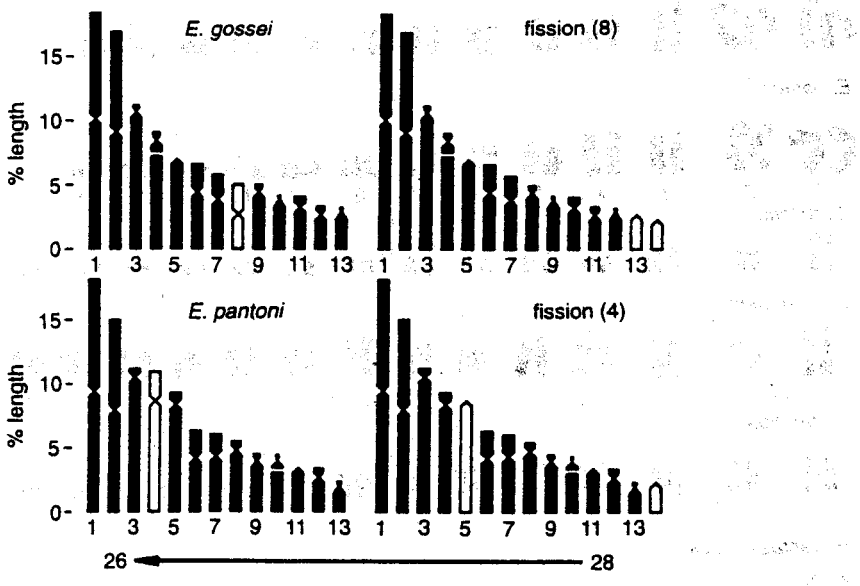


FIG. 3. Postulated fusion events that may have led to the independent origin of the 26-chromosome species *E. gossei* and *E. pantoni* from a 28-chromosome ancestral lineage. The chromosomes that are most dissimilar in the 26-chromosome species (8 in *E. gossei* and 4 in *E. pantoni*) may have resulted from fusions of different chromosomes in similar 28-chromosome ancestors (13 and 14 to produce chromosome 8 in *E. gossei*; 5 and 14 to produce chromosome 4 in *E. pantoni*).

The allozyme (Hedges, 1989*b*) and immunological (Hass & Hedges, 1991) information provide important clues which help to resolve the path and mode of karyotype evolution. The 28-chromosome species (*E. pentasyringos* and *E. fuscus*) are not sister species. However, they are included along with the 24- and 26-chromosome species in the *gossei* group (Hedges, 1989*a*). *Eleutherodactylus luteolus* clusters with 30-chromosome *E. grabhami* and 32-chromosome *E. sisyphodemus*. These three species are placed in the *luteolus* group (Hedges, 1989*a*). The molecular data clearly place *E. johnstonei* outside of the Jamaican radiation.

The karyotype in 28-chromosome *E. fuscus* apparently was derived from a 26-chromosome *gossei*-like karyotype through a fission of chromosome 1 (Fig. 4). The karyotype in 24-chromosome *E. junori* appears to have been derived from a 26-chromosome *gossei*-like karyotype through a fusion of chromosomes 5 and 9. *Eleutherodactylus pentasyringos* apparently obtained a 28-chromosome karyotype from a *pantoni*-like 26-chromosome lineage through a fission of chromosome 2 (Fig. 5). These postulated events would explain the size difference in chromosome 1

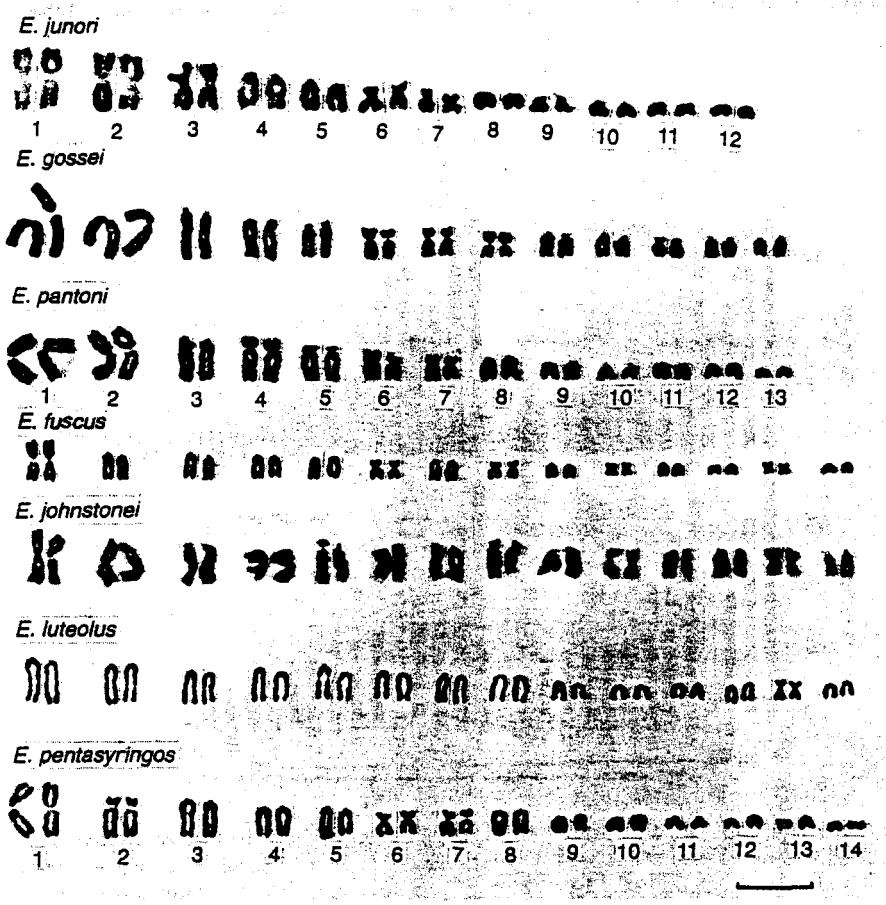


PLATE I. Karyotypes of 24, 26 and 28-chromosome species of Jamaican *Eleutherodactylus*. The scale represents 10 μm and is the same for all seven karyotypes.

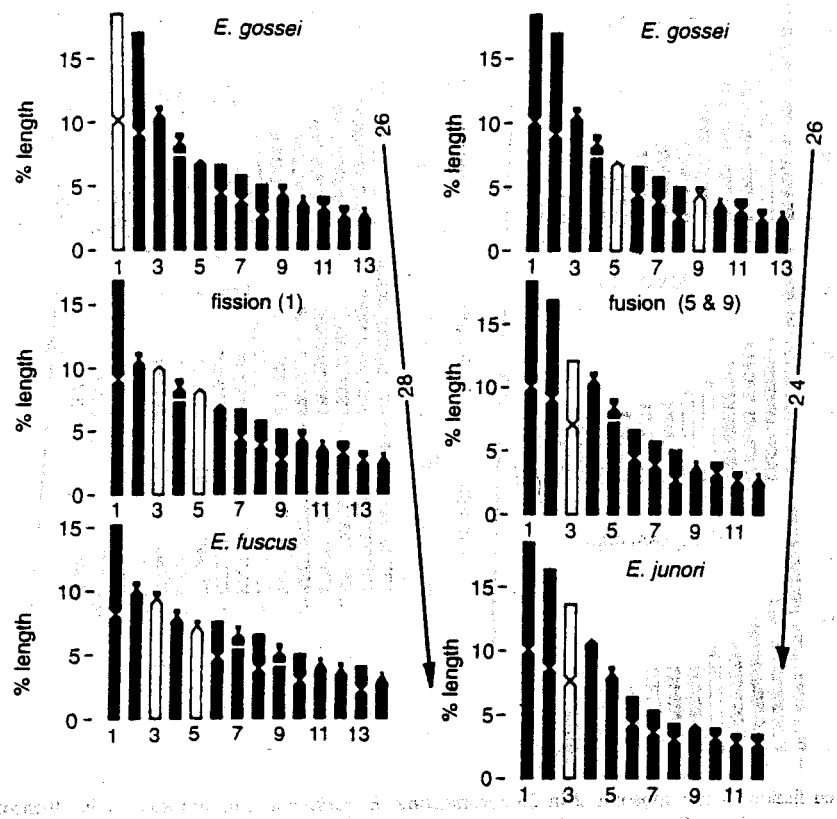


FIG. 4. Postulated fission and fusion of a 26-chromosome karyotype, similar to that of *E. gossei*, that would produce similar idiograms to those observed in 28-chromosome *E. fuscus* and 24-chromosome *E. junori*.

in *E. fuscus* and *E. pentasyringos* and the similar chromosomes 4 in *E. pantoni* and 2 in *E. pentasyringos*. This later chromosome probably arose in conjunction with the 26-chromosome *pantoni*-like karyotype (Fig. 3).

30-chromosome species
(Plate II, Fig. 6)

Eleutherodactylus glaucoreius appears most similar to *E. cavernicola* based on the two metacentric or submetacentric chromosomes (8, and 9 or 10), a secondary constriction on chromosome 12 or 13, and 12 telocentric pairs of chromosomes. *Eleutherodactylus cundalli* also has 12 telocentric chromosome pairs and a large submetacentric to subtelocentric chromosome (1) which could be homologous to a similar chromosome (1) in *E. cavernicola*. Together, these three species are placed in the *cundalli* group by Hedges (1989*a*). *Eleutherodactylus jamaicensis* has three fewer telocentric pairs and only two pairs of chromosomes in *E. grabhami* are telocentric. Chromosome pairs 2, 3 and 9 in *E. grabhami* might be homologous to respective chromosome pairs 1, 3 and 9 in *E. jamaicensis*. The latter species is placed in its own group, the *jamaicensis* group (Crombie, 1977; Hedges, 1989*a*).

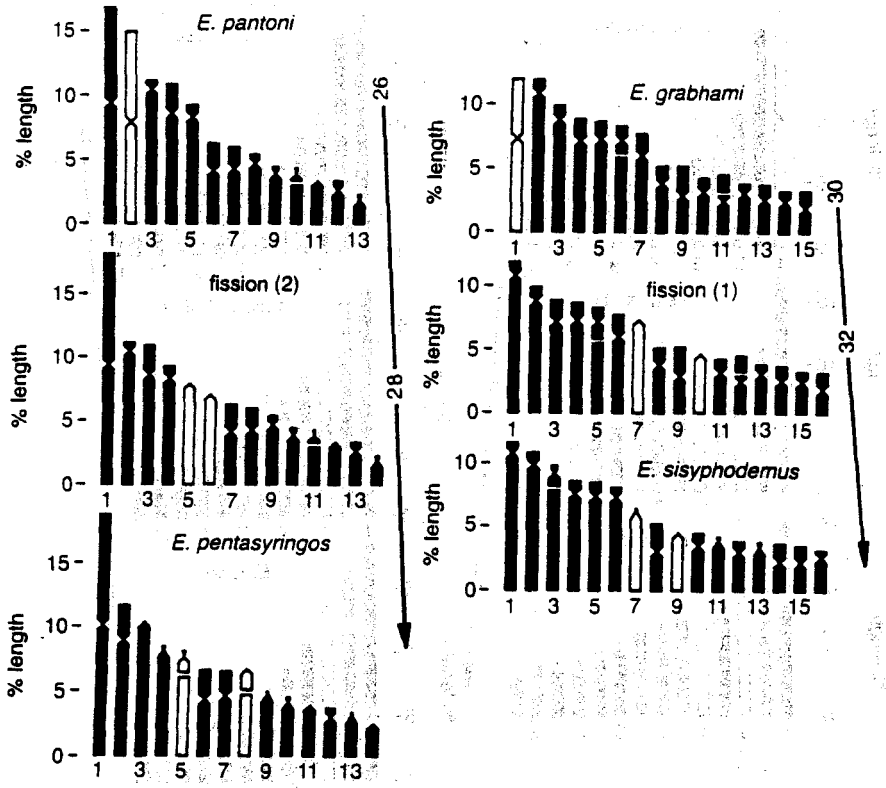


FIG. 5. Postulated fission of chromosome 2 in 26-chromosome *E. pantoni* and chromosome 1 in 30-chromosome *E. grabhami* that would produce idiograms similar to those of 28-chromosome *E. pentasyringos* and 32-chromosome *E. sisyphodemus*, respectively.

The allozyme analyses (Hedges, 1989*a*, *b*) group the 30-chromosome species which have the most similar karyotypes (*E. cavernicola*, *E. glaucoreius* and *E. cundalli*), whereas *E. jamaicensis* has electrophoretic similarities with some 32-chromosome species (especially *E. orcutti* of the *nubicola* group). Hypothetical fusion events from the 32-chromosome species or a fission of the metacentric chromosome (7) in *E. jamaicensis* does not provide a reasonable match with *E. orcutti*. *Eleutherodactylus grabhami* clusters with 28-chromosome *E. luteolus* and 32-chromosome *E. sisyphodemus* (Hedges, 1989*b*). A postulated fission of the distinctive chromosome (1) in *E. grabhami* produces a karyotype which has a number of similarities with *E. sisyphodemus* (Fig. 5).

32-chromosome species
(Plate III, Fig. 7)

Eleutherodactylus alticola, *E. nubicola*, *E. orcutti*, *E. griphus* and *E. andrewsi* all have 14 pairs of telocentric chromosomes and two pairs of metacentric or submetacentric chromosomes and are placed by Hedges (1989*a*) in the *nubicola* group. *Eleutherodactylus alticola* and *E. nubicola* share a

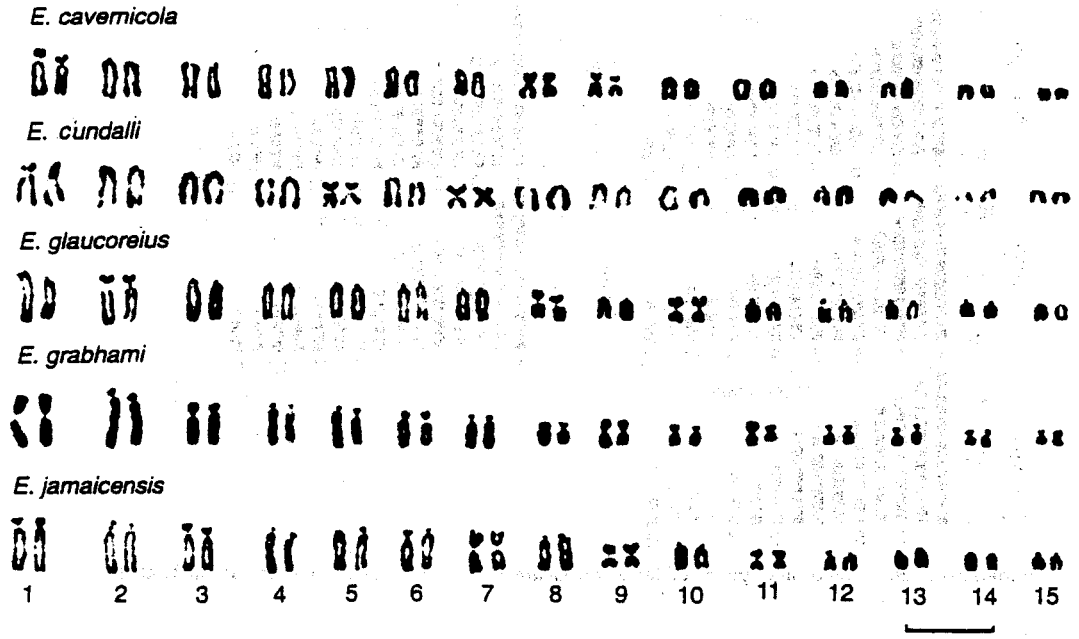


PLATE II. Karyotypes of 30-chromosome species of Jamaican *Eleutherodactylus*. The scale represents 10 μm and is the same for all five species.

unique metacentric chromosome (9) which has a secondary constriction on each arm and their two metacentric chromosomes are similar in size (7 or 8, and 9). *Eleutherodactylus orcutti*, *E. andrewsi* and *E. griphus* have a similar metacentric chromosome (13) but this chromosome bears a secondary constriction in *E. andrewsi*. *Eleutherodactylus sisyphodemus* has a very different karyotype containing four subtelocentric chromosome pairs (none in the other 32-chromosome species), only nine telocentric chromosome pairs, and three metacentric or submetacentric chromosome pairs. The 32-chromosome karyotype in *E. sisyphodemus* was apparently derived from a 28-chromosome *grabhami*-like karyotype (Fig. 5 and discussed above) and is therefore convergent in chromosome number with the other 32-chromosome species.

C-bands
(Plates IV and V)

We were successful in obtaining C-banded karyotypes for most of the Jamaican species. C-banded idiograms (Fig. 8) were constructed by incorporating bands, observed from the karyotypes (Plates IV and V) and from other C-banded metaphase spreads observed directly through the microscope. The locations of the C-bands varied considerably between species and provided little information which could be used to determine the relationships of species.

In some cases, the C-bands might serve as evidence to disprove homologies. The C-banded karyotypes show the secondary constrictions on pair 12 in *E. glaucoreius* and pair 13 in

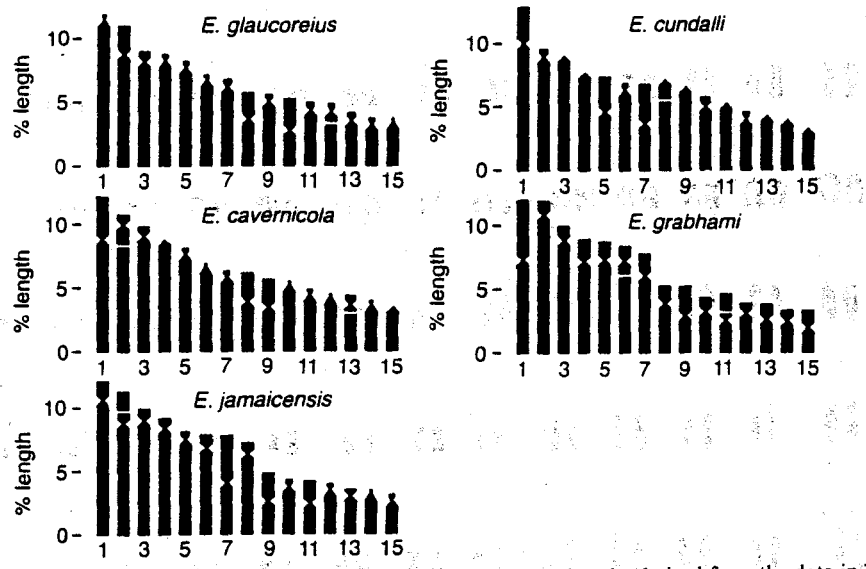


FIG. 6. Idiograms of 30-chromosome species of Jamaican *Eleutherodactylus* derived from the data in Table I.

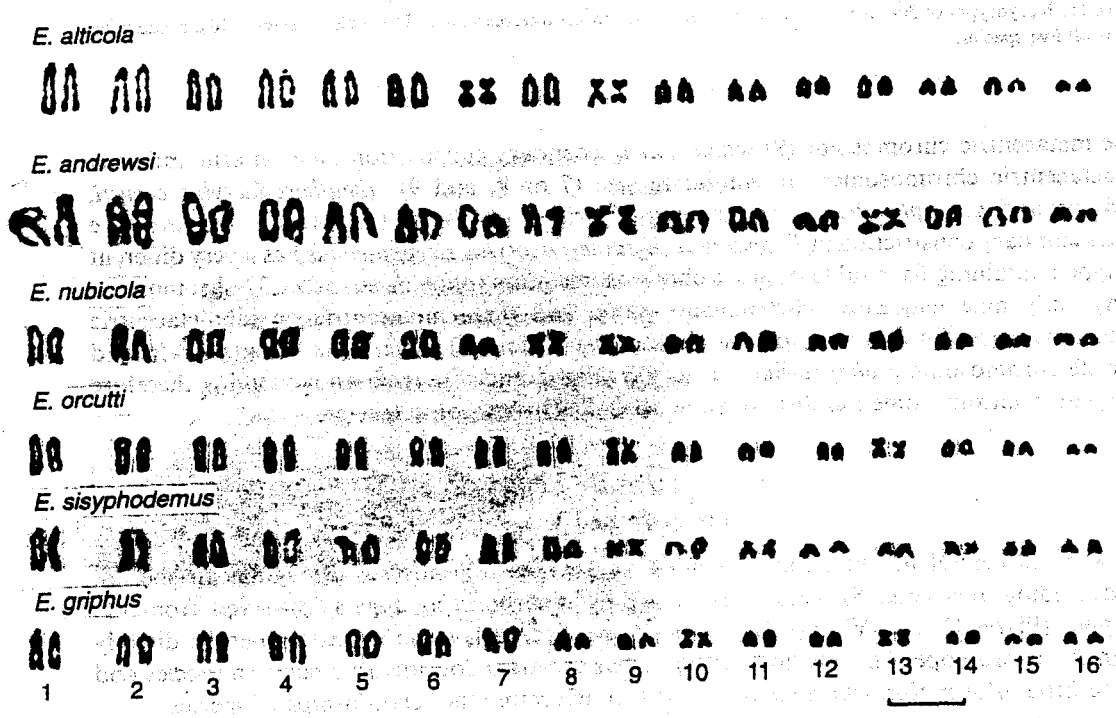


PLATE III. Karyotypes of 32-chromosome species of Jamaican *Eleutherodactylus*. The scale represents 10 μm and is the same for all six species.

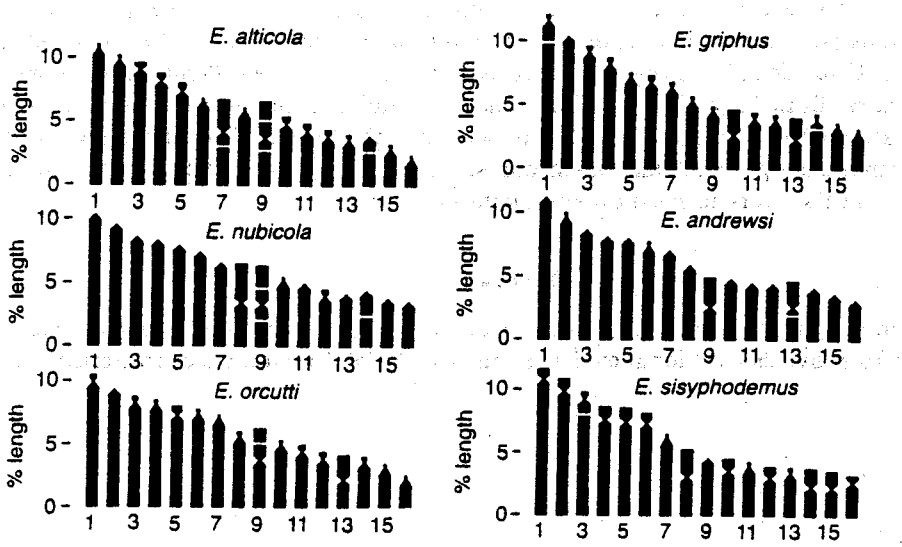


FIG. 7. Idiograms of 32-chromosome species of Jamaican *Eleutherodactylus* derived from the data in Table I.

E. cavernicola to be fundamentally different. None of the three C-banded 28-chromosome species demonstrated similar bands. The different banding patterns of chromosome 1 in *E. fuscus* and *E. pentasyringos* support the contention (above) that these chromosomes relate to respective chromosomes 2 and 1 in a putative 26-chromosome ancestor.

The position of the nucleolus organizing region (NOR) is at C-band associated secondary constrictions in a number of anuran species (Schmid, 1982). *Eleutherodactylus fuscus* was the only

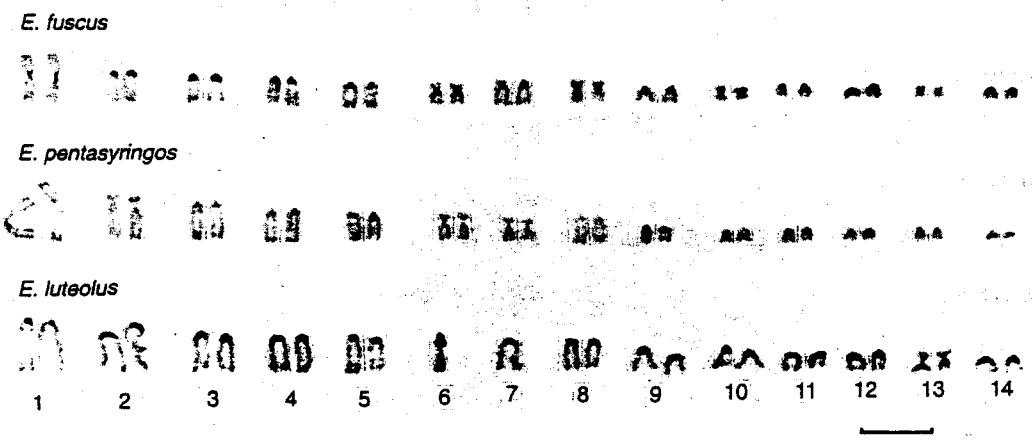


PLATE IV. C-banded karyotypes of three 28-chromosome species of Jamaican *Eleutherodactylus*. The C-banded karyotype of *E. luteolus* does not represent a complete chromosome complement. Only one homologue for chromosome pairs 6 and 7 was found in this particular metaphase spread. The scale represents 10 μm for the three karyotypes.

species which did not have a C-band associated secondary constriction and some species had two such constrictions (*E. griphus*, *E. luteolus* and *E. grabhami*). Interstitial 'dot-like' C-bands are present in all the C-banded 32-chromosome species and all the C-banded 30-chromosome species except *E. grabhami*. Both *E. griphus* and *E. alticola* have similar chromosomes 11 and 12 and the secondary constriction on chromosome 14 is associated with C-bands. The interstitial dot-like C-bands are found among the 30- and 32-chromosome species which are considered to be most closely related, but they vary in position and quantity.

Discussion

The variation in chromosome number and morphology found among the Jamaican species of *Eleutherodactylus* provides additional evidence that karyotypic variation is characteristic of the

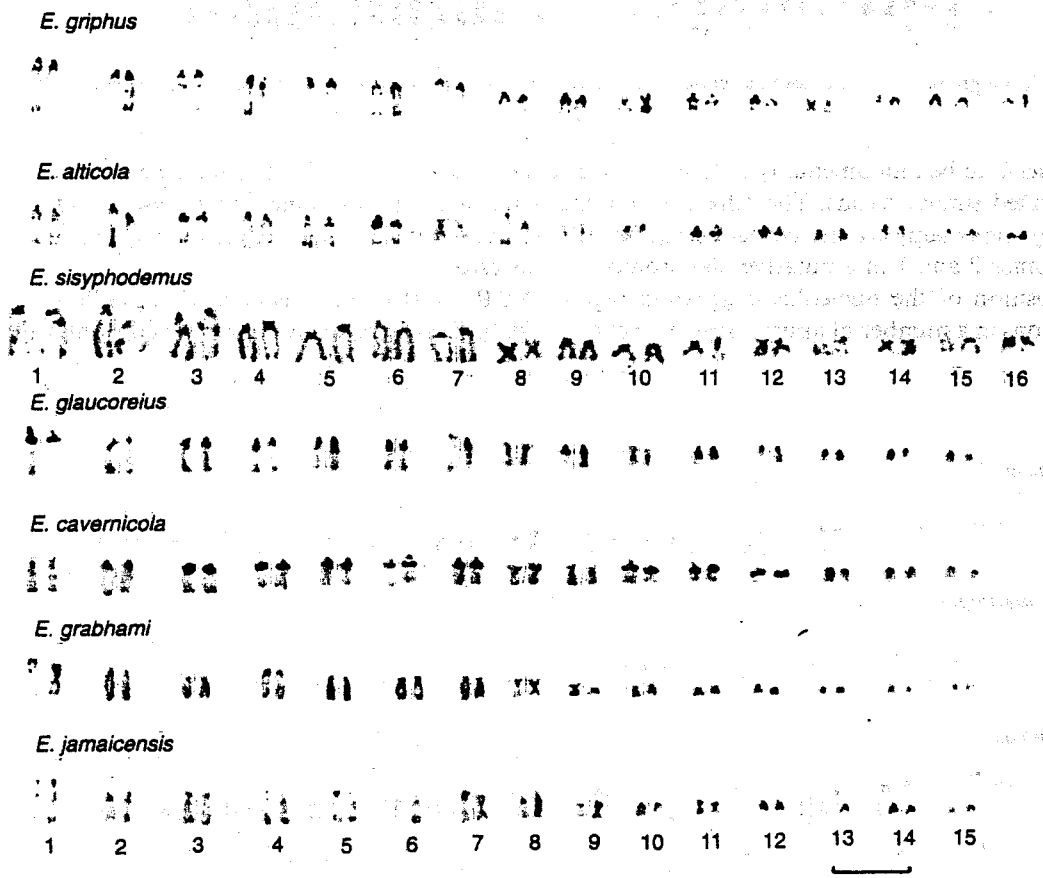


PLATE V. C-banded karyotypes of three 16-chromosome species and four 15-chromosome species of Jamaican *Eleutherodactylus*. Variation in staining intensity and the presence of interstitial C-bands are most easily observed in *E. cavernicola*. The scale represents 10 μm for all seven karyotypes.

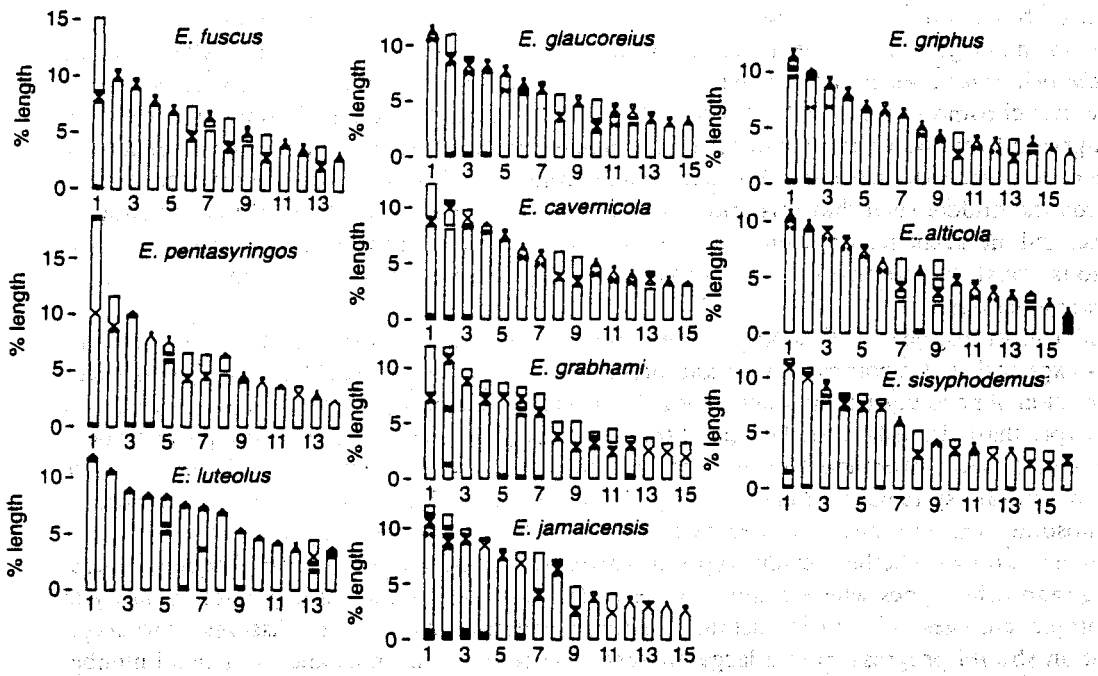


FIG. 8. C-banded idiograms of 28, 30 and 32-chromosome species of Jamaican *Eleutherodactylus*. The positions of the C-bands were determined directly from the C-banded karyotypes (Figs 3 and 4). Secondary constrictions may or may not be associated with C-bands. Only one of the three *E. alticola* secondary constrictions has associated C-bands (14). Telomeres may be intensely stained (*E. jamaicensis*, 1–4) or weakly stained (*E. grabhami*, 5–7). Interstitial C-bands often have a 'dot-like' appearance (*E. cavernicola*, 10–12) or appear as a bar across all chromatids (*E. grabhami*, 2). Considerable variation is observed in the position of C-bands.

genus and is not restricted to a few species or a particular geographical range. Karyotypic variation has been encountered among species of this genus inhabiting other islands in the Caribbean (Bogart, 1981*a*), and on the mainland in Central and South America (Bogart, 1973; DeWeese, 1976; Savage & DeWeese, 1981).

Karyotypic similarities and differences may be used to infer phylogeny if chromosome numbers, C-bands, sites of secondary constrictions or other 'markers' can be traced to a common ancestral karyotype in a monophyletic lineage (King, 1990). In the cosmopolitan toad genus *Bufo*, the 20-chromosome African toads appear to be a monophyletic group (Bogart, 1972) which would include the tetraploid 40-chromosome *B. asmarae* (Tandy *et al.*, 1982). All other species in the family Bufonidae that have been karyotyped have 22 chromosomes (Kuramoto, 1990).

Green (1986) constructed a phylogenetic tree of western North American *Rana* based on 13 karyotypic character states. Compared to *Eleutherodactylus*, the karyotypic variation in *Rana* involves rather subtle differences. Six of Green's characters were considered to have no polarity which indicated that the derived condition was not obvious when compared with the karyotype in the outgroup species (*R. pipiens*).

Generally, species with the lowest numbers of chromosomes have mostly metacentric chromosomes and those with high numbers have mostly telocentric chromosomes. It has been

assumed, based on this observation, that centric fusion and fission are common mutational events in many genera that demonstate chromosome number variation (King, 1990). Finding *Eleutherodactylus* species with 18 metacentric chromosomes ($n = 9$), as well as species with 36 telocentric chromosomes ($n = 18$; Bogart, 1970, 1973), supports this assumption. However, it is also apparent that other chromosomal mutational events have taken place in *Eleutherodactylus*. Pericentric inversions can move the centromere either away from the middle (metacentric) or towards the middle such that this mutation could convert telocentric chromosomes to subtelocentric, submetacentric, or even metacentric chromosomes. Translocations, insertions, and deletions must all be invoked to explain the chromosome variation which is evident in *Eleutherodactylus*.

Unlike most frog genera, *Eleutherodactylus* demonstrates chromosome variability, and therefore, it was expected that a comparison of karyotypes among the species would provide phylogenetic information. As evolutionary markers (King, 1990), closely related species should have more similar karyotypes than distantly related species if the mutational events resulting in karyotypic alterations accumulated slowly over time. Chromosome number has been used as a primary difference between taxa in anuran systematic studies (Lynch, 1971; Heyer, 1975; King, 1990); the number of chromosome arms also has been used by DeWeese (1976) in *Eleutherodactylus*.

It is not known whether certain types of chromosome mutation are more frequent, or less lethal, than other types which could provide useful information and some possible polarity to karyotypic changes. If centric fusions are more common than centric fissions, karyotypic evolution should progress from a large number of telocentric chromosomes to a small number of metacentric chromosomes, but if fusion and fission phenomena are equally likely, the same chromosome number could easily be derived by convergence in separate lineages. If telocentric and metacentric chromosomes are the only chromosomes which can participate in centric fusion or fission phenomena, the number of chromosome arms could be a very useful parameter to compare karyotypes (DeWeese, 1976; King, 1990). However, if telocentric chromosomes can be derived from a break distal to the centromere, the number of arms would be expected to vary in a lineage that demonstrates number variation. If it could be established that pericentric inversions were frequent events among telocentric chromosomes, then karyotypes possessing telocentric chromosomes might be the product of recent fission mutations. By comparing closely related, chromosomally distinctive populations of *Eleutherodactylus*, knowledge concerning the rates of certain types of mutations should be obtained. No other frog genus has demonstrated such a diversity of chromosome alterations.

Jamaican *Eleutherodactylus* are monophyletic (Hedges, 1989*a*, *b*) and therefore the observed karyotypic variation must have been derived from some ancestor that inhabited Jamaica only since the late Oligocene or early Miocene (25 Mya) when the island emerged (Robinson, Lewis & Cant, 1970; Horsfield, 1973; Comer, 1974; Arden, 1975; Buskirk, 1985). The initial colonization of modern Jamaica by *Eleutherodactylus* was probably from Cuba soon after Jamaica became emergent (Hedges, 1989*a*).

The ancestral chromosome number for Jamaican *Eleutherodactylus* is not directly apparent. Because of the observed chromosomal variation, outgroup comparisons are probably not very meaningful without additional information concerning inter-island relationships. Cuban and Hispaniolan members of the subgenus *Euhyas* are closest to the Jamaican radiation of *Eleutherodactylus* based on immunological (Hass & Hedges, 1991) and allozyme data (Hedges, 1989*a*, *b*). Cuban 32-chromosome members of the subgenus *Euhyas* (Bogart, 1981*a*) have karyotypes most similar to Jamaican species.

A possible chromosomal phylogeny of Jamaican *Eleutherodactylus* (Fig. 9) includes postulated karyotypic changes. This tree (see also Hedges 1989*b*; fig. 4) represents the best estimate of the relationships of Jamaican *Eleutherodactylus* showing congruence of allozymes, immunology, chromosomes, morphology and geography. From this figure, chromosome number change occurred at least nine times. Assuming Fig. 9 is a realistic representation of evolution among Jamaican *Eleutherodactylus*, observed karyotypic changes help to establish the types of mutation which are significant in evolutionary terms by comparing karyotypes in the monophyletic lineages.

It is evident, from the distinctively different karyotypes and the lineages defined by molecular data involving species with the same chromosome number, that chromosome numbers or chromosome arm numbers by themselves cannot be used to define *Eleutherodactylus* lineages without additional information. This was attempted by Savage (1987) for the genus *Eleuthero-*

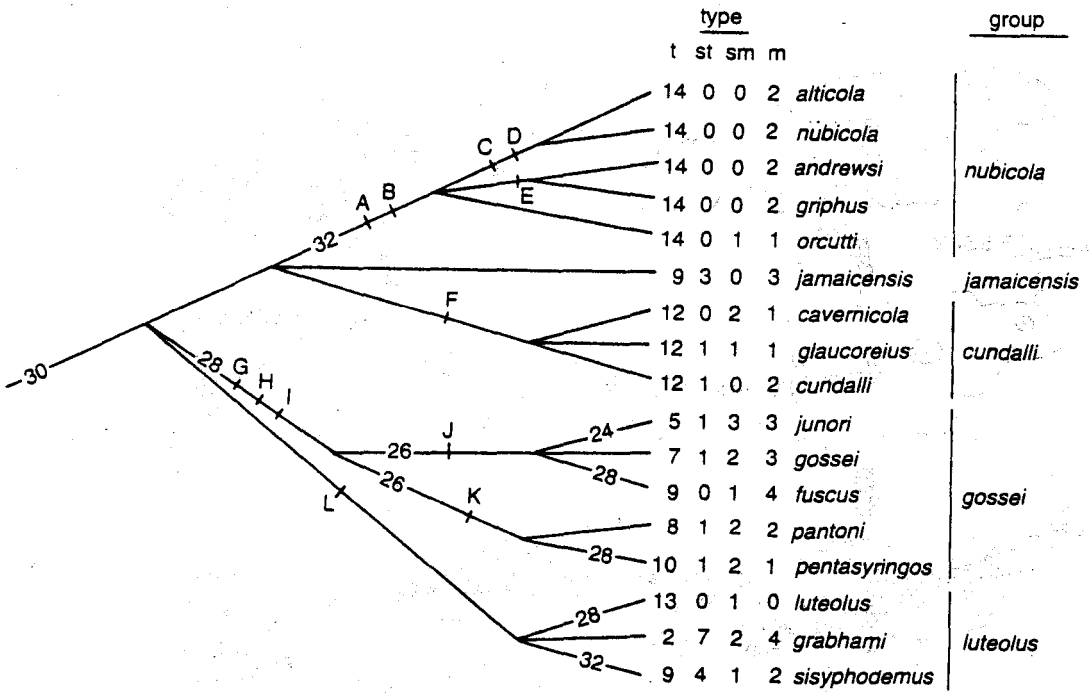
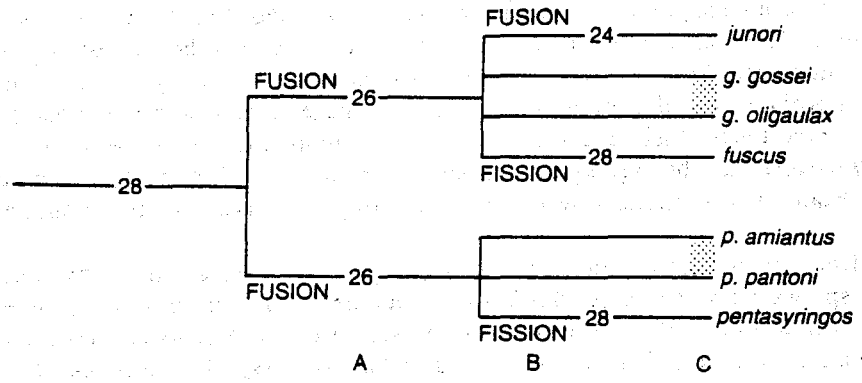


FIG. 9. Phylogenetic relationships of 17 native species of Jamaican *Eleutherodactylus* based largely on allozyme and albumin immunological data (Hedges, 1989*b*; Hass & Hedges, 1991). Hypothesized chromosomal number changes are mapped on the phylogeny, and karyotypic information is given for each species. This phylogeny suggests that nine independent chromosome number changes occurred in Jamaica since the mid-Miocene. Additional chromosome features that may provide supporting evidence for certain clades are indicated (A to L) on the tree. They are: (A) 14 telocentrics, (B) metacentric No. 9 (No. 10 in *E. griphus*), (C) two secondary constrictions on No. 9, (D) a larger metacentric (No. 7 or No. 8), (E) two small metacentrics; No. 9 (No. 10 in *E. griphus*) and No. 13, (F) 12 telocentrics, (G) metacentric No. 1 (= telocentrics No. 3 and No. 5 in *E. fuscus* through fission), (H) submetacentric No. 6, (I) submetacentric No. 7 (= metacentric No. 8 in *E. fuscus*), (J) metacentric or submetacentric No. 8 (= No. 10 in *E. fuscus*), (K) Nos. 5 and 8 in *E. pentasyringos* derived from No. 8 in *E. pantoni*, (L) Nos. 7 and 9 in *E. sisyphodemus* derived from No. 1 in *E. grabhami*. Types (arm length ratio abbreviations) are: t = telocentric, st = subtelocentric, sm = submetacentric, m = metacentric. Species group names are from Hedges (1989*a*).



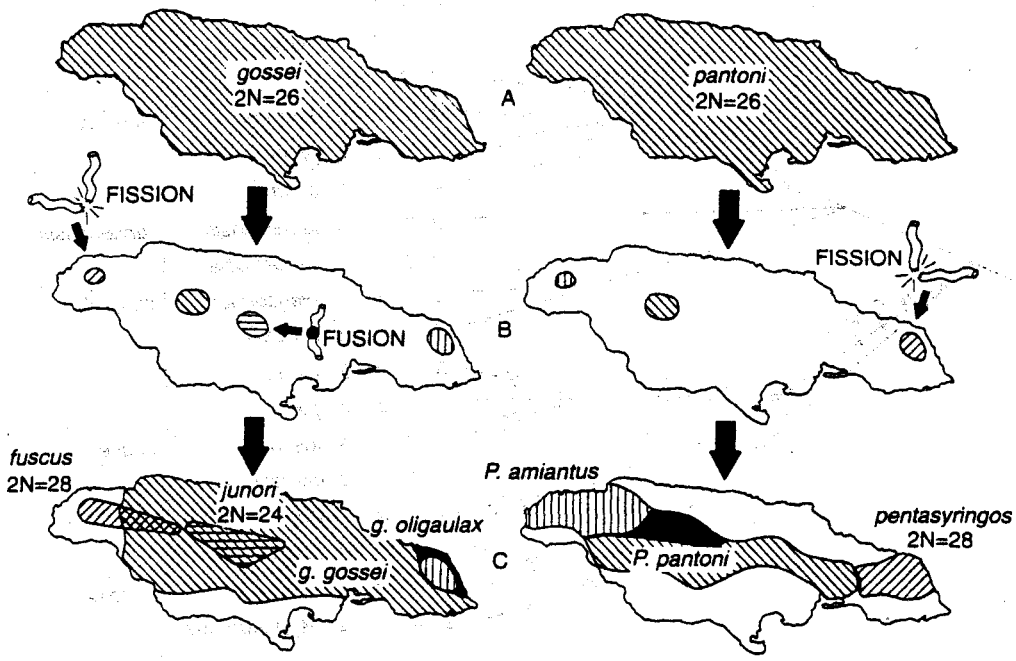


FIG. 10. Hypothesized sequence of events leading to speciation in the *gossei* group. *Eleutherodactylus gossei* and *E. pantoni* are widely distributed during equable climate. (A) Possibly late Miocene or early Pliocene (5 Mya) based on molecular data (Hedges, 1989*b*; Hass & Hedges, 1991). (B) Ranges contract during cooler and drier climate (possibly mid-Pliocene) forming at least four refuges: Dolphin Head Mountain, the Cockpit Country, the central highlands (in *E. gossei*), and the NE slopes of the Blue and John Crow Mountains (from west to east). Fixation of a chromosome fission in the Dolphin Head isolate of *E. gossei* and the eastern isolate of *E. pantoni*, and a fusion in the central highlands isolate of *E. gossei* occurs. (C) Present. Equable climates during Holocene promote range expansion and eventual contact of previously isolated populations. The subspecies *E. g. oligaulax* and *E. p. amiantus* probably represent the eastern and western isolates (respectively) which did not attain reproductive isolation and therefore intergrade (indicated by black) with the nominate subspecies (*E. g. gossei* and *E. p. pantoni*). Chromosome data are not available for *E. p. amiantus*. Distributional data are from Schwartz & Henderson (1991).

dactylus (additional information on jaw musculature was used for one subgenus, *Craugastor*). Aside from difficulties in drawing conclusions from such a limited data set (karyotypes were available for only 65 of 500 species), the relationships obtained by Savage (1987) showed only poor agreement with other types of information, such as morphology, allozymes, immunology and geography (see also discussion in Hedges, 1989*a*). The rate of karyotypic change in any given lineage appears to be as variable in frogs as it has been shown to be in some other vertebrates (Fredga, 1977; Baker & Bickham, 1980).

Speciation and chromosome evolution

In contrast to the five species in the *nubicola* group (2N = 32) and the three species in the *cundalli* group (2N = 30), speciation in the *gossei* and *luteolus* groups was accompanied by fissions and fusions resulting in number changes. The three species in the *luteolus* group are broadly sympatric and therefore it is difficult to infer the sequence of events that led to their origin. However, the karyotypic and distributional data suggest that speciation in the *gossei* group of five species involved fixation of chromosome variants in isolated populations (Fig. 10).

Both *E. pentasyringos* and *E. gossei oligaulax* are confined to extreme eastern Jamaica and are only slightly sympatric or parapatric with their closest relatives (*E. pantoni* and *E. gossei*, respectively). This distributional pattern is also seen in *E. glaucoreius* of the *cundalli* group as well as some reptile taxa (Schwartz & Henderson, 1991), suggesting that formerly continuous ranges became disrupted in eastern Jamaica, resulting in isolation and differentiation. The isolating mechanism may have been the uplift of the Blue Mountains during the late Miocene and Pliocene sea level changes (Haq, Hardenbol & Vail, 1987), or the cooler and drier climate during the Pleistocene (Pregill & Olson, 1981) resulting in forest refugia. The distributions of *E. fuscus* and *E. pantoni amiantus* in western Jamaica also suggest allopatric isolation and differentiation because their closest relatives (*E. gossei* and *E. p. pantoni*, respectively) are allopatric or only partially sympatric. However, the isolation of those taxa is not readily explained by a geological event; forest refugia seem a more likely mechanism.

Speciation in the *gossei* group probably began with the independent derivation of 26-chromosome *E. gossei* and *E. pantoni* from a 28-chromosome ancestor by the fusion of chromosomes 13 and 14 (in *E. gossei*) and 5 and 14 (in *E. pantoni*). This probably occurred in the Late Miocene (5–7 Mya) based on the molecular data. Subsequently, both species became sympatric and widely distributed (Fig. 10: A). In the Pliocene or Pleistocene, ranges contracted and at least three isolates formed: (1) western (Dolphin Head mountain), (2) west-central (Cockpit Country), and (3) eastern (John Crow mountains/Rio Grande valley). These three areas presently have the highest rainfall in Jamaica (Lack, 1976: fig. 4), and if historical rainfall patterns were similar, they would have been likely places for forest refugia during dry periods. The present distribution of *E. junori* in the central highlands suggests a fourth possible refugium for ancestral *E. gossei* populations.

The fixation of chromosome variants in these isolates appears to have been associated with the speciation events leading to *E. fuscus* and *E. junori* (from *E. gossei*) and *E. pentasyringos* (from *E. pantoni*). All three daughter species have different chromosome numbers and different advertisement calls from their putative parental species. *Eleutherodactylus junori* is completely sympatric and *E. fuscus* is partially sympatric with *E. gossei*; *E. pentasyringos* is only slightly sympatric with *E. pantoni* (Hedges & Thomas, 1989). There is no evidence of hybridization between any of these species. Although the eastern isolate of *E. gossei* and the western isolate of

E. pantoni underwent morphological differentiation, there was little or no accompanying vocal differentiation and both subspecies (*E. gossei oligaulax* and *E. pantoni amiantus*) presently intergrade with the nominate subspecies (Schwartz & Fowler, 1973). No chromosome data are available for *E. p. amiantus* but *E. g. oligaulax* has 26 chromosomes as in *E. g. gossei*. The above scenario assumes allopatric speciation based on present distributions (Hedges, 1989*b*; fig. 6), with the exception of *E. junori* (sympatric with *E. gossei*).

Futuyma & Mayer (1980) and Coyne (1984) argue that chromosome rearrangements are probably not responsible for conferring reproductive isolation during speciation. However, the association between chromosome number change and vocal differentiation in the *gossei* group is intriguing: perhaps gene rearrangements associated with the chromosome changes alter the advertisement call of a species, thus leading to reproductive isolation. Alternatively, both chromosome and call differences may be the result of a population bottleneck and are otherwise unrelated.

Rates of chromosome evolution

Using the time scale for Jamaican *Eleutherodactylus* evolution (Hedges, 1989*b*: fig. 4), our data suggest that chromosome numbers changed at least nine times during the last 13 million years since those species had a common ancestor. Wilson *et al.* (1974) and Bush *et al.* (1977) acknowledged that chromosome evolution also involves mutational events which do not result in a chromosome number change but, for their calculations of rates of chromosome evolution, these authors only used chromosome number changes and the number of chromosome arms. These mutational changes were considered to be the most obvious changes and the only consistent data for the many genera which they compared. Our findings suggest that Jamaican *Eleutherodactylus* karyotypes undergo a number change at a rate of one per 6·8 MY of divergence. Our value was obtained by using the 'phylogenetic' method of rate calculation (Wilson, Carlson & White, 1977; Maxson & Wilson, 1979). The number of karyotypic changes (chromosome number) that have occurred during the radiation of Jamaican *Eleutherodactylus* (9) was divided by the total time elapsed in all lineages (123 MY; Hedges, 1989*b*: fig. 4) to obtain a rate of 0·073 number changes per MY. This rate was multiplied by two to obtain a rate of 0·15 number changes per MY divergence between two lineages (one number change every 6·8 MY of divergence). This figure is about six times faster than Wilson *et al.*'s 70 MY estimate for frogs and 0·5 times as fast as the average mammalian rate (3·5 MY).

The actual mechanisms for chromosome change are of more evolutionary significance than correlations based on an unequal sampling of genera. Bush (1975) clearly distinguished attributes which would be expected to give rise to chromosome rearrangements during speciation but he categorized frogs as sharing similar biological attributes with the large mammals (Bush's Type 1a). Many frog species, and especially species of *Eleutherodactylus* would be more appropriately classified as Type 1b or Type II and would be expected to undergo speciation in a manner similar to small rodents, foxes or horses which have considerable chromosomal variation. According to Lande (1979), such variation is predictable when populations are fragmented into small demes which tolerate heterozygote disadvantage of chromosomal rearrangements. There is a direct positive correlation linking anuran species which have small clutch size, terrestrial reproduction, and territoriality with inter-specific chromosome variation (Bogart, 1981*b*, 1991).

Coyne (1984) correlated electrophoretically determined heterozygosity values against the taxa used by Wilson *et al.* (1974) and Bush *et al.* (1977) to calculate rates of chromosome evolution.

With only one exception (carnivorous mammals), he found a strong negative correlation: taxa with the lowest rates of chromosome evolution had the highest heterozygosity. Inbreeding and drift in small, isolated populations were considered by Coyne to be factors which could account for both low electrophoretically determined heterozygosity and high karyotypic variability. Coyne stated that his conclusions were preliminary owing to inadequate fossil data and the unequal compilation of chromosomal and electrophoretic data from various sources, "more correct analysis awaits acquisitions of such data from the same species in monophyletic groups."

Eleutherodactylus is a rapidly speciating genus which has demonstrated more chromosome variation than any other amphibian genus. A comparison of species which have different population or clutch sizes and reproductive modes should provide answers to questions relating to how rates of chromosomal and molecular evolution are influenced by these parameters. Such information also may help distinguish between models of speciation, and test the effects of parapatric versus allopatric demes, effective population sizes, and time required for fixation of different types of chromosome mutations.

Permission to collect frogs in Jamaica was provided by the Jamaican Natural Resource Conservation Department through the courtesy of Patrick Fairbairn and Ann Haynes. Mark Coggiano, Carla Hass, Cindy Mayer, Minocal Stephenson and Walton Stephenson assisted with the field work. Richard Highton and Allan Wilson kindly read an earlier draft of the manuscript. SBH especially thanks Richard Highton (Department of Zoology, University of Maryland) for providing facilities, advice and encouragement. Financial support for SBH was provided by Claydene Hass and grants from the National Science Foundation (BSR 8307115 to Richard Highton; and BSR 8906325 and BSR 9123556 to SBH), and for JPB by Natural Sciences and Engineering Research Council of Canada (NSERC) grant A9866.

REFERENCES

Arden, D. D. (1975). The geology of Jamaica and the Nicaraguan Rise. In *Ocean basins and margins*. 3: *Gulf Coast, Mexico and the Caribbean*: 617–661. Nairn, A. E. M. & Stehli, F. G. (Eds). New York & London: Plenum Press.

Baker, R. J. & Bickham, J. W. (1980). Karyotype evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. *Syst. Zool.* **29**: 239–253.

Blommers-Schlösser, R. M. A. (1978). Cytotaxonomy of the Ranidae, Rhacophoridae, Hyperoliidae (Anura) from Madagascar with a note on the karyotype of two amphibians of the Seychelles. *Genetica* **48**: 23–40.

Bogart, J. P. (1970). Los cromosomas de anfibios del genero *Eleutherodactylus*. *Act. IV Congr. Latin. Zool.* **1**: 65–78.

Bogart, J. P. (1972). Karyotypes. In *Evolution in the genus Bufo*: 171–195. Blair, W. F. (Ed.). Austin, Texas: University of Texas Press.

Bogart, J. P. (1973). Evolution of anuran karyotypes. In *Evolutionary biology of the anurans*: 337–349. Vial, J. L. (Ed.). Columbia, Missouri: University of Missouri Press.

Bogart, J. P. (1981*a*). Chromosome studies in *Sminthillus* from Cuba and *Eleutherodactylus* from Cuba and Puerto Rico (Anura: Leptodactylidae). *Life Sci. Contr. R. Ont. Mus.* No. 129: 1–22.

Bogart, J. P. (1981*b*). How many times has terrestrial breeding evolved in anuran amphibians? *Monit. zool. ital. (N.S.)* (Suppl.) **15**: 29–40.

Bogart, J. P. (1991). The influence of life history on karyotypic evolution in frogs. In *Amphibian cytogenetics and evolution*: 233–258. Green, D. M. & Sessions, S. K. (Eds). New York & London: Academic Press.

Bogart, J. P. & Tandy, M. (1981). Chromosome lineages in African ranoid frogs. *Monit. zool. ital. (N.S.)* (Suppl.) **15**: 55–91.

Bush, G. L. (1975). Modes of animal speciation. *A. Rev. Ecol. Syst.* **6**: 339–364.

Bush, G. L., Case, S. M., Wilson, A. C. & Patton, J. L. (1977). Rapid speciation and chromosomal evolution in mammals. *Proc. natn. Acad. Sci. USA* **74**: 3942–3946.

Buskirk, R. (1985). Zoogeographic patterns and tectonic history of Jamaica and the northern Caribbean. *J. Biogeogr.* **12**: 445–461.

Comer, J. B. (1974). Genesis of Jamaican bauxite. *Econ. Geol.* **69**: 1251–1264.

Coyne, J. A. (1984). Correlation between heterozygosity and rate of chromosome evolution in animals. *Am. Nat.* **123:** 725–729.

Crombie, R. I. (1977). A new species of frog of the genus *Eleutherodactylus* (Amphibia: Leptodactylidae) from the Cockpit Country of Jamaica. *Proc. biol. Soc. Wash.* **90:** 194–204.

DeWeese, J. E. (1976). *The karyotypes of Middle American frogs of the genus* ***Eleutherodactylus*** *(Anura: Leptodactylidae): A case study of the significance of the karyologic method.* PhD diss., University of Southern California.

Duellman, W. E. (1993). *Amphibian species of the world: additions and corrections. Univ. Kans. Mus. nat. Hist. Spec. Publ.* No. 21: 1–372.

Fredga, K. (1977). Chromosomal changes in vertebrate evolution. *Proc. R. Soc.* (*B*) **199:** 377–397.

Futuyma, D. J. & Mayer, G. C. (1980). Non-allopatric speciation in animals. *Syst. Zool.* **29:** 254–271.

Goin, C. J. (1947). Studies on the life history of *Eleutherodactylus ricordii planirostris* (Cope) in Florida. *Univ. Fla Publ. biol. Sci. Ser.* **4**(2): 1–66.

Green, D. M. (1986). Systematics and evolution of western North American frogs allied to *Rana aurora* and *Rana boylii*: karyological evidence. *Syst. Zool.* **35:** 273–282.

Green, D. M., Bogart, J. P., Anthony, E. H. & Genner, D. L. (1980). An interactive microcomputer-based analysis system for phylogenetic cytotaxonomy. *Comput. Biol. Med.* **10:** 219–227.

Haq, B. V., Hardenbol, J. & Vail, P. R. (1987). Chronology of fluctuating sea levels since the Triassic. *Science, Wash.* **235:** 1156–1167.

Hass, C. A. & Hedges, S. B. (1991). Albumin evolution in West Indian frogs of the genus *Eleutherodactylus* (Leptodactylidae): Caribbean biogeography and a calibration of the albumin immunological clock. *J. Zool., Lond.* **225:** 413–426.

Hedges, S. B. (1989*a*). Evolution and biogeography of West Indian frogs of the genus *Eleutherodactylus*: slow-evolving loci and the major groups. In *Biogeography of the West Indies: past, present and future*: 305–370. Woods, C. A. (Ed.). Gainesville, Florida: Sandhill Crane Press.

Hedges, S. B. (1989*b*). An island radiation: allozyme evolution in Jamaican frogs of the genus *Eleutherodactylus* (Leptodactylidae). *Caribb. J. Sci.* **25:** 123–147.

Hedges, S. B., Hass, C. A. & Maxson, L. R. (1992). Caribbean biogeography: Molecular evidence for dispersal in West Indian terrestrial vertebrates. *Proc. natn. Acad. Sci. USA* **89:** 1909–1913.

Hedges, S. B. & Thomas, R. (1989). Supplement to West Indian amphibians and reptiles: a checklist. *Milw. Publ. Mus. Contr. Biol. Geol.* **77:** 1–11.

Heyer, W. R. (1975). A preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. *Smithson. Contr. Zool.* No. 199: 1–55.

Horsfield, W. T. (1973). Late Tertiary and Quaternary crustal movements in Jamaica. *J. geol. Soc. Jamaica* **13:** 6–13.

Kezer, J. & Sessions, S. K. (1979). Chromosome variation in the plethodontid salamander *Aneides ferreus*. *Chromosoma* **71:** 65–80.

King, M. (1990). *Animal cytogenetics. 4 Chordata. 2 Amphibia.* Berlin: Gebrüder Borntraeger.

Kuramoto, M. (1990). A list of chromosome numbers of anuran amphibians. *Bull. Fukuoka Univ. Educ.* **39**(III): 83–127.

Lack, D. (1976). *Island biology*. Berkeley, California: University of California Press.

Lande, R. (1979). Effective deme sizes during long-term evolution estimated from rates of chromosomal rearrangement. *Evolution* **33:** 234–251.

Lynch, J. D. (1971). Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs. *Misc. Publs Mus. nat. Hist. Univ. Kans.* No. 53: 1–238.

Lynch, J. D. (1976). The species groups of the South American frogs of the genus *Eleutherodactylus* (Leptodactylidae). *Occ. Pap. Mus. nat. Hist. Univ. Kans.* No. 61: 1–24.

Lynn, W. G. & Dent, J. N. (1943). Notes on Jamaican amphibians. *Copeia* **1943:** 234–242.

Maxson, L. R. & Wilson, A. C. (1979). Rates of molecular and chromosomal evolution in salamanders. *Evolution* **33:** 734–740.

Morescalachi, A. (1973). Amphibia. In *Cytotaxonomy and vertebrate evolution*: 233–348. Chiarelli, A. & Capanna, E. (Eds). New York & London: Academic Press.

Pregill, G. K. & Olson, S. L. (1981). Zoogeography of West Indian vertebrates in relation to Pleistocene climatic cycles. *A. Rev. Ecol. Syst.* **12:** 75–98.

Robinson, E., Lewis, J. F. & Cant, R. V. (1970). Field guide to aspects of the geology of Jamaica. In *International Field Institute guidebook to the Caribbean island-arc system*: 3–9. Donnelly, T. W. (Ed.). American Geol. Inst./Nat. Sci. Foundation.

Savage, J. M. (1987). Systematics and distribution of the Mexican and Central American rainfrogs of the *Eleutherodactylus gollmeri* group (Amphibia: Leptodactylidae). *Fieldiana, Zool. (N.S.) No. 33: 1–57.*

Savage, J. M. & DeWeese, J. E. (1981). The status of the Central American leptodactylid frogs *Eleutherodactylus melanostictus* (Cope) and *Eleutherodactylus platyrhynchus* (Gunther). *Proc. biol. Soc. Wash.* **93:** 928–942.

Schmid, M. (1982). Chromosome banding in amphibia. Analysis of the structure and variability of NORs in Anura. *Chromosoma* **87:** 327–344.

Schwartz, A. (1969). The Antillean *Eleutherodactylus* of the *auriculatus* group. *Stud. Fauna Curaçao* **30:** 99–115.

Schwartz, A. (1976). Variation and relationships of some Hispaniolan frogs (Leptodactylidae: *Eleutherodactylus*) of the *ricordi* group. *Bull. Fla St. Mus. biol. Sci.* **21:** 1–46.

Schwartz, A. & Fowler, D. (1973). The Anura of Jamaica: a status report. *Stud. Fauna Curaçao* **43:** 50–142.

Schwartz, A. & Henderson, R. W. (1991). *Amphibians and reptiles of the West Indies: descriptions, distributions, and natural history.* Gainesville, Florida: University of Florida Press.

Sessions, S. K. (1982). Cytogenetics of diploid and triploid salamanders of the *Ambystoma jeffersonianum* complex. *Chromosoma* **84:** 599–621.

Sumner, A. T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Expl Cell Res.* **75:** 304–306.

Tandy, M., Bogart, J. P., Largen, M. J. & Feener, D. J. (1982). A tetraploid species of *Bufo* (Anura, Bufonidae) from Ethiopia. *Monit. zool. ital.* (N.S.) (Suppl.) **17:** 1–79.

Wilson, A. C., Carlson, S. S. & White, T. J. (1977). Biochemical evolution. *A. Rev. Biochem.* **46:** 573–639.

Wilson, A. C., Sarich, V. M. & Maxson, L. R. (1974). The importance of gene rearrangement in evolution: evidence from studies on rates of chromosomal, protein, and anatomical evolution. *Proc. natn. Acad. Sci. USA* **71:** 3028–3030.