fluorescence intensity between normal and heterozygous cells.

In blood of individuals homozygous for acatalasemia a pseudomosaicism can be observed (Figure 1), similar to that seen by applying the elution technique[3]. However, using this procedure, it becomes evident that the catalase positive cells do not represent a uniform entity, but seem to be composed of various stages of intermediate fluorescence intensity. In total blood the number of fluorescent (= catalase positive) cells varies between 1–2%, a figure which is slightly higher than that previously obtained using the BETKE staining technique (0.5–1.0%)[3]. Fractionation experiments have revealed that the number of catalase positive cells, as visualized by fluorescent anticatalase, steadily decreases from the top to the bottom fraction. As shown in Figure 2, in each fraction a correlation between the fluorescent cell count and the number of reticulocytes is found. However, throughout this study the number of catalase positive cells has been found to be slightly higher than the reticulocyte count. Members of all 3 Swiss acatalasemia families (type III) have been investigated[2]. They all led to similar results. This procedure of localizing catalase in single cells was compared with an analogue technique visualizing another red cell constituent. For this purpose HbF was stained with a specific fluorescent antibody. The percentage of the HbF-positive cells remains constant, whereas a steady decrease in the number of fluorescent, catalase positive cells is observed from the top to the bottom fraction.

The uneven distribution of residual catalase activity previously found with the elution method is confirmed with the fluorescent antibody technique. However, the identity of catalase positive cells and reticulocytes cannot be proved. The findings reported are consistent with the hypothesis that the low level of catalase activity ($\sim 1\%$) in blood of homozygotes is due to the synthesis of an unstable enzyme variant. A final proof of this concept will only arise from a structural analysis of the catalase variant[11].

Zusammenfassung. Im Blutausstrich lassen sich Erythrozyten von normalem und solche von stark vermindertem Katalasegehalt durch Verwendung von fluoreszierender Antikatalase unterscheiden. Mit dieser Methode konnte der früher erhobene Befund, wonach bei homozygoten Trägern des Enzymdefektes Akatalasie ein Pseudomosaizismus besteht, bestätigt werden. Bei der Untersuchung von Erythrozytenfraktionen verschiedener Dichte besteht eine Korrelation zwischen der Anzahl Retikulozyten und fluoreszierender Zellen. Dieser Befund passt zur Annahme, dass es sich bei der Katalaserestaktivität im Blut homozygoter Defektträger um eine instabile, jedoch antigenidentische Enzymvariante handelt.

T. HOSOI, H. SUTER,
S. YAHARA and H. AEBI

Medizinisch-chemisches Institut der Universität, 3000 Bern (Switzerland) and Kyoto First Red Cross Hospital, Higashiyama-Ku, Kyoto (Japan), 17 December 1968.

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Karyotypic Data for Five Species of Anguid Lizards

MCDOWELL and BOGERT[1] defined an infra-order of lizards, the Anguimorpha, with 2 major phyletic branches, the Diploglossa which retained the more usual and presumably more primitive characters, and the Platynota. Diploglossa includes the wide-spread and species-rich Anguidae, considered to be the most primitive family, and 2 other small extant families. Three subfamilies of Anguidae are recognized[1]: Diploglossinae, presumably the most primitive; Gerrhonotinae, whose ancestry is probably derived from a primitive diploglossine; and Anguinae which is considered close to and derived from Gerrhonotinae. The Gerrhonotinae, containing *Gerrhonotus* and *Ophisaurus*, show a great deal of chromosome variation[2]. We know of no previous chromosome data for the neotropical Diploglossinae.

Previous taxonomic studies on American gerrhonotine lizards, excluding *Ophisaurus*, appear to conflict. TIHEN[3] divided the group into 5 genera: *Gerrhonotus* was monotypic (*G. liocephalus*); *Elgaria* included the species *coerulea, multicarinata, cedrosensis, kingi,* and *paucicarinata*, distributed in Mexico and the western and southwestern United States; and *Barisia* was comprised of 9 species (including *monticola*) that occur in Mexico and Central America. STEBBINS[4] re-examined the species that TIHEN assigned to *Elgaria, Barisia,* and *Gerrhonotus*, and recommended the recognition of a single genus *Gerrhonotus* with 2 subgenera: *Gerrhonotus* (including *liocephalus, multicarinatus, cedrosensis, kingi,* and *paucicarinatus*) and subgenus *Barisia* (including *coeruleus* of western North America, *monticolus* of Costa Rica, and 7 other Mexican and Central American species). A recent osteological study by CRILEY[5] failed to confirm either of these alternative classifications. There were few consistent differences among any of the gerrhonotine lizards. We studied 4 species of *Gerrhonotus* and *Diploglossus costatus* to determine if chromosome data would be useful in clarifying the relationships of these anguid lizards.

Mitotic and meiotic chromosome spreads were obtained from direct testis preparations[6] of 2 *G. multicarinatus multicarinatus* from Napa County, California, 4 *G. coeruleus principis* from Humboldt County, California, 2 *G. monticolus* taken near the summit of Volcan Irazu, Costa Rica, and 4 *G. paucicarinatus* from near La Paz, Baja California. *D. costatus* is a live bearing species. A pregnant female of the subspecies *oreistes*, collected near Kenscoff, Haiti, was injected with Colcemid (Ciba, 0.2 ml of 1 mg/ml solution) 24 h before sacrifice. Two sibling embryos were

[1] S. B. MCDOWELL and C. M. BOGERT, Bull. Am. Mus. nat. Hist. *105*, 1 (1964).
[2] R. MATTHEY, *Les Chromosomss des Vertébrés* (Librairie de l'Université, F. Rouge, Lausanne 1949).
[3] J. A. TIHEN, Am. Midl. Nat. *41*, 580 (1949).
[4] R. C. STEBBINS, Am. Mus. Novit. *1883*, 1 (1958).
[5] B. B. CRILEY, Am. Midl. Nat. *80*, 199 (1968).
[6] G. C. GORMAN, L. ATKINS and T. HOLZINGER, Cytogenetics *6*, 286 (1967).

removed from the oviduct. One was placed in a hypotonic sodium citrate solution and immediately minced; the second was kept in tissue culture medium with Colcemid for an additional 2 h. Cells were prepared in the same manner that is used for testis. Both embryos had numerous mitotic metaphase spreads.

Mitotic metaphase cells of the 2 *G. multicarinatus* have 48 chromosomes, with a rather marked break in size between the largest 11 acrocentric pairs (or macrochromosomes) and the smallest 13 pairs (microchromosomes). This is illustrated in Figure 1 A. *G. paucicarinatus* has $2n = 46$ and is similar to *multicarinatus* except for 1 large pair of metacentric chromosomes. In metaphase II one can clearly see a single metacentric and 9 acrocentric macrochromosomes, and 13 microchromosomes (Figure 2). *G. coeruleus* has fewer chromosomes, $2n = 38$. Like *multicarinatus* and *paucicarinatus* there are 13 pairs of microchromosomes, but *G. coeruleus* has 5 pairs of metacentric and 1 pair of subacrocentric macrochromosomes (Figure 1 B). *G. monticolus* has a diploid number of 30. There are 9 pairs of macrochromosomes, 1 intermediate sized pair, and 5 pairs of microchromosomes. The mitotic metaphase karyotype illustrated in Figure 1 C shows that the 2 largest pairs of macrochromosomes are metacentric, pairs 3–9 are acrocentric or sub-acrocentric, and the intermediate pair is metacentric. In mitosis, the intermediate appears to be the largest of the microchromosomes (Figure 1 C), but in diakinesis (Figure 1 D) it appears to be the smallest of the macrochromosomes. We consider it a microchromosomal element.

Diploglossus costatus has $2n = 36$. There are 12 metacentric macrochromosomes and 24 microchromosomes. Figure 3 A of a mitotic spread shows that the microchromosomes are virtually all metacentric. Figure 3 B illustrates clearly the morphology of the macrochromosomes prepared from another cell. The Table summarizes the data.

The chromosomal differences between *G. multicarinatus*, *paucicarinatus*, and *coeruleus* could be explained simply by Robertsonian mechanisms of centric fusions or fissions. All have 22 macrochromosomal arms, and 26 microchromosomes. The karyotype of *G. monticolus* does not bear a simple relationship to the other 3 species, but the macrochromosomal complement again appears to have 22 arms, as there are 7 pairs of acrocentric or subacrocentric chromosomes (= 14 arms) and 2 pairs of metacentrics (= 8 arms). Loss of microchromosomes has been observed frequently in other lizards[6,7].

MATTHEY[2] reviewed data on intraspecific chromosome variation in *G. scincicauda* (= *G. multicarinatus scinci-*

[7] G. C. GORMAN and L. ATKINS, Syst. Zool. *16*, 137 (1967).

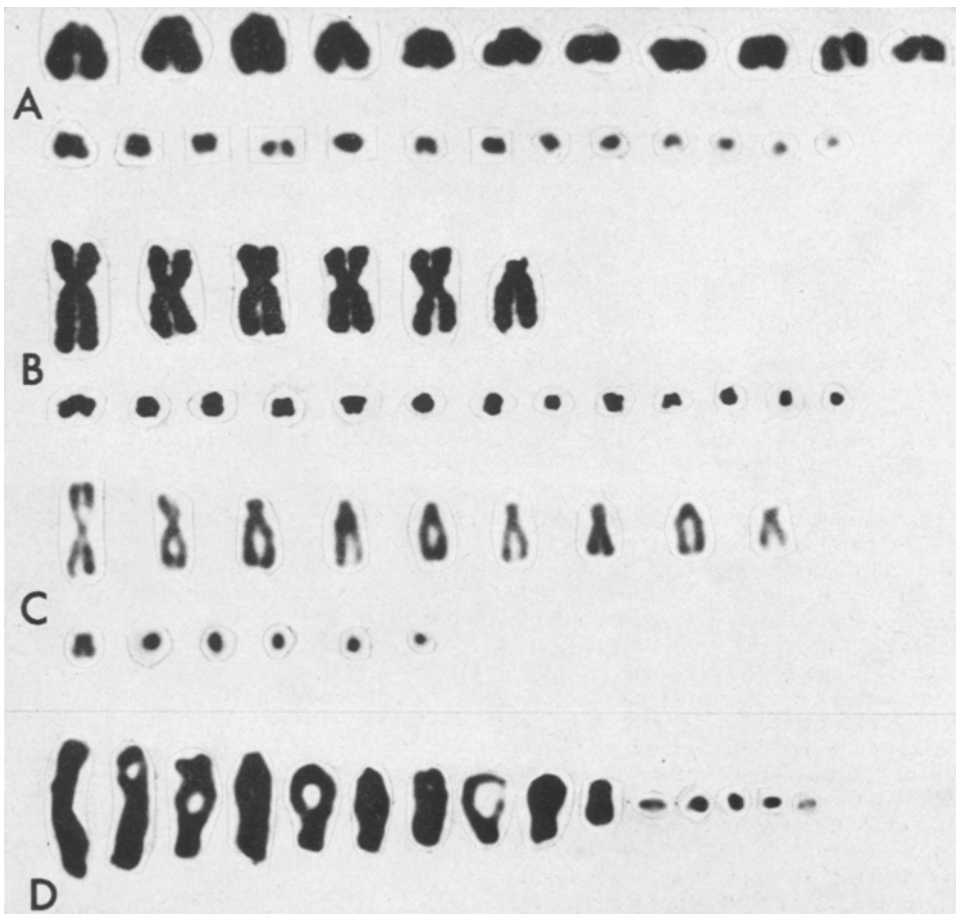

Fig. 1. *Gerrhonotus* chromosomes. (A) *G. multicarinatus* mitotic metaphase. Haploid karyotype showing 11 acrocentric macrochromosomes and 13 microchromosomes. (B) *G. coeruleus* mitotic metaphase. Haploid karyotype showing 5 metacentric and 1 subacrocentric macrochromosomes, and 13 microchromosomes. (C) *G. monticolus* mitotic metaphase. Haploid karyotype showing 2 metacentric and 7 acrocentric macrochromosomes, and 6 microchromosomes. The largest of the microchromosomes is metacentric, and somewhat intermediate in size. (D) *G. monticolus* diakinesis. There are 9 macrobivalents, 5 microbivalents, and 1 of intermediate size.

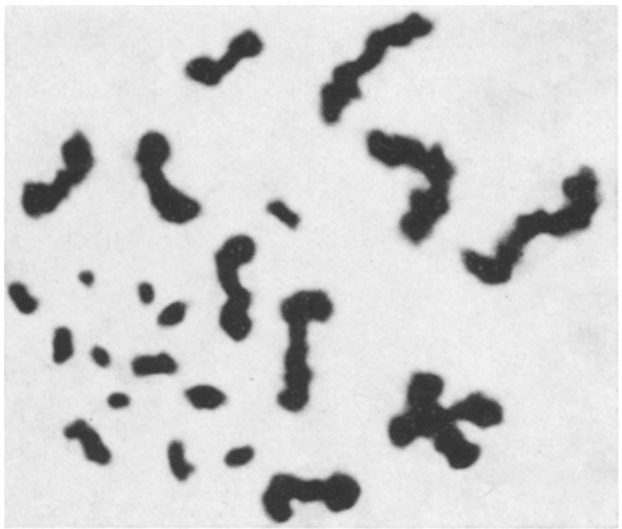

Fig. 2. *Gerrhonotus paucicarinatus*. Metaphase II showing a single metacentric and 9 acrocentric macrochromosomes, and 13 microchromosomes.

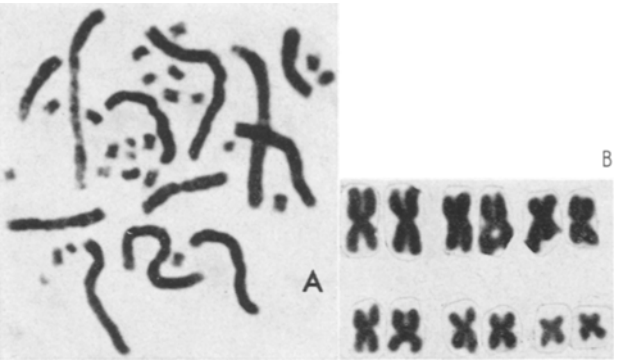

Fig. 3. *Diploglossus costatus* embryo. (A) Mitotic metaphase cell which clearly shows the metacentric nature of some of the microchromosomes. (B) The metacentric macrochromosomes of a metaphase cell in a second embryo.

Chromosome data for 5 species of anguid lizards

Species	Macrochromosomes		Microchromosomes		
	Meta-centric	Acro-centric		2*n*	N.F.
Gerrhonotus multicarinatus	0	22	26	48	48
G. paucicarinatus	2	18	26	48	48
G. coeruleus	10	2	26	38	48
G. monticolus	4	14	12	30	34
Diploglossus costatus	12	0	24	36	48

N.F. is the 'nombre fondamental' of MATTHEY[2] and represents the total number of chromosome arms in the karyotype. All microchromosomes have a value of 1, although some are clearly metacentric.

cauda). He reported karyotypes with 20 macrochromosomes including 2 metacentrics, 21 macrochromosomes with 1 metacentric, and he postulated a karyotype with 22 macrochromosomes, all acrocentric. These karyotypes all had 24 microchromosomes. Further, MATTHEY reported that *G. kingi* had $2n = 45$ and the same karyotype as *G. scincicauda* (= *G. multicarinatus*). We believe that a pair of microchromosomes may have been missed in the counts of *G. scincicauda* and probably in *G. kingi*, possibly the difficulty to resolve smallest pair shown in Figure 1 A[8]. OHNO[9] illustrated a mitotic metaphase of *G. multicarinatus* with 1 large metacentric, 20 large acrocentrics, and 26 microchromosomes ($2n = 47$), and mentioned that this species has 46, 47, and 48 chromosomes involving Robertsonian variants in a single population.

The karyotypes of *G. multicarinatus* and *G. paucicarinatus* are very similar. The diploid number of $2n = 46$ with 1 large pair of metacentrics found in the *G. paucicarinatus* examined falls within the reported intraspecific variation of *G. multicarinatus*. Thus there appear to be similar chromosome complements in *G. multicarinatus*, *G. kingi*, and *G. paucicarinatus*, which both TIHEN and STEBBINS considered closely related.

The reduced number of macrochromosomes in *G. coeruleus* bears a simple Robertsonian relationship to *G. multicarinatus* and *G. paucicarinatus*, but data on more species are needed before we can attempt to postulate the direction of evolutionary change – whether fusion toward a *coeruleus* karyotype, or fission away from it. The karyotype of *G. monticolus* is markedly different from any other known gerrhonotine lizard. This may represent a separate evolutionary trend. Understanding the relationships of *G. monticolus* to the other gerrhonotine lizards awaits analysis of other Central American and Mexican species. Our data indicate that chromosomes may be useful for the classification of the species of *Gerrhonotus* and for interpretation of their evolutionary relationships.

D. costatus has 12 metacentric macrochromosomes and 24 microchromosomes, a karyotype known in many lizard families. This may well represent a primitive character state[10], thus supporting the phylogeny of MCDOWELL and BOGERT[1,11].

Zusammenfassung. Die Chromosomensätze von 5 Eidechsenarten der Familie Aguidae, davon vier vom Genus *Gerrhonotus* und eine vom Genus *Diploglossus* werden beschrieben. *D. costatus* ist $2n = 36$ mit 12 grossen metazentrischen Chromosomen und 24 Mikrochromosomen. *G. multicarinatus* ist $2n = 48$, *G. paucicarinatus* $2n = 46$ und *G. coeruleus* $2n = 38$. Diese 3 Arten haben einen identischen «nombre fondamentale» von 48 und alle haben 26 Mikrochromosomen. *G. monticolus* besitzt $2n = 30$. Die Anzahl der Mikrochromosomen ist auf 12 reduziert.

R. B. BURY, G. C. GORMAN and J. F. LYNCH

Museum of Vertebrate Zoology, University of California, Berkeley (California 94720, USA), 31 October 1968.

[8] We say this because of the seeming conservativeness of the 13 pairs of microchromosomes.

[9] S. OHNO, *Sex Chromosomes and Sex-linked Genes. Monographs on Endocrinology* (Springer-Verlag, Berlin 1967).

[10] G. C. GORMAN, manuscript in preparation.

[11] This work was supported in part by NSF Grant No. GB-6944. We thank T. PAPENFUSS for providing some of the specimens used in the study.