from external features and skeletal anatomy[5] that *L. archeyi* and *L. hamiltoni* have a closer affinity with each other than either does with *L. hochstetteri*. A neotenic basis for the apparent relationship between *L. archeyi* and *L. hamiltoni* has previously been suggested[5].

The degree of importance that should be attached to variations in relative lengths of specific chromosomes of *L. archeyi* and *L. hamiltoni* is questionable at this stage in view of the limited and suboptimal preparations on which the measurements of *L. archeyi* chromosomes were of necessity based[2]. It is desirable that further material of this species should be examined, particularly with regard to the detailed morphology of the chromosomes bearing the secondary constrictions. On present evidence (Figure 2), it appears to be theoretically feasible to derive a karyotype very similar to that of *L. archeyi* by postulating a translocation of the secondary constriction and telomere from Pair 9 of *L. hamiltoni* (Figure 2) to Pair 6 of the same species (Figure 2). Whatever the evolutionary basis of the apparent relationship between *L. archeyi* and *L. hamiltoni* may have been, it is now obscured by the total geographical separation of these 2 species. However, the combined information now available regarding the chromosome patterns of *Leiopelma* suggests that the time may now be appropriate for a taxonomic reassessment of the genus as a whole, combining evidence from all available areas of investigation.

Characteristics of early meiosis in male anurans have been tabulated by MORESCALCHI[7]. Those of the Ascaphidae and Discoglossidae are listed as being generally different from those of 'higher' anuran families. Diakinesis in *L. hamiltoni* appears to conform with the pattern for other ascaphids and discoglossids in some respects, but also has similarities to that of the more advanced families. The scarcity of diakinesis stages supports the contention that this stage is short in ascaphids and discoglossids. On the other hand, the possession of 2 terminal chiasmata in most bivalents in *L. hamiltoni* is an exception to the generalization that ascaphids and discoglossids have more than 2 chiasmata in large bivalents and that chiasma terminalization is normally never total. MORESCALCHI[7] referred to 'the presence, but not constant' of ring bivalents in *Discoglossus* and less commonly in *Alytes*. *L. hamiltoni* can be added to this group, although it should be noted that such an inclusion is based on a single spread.

MORESCALCHI[7–9] has suggested that bivalent morphology and behaviour of ascaphids and discoglossids represent primitive anuran conditions similar to the urodelan type. In urodeles the number, localization and terminalization of chiasmata between and even within species may vary and it would appear that there is also variation in these aspects of male meiosis in the genus *Leiopelma*.

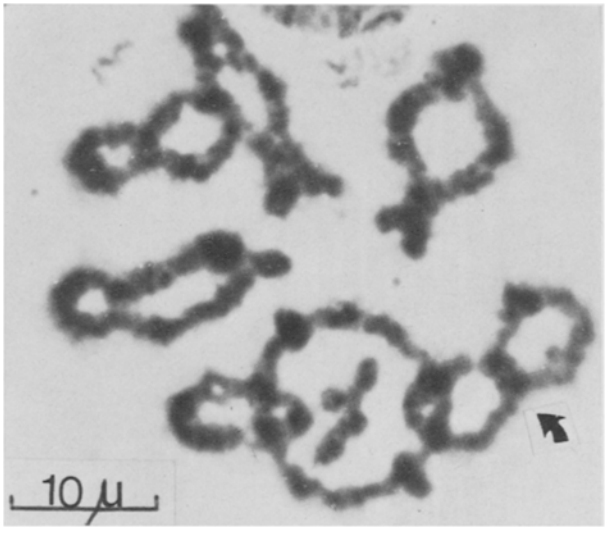


Fig. 3. Late prophase (diakinesis) of first meiotic division from testis squash of *L. hamiltoni*. The arrow indicates an area of overlapping of 2 medium-sized bivalents.

Résumé. Le caryotype de la grenouille couramment classée sous le nom de *Leiopelma hamiltoni* MCCULLOCH, de l'île de Maude, en Nouvelle Zélande, est décrit pour la première fois. A l'état diploïde, le nombre des chromosomes est de 18. La plus petite des paires est acrocentrique et présente de petits satellites terminaux. On n'observe pas de microchromosomes. Pendant la diacinèse de la meïose mâle on peut observer des chromosomes bivalents avec 2 chiasmas terminaux. L'évidence caryologique est en accord avec les indications précédentes concernant l'aspect extérieur et l'anatomie du squelette: *L. hamiltoni* ressemble davantage à *L. archeyi*, qu'à *L. hostetteri*. On suggère la nécessité d'une réévaluation taxonomique du genre.

E. M. STEPHENSON[10], E. S. ROBINSON and N. G. STEPHENSON[11]

School of Biological Sciences, A08, The University of Sydney, Sydney (N.S.W. 2006, Australia); and School of Biological Sciences, Macquarie University, North Ryde (N.S.W. 2113, Australia), 4 June 1974.

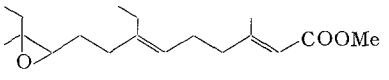
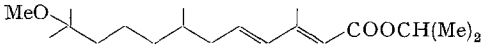
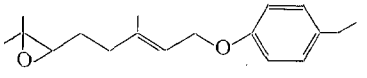
[7] A. MORESCALCHI, in *Cytotaxonomy and Vertebrate Evolution* (Eds A. B. CHIARELLI and E. CAPANA; Academic Press: London and New York 1973), p. 233.
[8] A. MORESCALCHI, Experientia *24*, 964 (1968).
[9] A. MORESCALCHI, Boll. Zool. *37*, 1 (1970).
[10] Present address: Strangeways Research Laboratory, Wort's Causeway, Cambridge CB1 4RN (England).
[11] Acknowledgments. We thank the Hon. Minister for Internal Affairs, Wellington, New Zealand, for permission to obtain the animals used in this study. We are deeply grateful to Mr. I. CROOK for collecting the frogs and transporting them to Australia. We thank Dr. L. MOFFAT for unpublished evidence that tissues from colchicine-treated animals could later be cultured satisfactorily for chromosome studies.

Activity of Some Juvenoids in Chironomid Larvae

LAUFER et al.[1,2] reported that synthetic C_{18} *Cecropia* juvenile hormone and a mixture of derivatives of the farnesenic acid prevent metamorphosis in chironomids. The present study extends their observations and compares activities of some compounds which have been described as potent juvenoids for various Diptera.

Our tests were performed on last instar larvae of *Chironomus annularis* Meig. and *Ch. dorsalis* Meig., which were collected from an outdoor water container in Prague[3]. Groups of 10–15 larvae were kept in 50 ml of tap water in Petri dishes (diameter 12 cm) at room temperature. The larvae were fed with nettle powder and the water was

Activities of 3 potent juvenoids on *Chironomus annularis* and *Ch. dorsalis* (undistinguished)

	Compound	Effective dosage (ppm)
I		0.01
II		0.1
III		0.01
II formulated		0.001

The effective dosages indicate concentration of juvenoids in water which caused development of larval-pupal intermediates in 50% of treated larvae. The remaining 50% of larvae pupated (dead larvae were discarded).

daily stirred. The last larval instar lasted under these conditions about 20 days. The mortality of larvae was very high, apparently due to some infection, and amounted in extreme cases up to 60%. The juvenoids[4] were added to water in 5 µl of acetone. Standard assays were performed in 3 replicas with larvae which were approximately in the middle of the last instar.

All compounds tested were toxic at the dose 0.1 µl/ml water, i.e. in the 100 ppm concentration. In order to ensure that pharmacological toxicity will not interfere with the tests of juvenilizing activity, a limit of 1 ppm was set as the maximum concentration to be used. 7 out of 10 assayed compounds elicited no significant morphological effect at this concentration and were eliminated from further tests. The discarded substances included ethyl 3,7,11-trimethyl-5-oxa-6,7-epoxy-dodec-2-enoate and 1-/(3,7-dimethyl-6,7-epoxy)octyl/-4-ethylphenyl ether, which are highly active on some Diptera[5]. Methyl farnesoate, ethyl 3,7,11-trimethyl-11-chloro-dodec-2-enoate and methyl 3,7,11-trimethyl-7,11-dichloro-dodec-2-enoate, which are possible components of the mixture of juvenoids used in an earlier study on chironomids[2], were also excluded. The Table shows activities of those compounds which passed the elimination test and were assayed further.

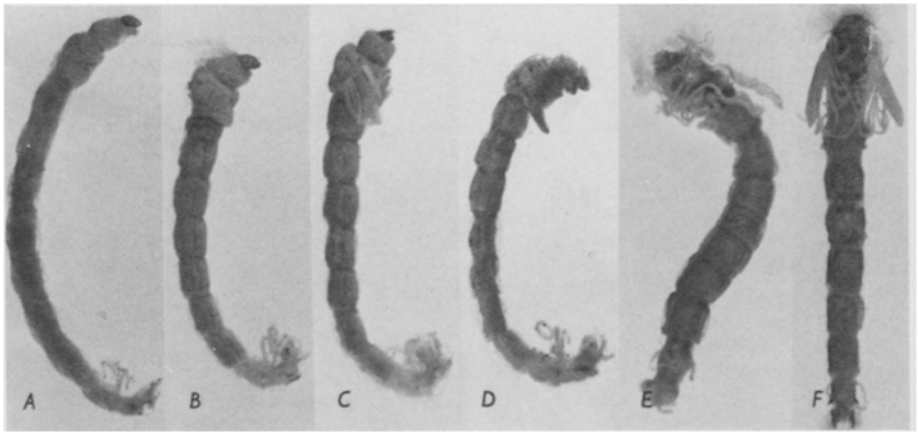
In an attempt to improve the distribution and stability of juvenoids in water, we have used the formulated isopropyl 3,7,11-trimethyl-11-methoxy-dodeca-2,4-dienoate (compound II in the Table). The liquid formulation FZ-51547 was developed by Zoecon Corporation for the use of compound II against mosquitoes and was kindly made available to us. The formulated compound showed a 100 times higher activity than the same compound administered in acetone. It must be noted, however, that a 10 ppm concentration of the emulsifying lotion alone was toxic to chironomid larvae.

The active compounds caused preservation of larval features in pupae. The morphological effects were classified with a scale ranging from 0 to 5 degrees. The occurrence of the maximum effect 5 – development of perfect superlarvae – was never observed. The affected specimens developed into intermediate forms between larva and pupa (effects 1–4, Figure). Some of the intermediates accomplished succesfully the ecdysis but all died shortly thereafter. Insects which formed externally normal pupae were considered as unaffected (effect 0). Some of them, however, perished as pharate adults with adult cuticle precociously tanned and sclerotized.

In one experiment we examined the action of 1 ppm of formulated compound II on larvae whose age was known within ± 2 days after the last larval ecdysis. The larval-pupal intermediates occurred in all age groups except after treatment of freshly ecdyzed larvae and prepupae. The freshly ecdyzed larvae died and the prepupae formed externally normal pupae.

In regard to the period of sensitivity to juvenoids and to the character of most of the induced effects, the chironomids seem to resemble the mosquitoes[6]. The effective doses found in our tests are difficult to compare with those reported for mosquitoes because our testing procedure was less standardized than the current mosquito assays. Nevertheless, it seems that compound II, whose activity

[1] H. Laufer and H. Greenwood, Am. Zool. *9*, 603 (1969).
[2] H. Laufer and T. K. H. Holt, J. exp. Zool. *173*, 341 (1970).
[3] Larvae of these 2 species could not be separated from one another. The species were kindly identified by Dr. J. Knoz of the University in Brno.
[4] The juvenoids were obtained through the courtesy of the Institute of Organic Chemistry and Biochemistry, ČSAV, Prague, and of the Zoecon Corporation, Palo Alto, California. The compounds were racemic mixtures; the aliphatic substances contained about $^2/_3$ of the 2-*trans* isomers.
[5] F. Sehnal and J. Žďárek, J. Insect Physiol., in print (1975).
[6] A. Spielman and V. Skaff, J. Insect Physiol. *13*, 1087 (1967).



Different effects produced by juvenoids applied to the last instar larvae of *Chironomus annularis* and *Ch. dorsalis* (undistinguished); A) effect 4 (larval-like form showing considerable differentiation of wings and appendages but failing to ecdyse from the last larval exuvia); B) and C) effect 3 (forms intermediate between larva and pupa with partly ecdyzed pupal-like thorax); D) and E) effect 2 (intermediates with pupal-like head and thorax and larval-like abdomen); F) effect 1 (pupal-like form with distorted wings and appendages and with remnants of larval gills and anal papillae.

on different insects was studied by several authors, is more active on mosquitoes than on chironomids. The unformulated compound affects 50% of treated mosquito larvae at the concentration equal to or lower than 0.0001 ppm[7,8] but 50% of treated chironomid larvae at concentrations 0.01 ppm (*Chironomus stigmaterus*)[9], 0.05 ppm (*Ch. stigmaterus* and *Tanypus grodhausi*)[10], and 0.1 ppm (present study), respectively. Field application of formulated compound II against mosquitoes reportedly caused only a few chironomids to die[9]. Hence, midges should not be seriously endangered by the use of compound II against mosquitoes providing that the minimal concentrations effective on mosquitoes are used.

Zusammenfassung. Applikation aktiver Juvenoidverbindungen während des letzten Larvenstadiums von *Chironomus annularis* Meig. und *Ch. dorsalis* Meig. verursachte die Entwicklung von Übergangsformen zwischen Larve und Puppe. Der formulierte 3,7,11-Trimethyldodeca-2,4-dien-Carbonsäure-Isopropylester beeinflusste 50% der Tiere bei einer Konzentration von 0,001 ppm.

I. GELBIČ and F. SEHNAL[11]

Entomological Institute, Czechoslovak Academy of Sciences, Viničná 7, CS-12800 Praha 2 (Czechoslovakia), 26 April 1974.

[7] C. H. SCHAEFER and W. H. WILDER, J. econ. Ent. *65*, 1066 (1972).
[8] C. H. SCHAEFER and W. H. WILDER, J. econ. Ent. *66*, 913 (1973).
[9] T. MIURA and R. M. TAKAHASHI, J. econ. Ent. *66*, 917 (1973).
[10] R. L. NORLAND, Proc. 41st Ann. Conf. of Calif. Mosquito Control Ass. (1973), p. 118.
[11] Critical reading of the manuscript by Prof. Dr. W. LOHER is gratefully acknowledged.

Mineralogical Differences in Populations of *Thais lapillus* Linné (Gastropoda: Prosobranchiata)[1]

Bivalve mineralogy has been shown to be influenced by the external environment[2–4]. But, conflicting evidences have also been presented by EISMA[5] and KENNEDY, TAYLOR and HALL[6] who believe that the primary control is 'clearly genetic'; KOBAYASHI's finding[7] that crystal structures in bivalves depend on the number of protein fractions in the extrapallial fluid, tends to support this. However, it has been pointed out by WISE[8] that statements concerning bivalve mineralogy may not be applicable to gastropods.

In this study, the polymorphic forms of calcium carbonate in the shells of *Thais lapillus* from 5 North American populations were analyzed as part of an overall effort designed to understand intraspecific variations in this species. Relative amounts of calcite versus aragonite expressed as mean ratios were used to compare the populations.

Materials and methods. The populations studied were obtained from Acadia Park, Owl's Head, Pemaquid Point and Cape Elizabeth, all in Maine, and Watch Hill, Rhode Island. These collecting sites range from latitude 44.20 *N* to 41.18 *N*.

Clean shells were initially measured (maximum height), then cracked open to remove soft parts, and ground individually by hand in a ceramic mortar to obtain a fine powder. The powder was then thinly smeared on clean glass slides with the aid of acetone and a glass rod. A total of 60 shells, each prepared on a separate slide, was examined. All samples were processed within 24 h after the snails were sacrificed.

A GE XRD-5 X-ray diffraction unit with a recorder and goniometer was used for determining the calcite/aragonite ratio. The apparatus consisted of a 1° beam slit and an 0.2° pick-up slit. A single nickel filter with copper radiation was employed. The apparatus was operated at 50 kv and 20 mA. Goniometer speed was set for 2 degrees per min for all runs. The intensities of the X-ray reflections were recorded on a chart using either 2000 or 5000 γ-radiation counts per sec, depending on the quantities presented in any given sample. Samples were scanned between 26° and 32° only, since this was sufficient to produce major calcite and aragonite peaks.

Calcite/aragonite ratios were calculated for each sample by dividing the area under the major calcite peak (3.03 Å) by the area under the major aragonite peak (3.40 Å). By using the ratio in this manner, the problems resulting from variation in absolute amounts of the minerals from one sample to the next are eliminated. Also, as a check, 2 slides were prepared from the same powdered shell. The ratios obtained (5.38 and 5.40) showed excellent agreement. The variation appears to be well within the limit of less than 4% errors in measurement using X-ray diffraction[9].

Results. Calcite/aragonite ratios obtained from analysis of the 5 populations are listed in the Table. Two different samples from Owl's Head were used. The first contained small, thin-shelled immatures, the second contained large, thick-shelled adults. These 2 groups were tested in an attempt to determine the influence of shell size, thickness, and maturity on the calcite/aragonite ratio. Since comparison of the means showed that there was no significant difference between the two series ($p < 0.1$), they were combined and treated as a single group. A Student-Newman-Keuls Test[10] was used to compare the mean ratios of the 5 populations (Table). The difference in the calcite/aragonite ratio of the shells from either Watch Hill or Owl's Head was highly significant when compared with the values obtained for the Cape Elizabeth, Acadia Park or Pemaquid Point populations ($p < 0.05$). The 3 populations of small-sized snails could not be separated on the baiss of these data, nor could the 2 populations of larger-sized snails.

[1] Contribution No. 98 from Marine Research Laboratory, University of Connecticut. Part of a thesis submitted to the Graduate Faculty of the University of Connecticut by the senior author in partial fulfillment of the requirements for the degree of Master of Science.
[2] H. A. LOWENSTAM, J. Geol. *62*, 284 (1954).
[3] J. R. DODD, J. Paleont. *38*, 1065 (1964).
[4] Y. KITANO, N. KANAMORI and A. TOKUYAMA, Am. Zool. *9*, 681 (1969).
[5] D. EISMA, J. Geol. *74*, 89 (1966).
[6] W. J. KENNEDY, J. D. TAYLOR and A. HALL, Biol. Rev. *44*, 499 (1969).
[7] S. KOBAYASHI, Biol. Bull. *126*, 414 (1964).
[8] S. W. WISE JR., Science *167*, 1486 (1970).
[9] J. BOUILLON, Annls Soc. r. zool. Belg. *89*, 229 (1958).
[10] R. R. SOKAL and F. J. ROHLF, *Biometry* (W. H. Freeman & Co., San Francisco, Calif. 1969), p. 776.
[11] R. L. PYLE, *Serial Atlas of the Marine Environment* (Ed. W. WEBSTER; Am. Geogr. Soc., N. Y. 1962), folio I, plates 2–13.