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Divergent male androgen patterns in two sympatric species of *Leptodactylus* from subtropical South America

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Abstract. *Leptodactylus ocellatus* L. is sympatric with *L. chaquensis* Cei on the banks of the Parana river, yet does not show evident gametogenic discontinuity or cyclical variation of secondary sex characters compared with the striking and well-defined seasonal rhythm found in *L. chaquensis*. The endocrine mechanisms regulating the seasonal reproductive cycle in the male of both species in their sympatric area have been studied through assessment of plasma androgens, related to the morphological examination of testis. A sudden intense spermatogenic activity was recorded in the testes of *L. chaquensis* in spring months (September-October), but this ceased in late November, and was followed by a strikingly long summer rest. Conversely, few morphological changes in *L. ocellatus* were found, since the mating period is more irregular and extensive. The androgen plasma changes paralleled the morphological observations. The plasma androgens behaved differently in the two species, since in *L. chaquensis* the androgen peak values occurred at the end of the intense spermatogenic activity, accompanied by dramatic increase of testicular weight and spermiation, while in *L. ocellatus*, the highest androgen plasma levels, occurring in August, seemed to indicate a precocious reproductive activity in this species. These data are discussed, in view of the pivotal role played by androgens in regulating the discontinuous reproductive cycle of *L. chaquensis*.

Introduction

The genus *Leptodactylus* is widespread in mesic and xeric environments of subtropical and tropical southern South America: its remarkable evolutionary differentiation is shown by the several species groups found throughout the whole area of its neotropical distribution (Cei et al., 1967). A general report on different physiological thresholds to high temperatures and the specific sensitivity of the pituitary and germinal epithelia in *Leptodactylus ocellatus* L. and *Leptodactylus chaquensis* Cei from northern and northeastern Argentina was given and discussed several years ago (Cei, 1980).

The physiological peculiarities of the reproductive cycle have been the first kind of evidence leading to the biological comparative screening of the “sibling” species of the *Leptodactylus* complex of the “pachypus” group. *Leptodactylus ocellatus* is sympatric with *L. chaquensis* on the banks of the Parana river, and does not show evident gametogenic discontinuity or cyclical variation of secondary sex characters, compared with the striking and well-defined seasonal rhythm found in *L. chaquensis* (Cei, 1948, 1949, 1950). A remarkable differentiation of annual gametogenic activity has been demonstrated in the xerophilous *L. chaquensis* from the Chaco area in northwestern Argentina, the male showing sudden intense spermatogenic activity in the spring months (September-October) which ceases in late November, followed by a strikingly long summer rest. Conversely, few seasonal variations in the reproductive organs are found in *L. ocellatus*, since its mating period is more irregular and extensive; the size and shape of its testes appear approximately unchanged the whole year long, on contrast to the situation in *L. chaquensis* (Cei, 1948).

Leptodactylus ocellatus and *chaquensis* usually live in allopatric ranges, but a narrow zone of sympatry is known, alongside the Parana river and in the internal swamps of Corrientes, Argentina. *L. chaquensis* is a very competitive species, often overcoming the closely related *L. ocellatus*, when accidental interventions or ecological changes may favor its peculiar trends or adaptability.

In order to have a better understanding of endocrine function related to the male reproductive cycle of both species, particularly in their sympatric area, changes in plasma androgens have been evaluated in relation to morphological examination of the testes.

Materials and methods

Animals

Wild populations of *L. chaquensis* and *L. ocellatus* were studied alongside the Parana river and in the internal swamps of Corrientes, Argentina. Ten males of *L. chaquensis* and ten of *L. ocellatus* were captured at intervals over two years (1989-1990 and 1991-1992) during the main representative phase of their annual reproductive cycle. Each animal was anaesthetized with 3-amino benzoic acid ethyl ester (Sigma, St. Louis, MO; 10 g/l tap water) within 5 min after capture, and blood was immediately collected in a heparinized syringe by cardiac puncture. Blood samples were stored in ice until processed; after centrifugation, plasma was frozen on dry ice and stored at -70°C until assay. Each animal was weighed and the testes were removed and weighed.

Histological procedures

The testes were immersed in Bouin's fixative, dehydrated with ethanol, and subsequently embedded in paraffin in a vacuum stove. Seven-micrometer sections were stained with

hematoxylin and eosin. The inserts of figs 1 and 2 represent reproductions made from the original slides.

Plasma hormone determinations

Plasma samples, taken from the males, were extracted with ether; subsequently, radio-immunological analyses (RIA) of androgens were carried out as previously described by Polzonetti-Magni et al. (1984); a sensitivity of 5 pg (intraassay variation, 5.5%; interassay variation, 10%) was observed.

Steroid antiserum was provided by Dr. G. Bolelli (Physiopathology of Reproduction Service, University of Bologna, Italy); tritium-labelled steroid was purchased from Amersham International (Buckinghamshire, England) and authentic steroids were obtained from Sigma. The testosterone antibody cross-reacted (> 80%) with 5 α -dihydrotestosterone and, since the two steroids were not separated, the data are expressed as "androgens".

Statistical analysis

Results were analyzed by ANOVA with a statistical software package, Stat View 512+™ (Brain Power Inc., USA), operating on a Macintosh Plus computer (Apple, USA). A probability level of < 0.05 was taken to indicate a statistically significant difference between means. Results are expressed as mean \pm SD of data from ten replicates.

Results

Figure 1 shows the annual trend of androgens in *L. chaquensis* correlated with the most significant changes of testicular morphology. Plasma androgen levels were low from April until October, when they suddenly increased significantly ($P < 0.01$) reaching peak values (4.5 ng/ml) in November, concomitant with the mating period. After that, androgen levels decreased significantly ($P < 0.01$), reaching baseline levels (0.5 ng/ml) in the fall months. The morphological changes in the testes were consistent with the presence of only primary spermatogonia in the epithelium of the reduced tubules. In early spring, a great number of groups of secondary spermatogonia and all the following stages of gametogenic processes were shown, soon followed by large numbers of sperm bundles in dilated tubules in November. The summer rest was characterized by release of the residual of a new spermatogenesis, which soon proved abortive. In *L. ocellatus* (fig. 2), baseline levels (also about 0.5 ng/ml) of plasma androgens were found from October until July; after that, they suddenly increased ($P < 0.01$) to a high value in August (5.7 ng/ml). They had decreased to baseline level by the next sampling period in October. Testicular activity did not change as evidenced by the picture of the testis taken in the fall-winter and summer months.

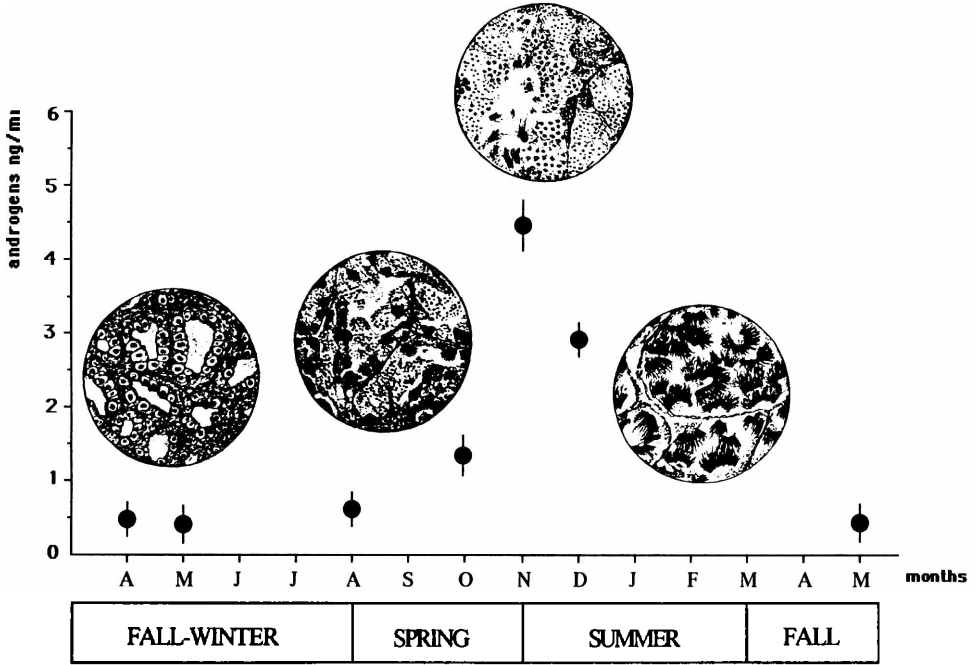


Figure 1. Seasonal variations of plasma androgens levels and cyclical changes of testis in *Leptodactylus chaquensis*. Each point is the mean of 10 determinations \pm SD.

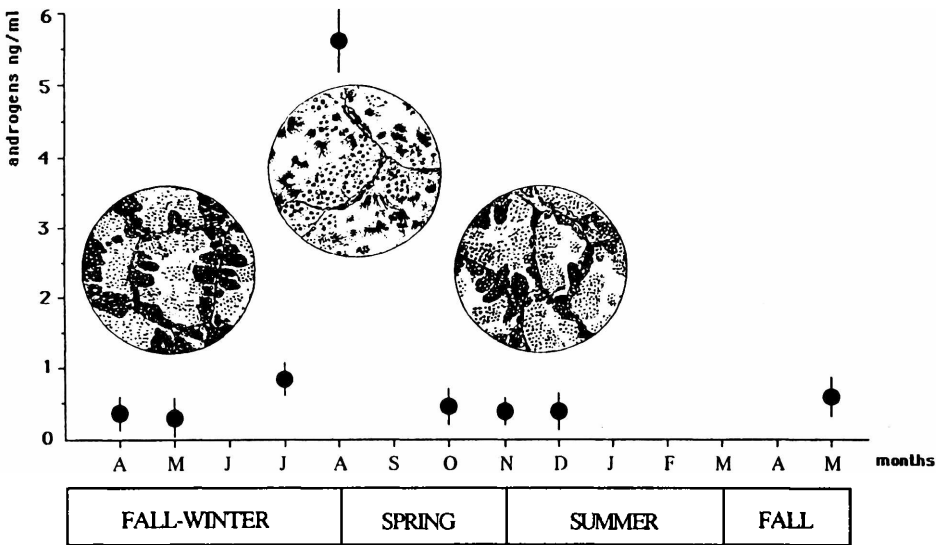


Figure 2. Seasonal variations of plasma androgens levels and cyclical changes of testis in *Leptodactylus ocellatus*. Each point is the mean of 10 determinations \pm SD.

Discussion

This study aimed to stress the divergent androgen patterns in the males of the two sympatric species. The influence of internal and external factors in the regulation of hypophysial and gonadal activity must be very different in the two species. Comparative observations carried out by Rengel (1950) on specimens of both forms kept at a temperature varying from 31°C to 36°C demonstrated that spermatogenetic activity of *L. chaquensis* exposed to this range of temperature ceased completely after 20 days; conversely, an increase of temperature in the same period did not appear to affect gametogenesis of *L. ocellatus*.

As previously demonstrated by Cei (1948), a striking seasonal rhythm in the development of testicular function was shown in *L. chaquensis*, together with a dramatic growth and reduction of some skeletal bones (humerus), and alternate seasonal variations of serum calcium values in parallel with the morphological changes in the testes. *Leptodactylus ocellatus* living in the same area, on the other hand, did not show evident discontinuity or cyclic variations in testicular osteoclastic activity, or in seasonal calcium values (Choen, 1962a, b, 1963).

The peak androgen values in *L. chaquensis* occur at the end of intense spermatogenetic activity, accompanied by a dramatic increase of testicular weight and spermiation. The reduction in spermatogenetic activity in the summer months is in agreement with the low levels of plasma androgens. The highest androgens plasma levels, which occur in *L. ocellatus* in August, seem to indicate a precocious reproductive activity in this species.

Taken together, these findings suggest that the plasma androgen profiles in male *Leptodactylus* living in the same area complement and supplement the knowledge about their divergent reproductive strategies. These different strategies were already described by Cei (1980), on the basis of ecological and ethological observation, for areas in which the two species are allopatric. The physiological role played by androgens in these species seems to be very similar to that demonstrated in other anuran species living in temperate areas, in which high androgen plasma levels have been found to be correlated with the regulation of testicular activity and development of secondary sexual characters in the seasonally anuran breeders (Polzonetti-Magni et al., 1984; Lofts, 1987).

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