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Changes in Plasma Sex Steroids in Females of Two Sympatric *Leptodactylus* from Subtropical South America

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Leptodactylus ocellatus is a well-known neotropical anuran in Argentina and Brazil, typically inhabiting marshes and streams, from sea level to 1000–1200 m in the Pampean mountains.

Leptodactylus ocellatus is sympatric with *L. chaquensis* in Paraguay and on the Parana borders. Previous work (Cei, 1948, 1949, 1950, 1980; Cei et al., 1955) with these two sibling species, *L. ocellatus* and *L. chaquensis*, revealed diversity in their reproductive cycles. *Leptodactylus ocellatus* exhibits less gametogenetic discontinuity and variety of secondary sex characters; the annual ovarian cycle of *L. chaquensis*, involves a fall-winter period of atresia for the residual mature oocytes from the former cycle, and at the same time a progressive auxocytic activity. The spring-summer period of mating and egg-laying of *L. chaquensis* is followed by resorption of retained oocytes. Moreover, in both sexes, a striking seasonal rhythm in the development of secondary sex characters has been found, together with dramatic growth of skeletal humeri and significant variation in serum calcium values (Cohen 1962a, b, 1963).

Endocrine regulatory mechanisms involved in reproduction in these species are unknown. We assessed the plasma sex steroid changes during the pre-reproductive and reproductive periods. In females of both species living in sympatry, plasma androgens, estradiol-17β, and progesterone were assessed in relation with the ovarian changes, evaluated through the gonadosomatic index (GSI).

Wild populations of *L. chaquensis* and *L. ocellatus* were studied alongside the Parana river in the Laguna Brava swamps of Corrientes, Argentina. In this zone and in other areas of sympatry, the mean monthly temperature in winter does not fall below 16 C, while in summer months it is about 26 C. The mean rainfall is 80–100 mm in the driest months, and 150–210 mm in the wettest months (September–October and April–May, respectively). The climate in the riparian Parana environment is mild and relatively moist.

Ten females of both species were captured at each sampling at intervals over two years (1989–1990 and 1991–1992) during their pre-reproductive and reproductive periods. Each animal was anesthetized with 3-amino benzoic acid ethyl ester (Sigma, St. Louis, MO; 10g/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 ovaries were removed and weighed. The gonadosomatic indexes (GSI) were calculated as a ratio of gonadal weight to body weight.

Plasma Hormone Determinations.—Plasma samples from 3 or 4 individuals randomly assigned to each batch of assays, were extracted with ether. Subsequently, radioimmunological analyses (RIA) of androgens, estradiol-17β, and progesterone, were carried out in duplicate as described by Polzonetti-Magni et al. (1984).

The following sensitivities were observed: testosterone 5 pg (intra- and interassay coefficients of variation were 5.5% and 10%, respectively); estradiol-17β, 7 pg (intra- and interassay coefficients of variation were 4.5% and 7.5%, respectively); and progesterone, 7 pg (intra- and interassay coefficients of variation were 4% and 8%, respectively). Steroid antisera were provided by Dr. G. Bolelli (Physiopathology of Reproduction Service, University of Bologna, Italy); tritium-labelled steroids were purchased from Amersham International (Buckinghamshire, England) and authentic steroids were obtained from Sigma. The testosterone antibody was cross-reacted (>80%) with 5α-dihydrotestosterone and since the two steroids were not separated data are expressed as "androgens."

Statistical Analysis.—Plasma hormone data were analyzed by one-way analysis of variance (ANOVA) using Stat View 512+® (Brain Power Inc., USA). A probability level of 0.05 was taken to indicate a statistically significant difference between means. Results are expressed as mean ± SD of data from ten different female samples.

Baseline androgen levels were found over the summer and fall-winter months in *L. chaquensis*, and then peaked ($F_{1,18} = 466.78$; $P < 0.05$) at about 9 ng/ml in November, during the spring spawning season (Fig. 1). In *L. ocellatus*, the androgen trend was similar to that of *L. chaquensis*, but the androgen peak ($F_{1,18} = 275.31$; $P < 0.05$) values (8.5 ng/ml) occurred in early spring (August). In *L. chaquensis* estradiol-17β levels of the three samples in the spring were significantly higher ($P < 0.05$) than those in fall-winter and the summer (Fig. 2). The GSI behaved similarly to the estradiol-17β, showing the highest values in November ($F_{1,18} = 37.09$; $P < 0.05$). In *L. ocellatus*, the lowest levels of estradiol-17β were found only in November and significantly higher ($F_{1,18} = 32.40$; $P < 0.05$) peak values of about 1000 pg/ml were found in August at the beginning of spring. Again, in *L. ocellatus* the GSI paralleled estradiol-17β, reaching its zenith in August. In *L. chaquensis*, increasing plasma titers of progesterone were found in the fall-winter months reaching peak ($F_{1,18} = 12.64$; $P < 0.05$) values (1800 pg/ml, Fig. 3) at the beginning of the spring spawning season. On the contrary, no significant changes of plasma progesterone were observed in *L. ocellatus*.

Ecologically, *L. ocellatus* and *L. chaquensis* are active and vigorous frogs, feeding on a number of small vertebrates and arthropods. Remarkable develop-

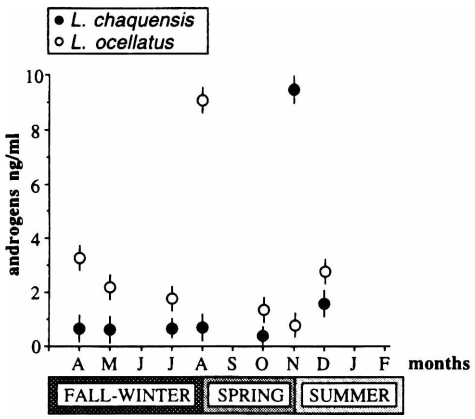

FIG. 1. Seasonal variations of plasma androgens levels in female *Leptodactylus chaquensis* and *Leptodactilus ocellatus*. Each point is the mean of 10 determinations ± SD.

ment of sex characters of males in both species has been reported (Cei, 1980). During the breeding season eggs are laid in foam nests on the surface of the water, usually in shallow ponds or lagoons. Given the sheltering effects of the foam nest, eggs and developing larvae are suitably protected from desiccation, and likely from many predators, such as water insects, carnivorous tadpoles, or snakes. Tadpoles of both species are gregarious and peculiar parental care was reported (Cei, 1980). Some differences exist in their larval morphology: a larger tail in *L. ocellatus*, a more rounded snout in *L. chaquensis*, and different location of the sinistral spiracle.

Plasma sex steroids showed the highest values during the spring spawning season (August–November). However, in *L. ocellatus* androgen peak values occurred in early August, while in *L. chaquensis* they occurred in November. Moreover, in *L. chaquensis*, estradiol-17β was lowest in summer-winter months, and was highest in the spring spawning season (August–November). Such a striking difference was not recorded for *L. ocellatus*, in which a slight but significant fluctuation in estradiol-17β plasma levels was found between spring and winter months. The clear-cut seasonal pattern of the estradiol-17β plasma level in *L. chaquensis* may physiologically support the outstanding seasonal ovarian changes (GSI) found for these frogs. Similarly the GSI for *L. ocellatus* is in agreement with the above-reported annual trend of the estradiol-17β plasma level. Finally, throughout the whole year the progesterone levels in *L. ocellatus* fluctuated between 850 and 1500 pg/ml, while those in *L. chaquensis* peaked in August, the other seasonal values being low.

These findings suggest a difference in reproductive physiology that both species maintain under similar environmental conditions. Even though now sympatric on the Parana borders, *L. chaquensis* and *L. ocellatus* may have evolved from allopatric ancestors in distinctly different ecological settings. Extreme continental thermic conditions led in *L. chaquensis* to an autonomous or internal rhythm. Instead, more equable climatic parameters in the wider continental distribution of *L. ocellatus* (from 10° to 40° South Lat.) seem to have been effective in determining a more irregular and extensive mating period (Cei, 1980).

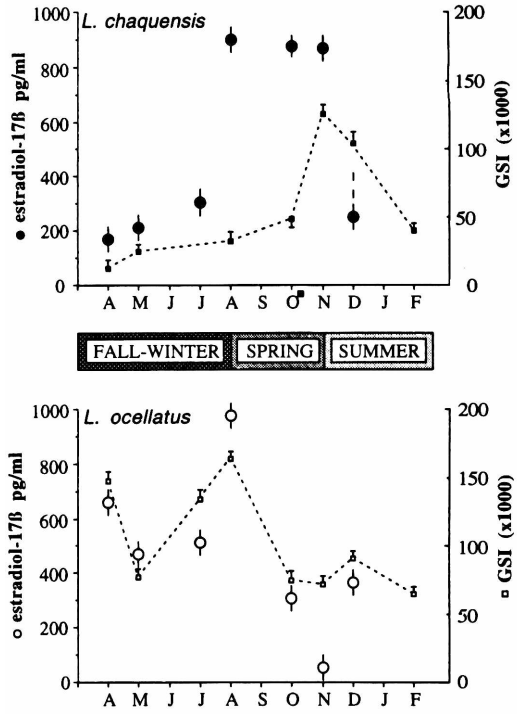

FIG. 2. Seasonal variations of gonadosomatic index (GSI) and plasma estradiol-17β levels in female *Leptodactylus chaquensis* and *Leptodactylus ocellatus*. Each point is the mean of 10 determinations ± SD.

The hypothalamus-hypophysial-gonadal axis relationship, well ascertained in other amphibians living both in temperate and tropical areas (Chieffi and Pier-

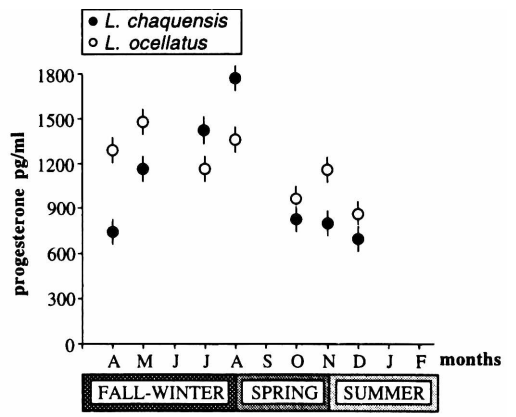

FIG. 3. Seasonal variations of plasma progesterone levels in female *Leptodactylus chaquensis* and *Leptodactylus ocellatus*. Each point is the mean of 10 determinations ± SD.

antoni, 1987; Rastogi and Iela, 1994), presumably regulates sex steroids in both *Leptodactylus*. In fact, androgens in female anurans seem to play a role, as in males, in inducing reproductive behavior, and are a source of estradiol-17β through aromatization (Chieffi and Pierantoni, 1987; Dubowsky and Smalley, 1993). The estradiol-17β increase, related to ovarian development and growth, seems to be involved in inducing liver vitellogenin synthesis. Vitellogenin synthesis is a hormonally controlled process, and vitellogenin synthesized by the liver is a precursor molecule of the yolk proteins, lipovitellin and phosvitin in all oviparous vertebrates so far studied (Wallace, 1985; Carnevali et al., 1994). Moreover, the increasing values of progesterone found in *L. chaquensis* during the spawning season may play a role in ovulation, as in other amphibians (Jones, 1987).

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