

REPRODUCTION IN A SPANISH POPULATION OF
ACANTHODACTYLUS ERYTHRURUS
(REPTILIA: LACERTILIA: LACERTIDAE)STEPHEN D. BUSACK^{1,2}

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ABSTRACT

Reproduction in male *Acanthodactylus erythrurus* from La Algaida, Cádiz Province, Spain, is characterized by spring spermiogenesis, copulation between June and August, and post-mating spermatogenesis that begins shortly after the testes are evacuated and continues through the fall. Vitellogenic growth of ovarian follicles begins in the spring, prior to mating. Vitellogenesis proceeds for a period of about one to two months, oviductal eggs are first seen in early June, and hatchlings first appear during August. Clutch size ranges between one and five ($\bar{x} = 3$) and larger females tend to produce larger clutches. No direct evidence for multiple clutches was found.

INTRODUCTION

Several aspects of the ecology of *Acanthodactylus erythrurus* at La Algaida, Spain, have been described (Busack, 1976; Pough and Busack, 1978; Busack and Jaksić, 1982), but no reproductive data have been published. We undertook this study of reproduction in the population to fill this gap in our knowledge of its life-history, and to compare reproductive parameters with those of the Moroccan population of this species (Bons, 1962, 1963, 1964, 1967, 1969, 1972).

MATERIALS AND METHODS

Specimens were collected from La Algaida (25.5 km [AIR] NW Jerez de la Frontera, Cádiz Province, Spain) between May 1970 and November 1971 (see Busack and Jaksić, 1982, for details). Each individual was preserved immediately in 10% formalin and later transferred to 70% ethyl alcohol for permanent storage. Busack and Jaksić (1982) determined that all males ≥ 61 mm snout-vent length (SVL), and all females ≥ 57 mm SVL, in this population were adult; only males and females of these SVLs or larger were used to assess seasonal reproductive changes.

Testes from individuals collected from March through October were prepared following procedures described by Berg (1953), sectioned, and examined microscopically for the cellular stages of spermatogenesis. Length of the left testis was measured *in situ* with an ocular micrometer, and testis volume in 140 adult males was determined by fluid displacement. Seminiferous tubule diameter was measured in 15 adult males collected from March–October, and the diameters of up to 30 tubule cross-sections were used to calculate average tubule diameter. We did not examine efferent ducts or epididymides for the presence of spermatozoa. In this species spermatozoa appear to pass rapidly through these structures (Bons, 1969), thus testis condition serves as a reliable indicator of the timing of mating.

Ovarian follicles were measured *in situ* with an ocular micrometer in 95 adult females. Larger, slightly elongated, follicles were measured along the axis of elongation, and follicles in which the

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diameter was ≥ 0.5 mm were recorded. The lengths of oviductal eggs were measured *in situ* with dial calipers accurate to 0.1 mm.

Estimates of clutch size are usually obtained by counting growing or preovulatory follicles present in ovaries during the breeding season (Fitch, 1970). Degeneration of large follicles has been observed in *A. erythrurus* (Bons, 1964) but atretic follicles were not conspicuous in females containing large yolky eggs examined during this study. By assuming that all vitellogenic follicles with a diameter ≥ 3 mm would be ovulated in a single clutch, we obtained an estimate of clutch size. Numbers of oviductal eggs and corpora lutea provided additional clutch size data.

Spearman rank correlation procedures were used to assess the relationship between lizard SVL and the number of developing follicles, corpora lutea, and oviductal eggs present. Resulting values of r_s were transformed according to Fisher's z transformation procedures and the probabilities presented are those for committing a Type I error in a two-tailed test.

Specimens examined.—The Carnegie Museum of Natural History (CM) 53245, 53282–53288, 53330, 53333, 53350, 53373, 53383, 53397, 53425, 53438, 53471, 53885–53886, 53888–53890, 53916, 54218–54220, 54223, 54225, 54256–54257, 54277, 54279, 54561, 54565, 54592, 54594, 54676, 54789, 54794, 54873, 55305–55328, 55337–55341, 55474, 55659, and 55699–55701.

RESULTS

Male Reproductive Cycle

Changes in both testis size and spermatogenic activity indicate a reproductive period from April to July. Testis length and volume reach a maximum between April and June and dramatically decrease during July. A gradual increase in size begins again in August and September and continues through October. By October, mean testis size had not yet reached that seen in the earliest spring collections (March), so we infer that additional testis growth took place during the winter when adults were inactive (Busack, 1976). Fig. 1 illustrates changes in testicular length and volume over the collecting period.

Histological examination confirmed that annual fluctuation in testis size and changes in diameter of seminiferous tubules are correlated with spermatogenic activity (Fig. 2). Maximal development of germinal epithelium and maximum tubule diameter occur from May through June. Seminiferous tubules contain several layers of primary spermatocytes in various stages of prophase, as well as numerous spermatids and spermatozoa (Fig. 2B), and spermatozoa are found only in tubule luminae during these months. Marked reductions in the height of the germinal epithelium and tubule diameter coincide with decrease in testis size in July, and seminiferous tubules often contain cellular debris (possibly the result of recent evacuation of spermatozoa) within the lumen. In most specimens the epithelium consists predominantly of a basal layer of spermatogonia and Sertoli cells (Fig. 2C). In a few specimens in which the tubule lumina contained cellular debris during June, primary spermatocytes were also abundant.

Proliferation of spermatogonia and development of primary spermatocytes occurs again during August. Although some August specimens contain only spermatogonia and Sertoli cells, others undergo active spermatogenesis (Fig. 2D). During the remaining months of the year, numbers of spermatogonia and spermatocytes present in seminiferous tubules increase. Large primary spermatocytes typically are distributed in basal clumps during September. Although some spermatocytes reach metaphase and telophase at this time, most spermatocytes are in earlier (leptotene and pachytene) stages of prophase during October. Although spermatogenesis resumes in some individuals as early as August, there are neither spermatozoa nor spermatids evident throughout the remainder of the year. The earliest appearance of spermatids is in April, just preceding the breeding season (Fig. 2A).

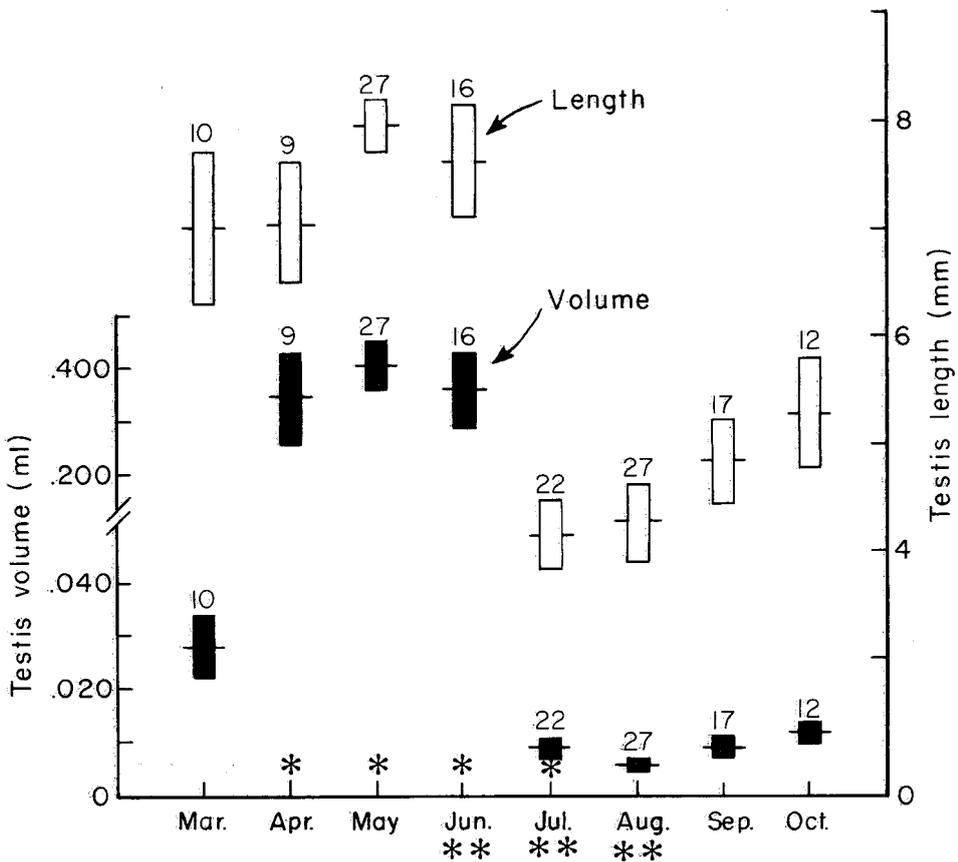


Fig. 1.—Seasonal changes in testis length and volume in adult male lizards. There were no statistically significant differences (Student's two-sample t-test) in testis length or volume between years, so monthly samples from both years are combined. Horizontal lines indicate monthly means, \pm two standard errors are indicated by the vertical bars, and sample sizes are indicated above the bars (* = months in which some individuals contained testicular spermatozoa, ** = months in which individuals showed evidence for the recent evacuation of spermatozoa).

Female Reproductive Cycle

Changes in maximum follicle size (Table 1) indicate a distinct seasonality in reproductive condition. Follicles measuring at least 2 mm in diameter are found during all months of the year in both ovaries of all adult females, and in most months 2 mm is the maximum size of any follicle (Table 1). Follicles larger than this are vitellogenic, and are found in 20 of 22 females collected between late April and early July. One to three vitellogenic follicles are distinct (2.4–4.5 mm) in each ovary by mid-May. By late May and June follicles markedly larger than all others are presumably destined for ovulation. The maximum diameter attained by a vitellogenic ovarian follicle is 9.5 mm, undoubtedly near size at ovulation. Vitellogenic follicles are absent in ovaries containing corpora lutea.

Because vitellogenic ovarian follicles appear in some individuals as early as 18 April, we infer that the period of vitellogenesis is about two months in duration.

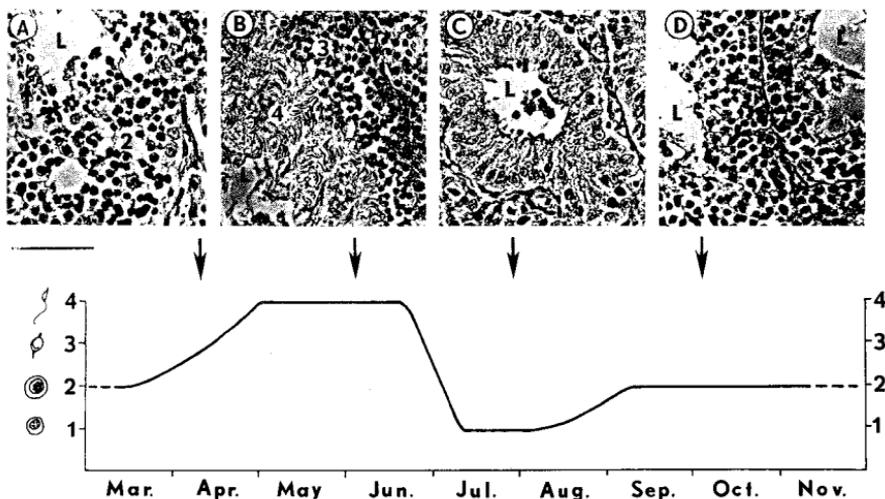


Fig. 2.—Seasonal changes in the seminiferous tubules of adult male *A. erythrurus*. Lower diagram illustrates the most differentiated stage of spermatogenesis in at least 50% of the individuals sampled. Numbered stages indicate the presence of: 1, spermatogonia and Sertoli cells only; 2, spermatogonia and primary spermatocytes; 3, developing spermatids in addition to the preceding cellular stages; 4, mature or nearly mature spermatozoa. Photomicrographs illustrate the appearance of the germinal epithelium in representative individuals collected on the dates indicated by the arrows. Scale bar below panel A = 0.05 mm; L identifies tubule lumen.

Oviductal eggs were first seen on 19 June. Shelled oviductal eggs measuring between 11.5 and 14.8 mm were found on 19 July, and the first hatchling lizards (SVL ≤ 31 mm) were found between 13 and 30 August during both years.

We found preovulatory follicles in 22 individuals, oviductal eggs in 6, and corpora lutea in 7 individuals. Estimates of mean clutch size derived from these data are 2.7 (range 1–5), 3.0 (2–4), and 2.7 (1–4), respectively. Females in Spain lay from one to a maximum of five eggs per clutch and the average clutch is about 3 eggs.

The relationship between SVL and number of vitellogenic follicles demonstrates probable differences in reproductive potential for lizards of different sizes. When all follicles with a minimum diameter of ≥ 3 mm are considered presumptive eggs, a significant relationship between SVL and clutch size is identified ($z = 1.12$, $N = 22$, $P \ll 0.05$). The number of corpora lutea, which probably provides a more accurate estimate of clutch size, also shows a statistically significant correlation with SVL ($z = 2.03$, $N = 7$, $P \ll 0.05$). No significant correlation between SVL and the number of oviductal eggs, however, was identified ($z = 0.64$, $N = 6$, $P > 0.2$).

DISCUSSION

Reproduction in *A. erythrurus* at La Algaída occurs in late spring and summer. Although we did not observe mating in the field, the reproductive condition of both males and females indicates breeding capability between late May and early July. Seasonal changes in testis size (length and volume) and spermatogenic activity indicate a single copulatory period for males annually. Males begin reproductive activity (spermatozoa present in seminiferous tubules) during April, and

Table 1.—*Monthly summary of follicle development and the distribution of oviductal eggs and corpora lutea among adult female A. erythrurus at La Algaída, Spain.*

Month	Number of adult females in which maximum developing follicle diameter (mm) was:					Number of oviductal eggs per individual			Number of corpora lutea per individual			
	1	2	3	4	≥5	2	3	4	1	2	3	4
Mar	—	4	—	—	—	—	—	—	—	—	—	—
Apr	—	14	1	—	—	—	—	—	—	—	—	—
May	—	2	—	4	5	—	—	—	—	—	—	—
Jun	—	—	4	4	3	—	4	1	—	2	1	1
Jul	3	5	—	1	—	1	—	—	1	—	—	1
Aug	14	6	—	—	—	—	—	—	—	—	1	—
Sep	3	6	—	—	—	—	—	—	—	—	—	—
Oct	3	11	—	—	—	—	—	—	—	—	—	—
Nov	—	2	—	—	—	—	—	—	—	—	—	—

reproductively capable individuals are present each month from April through mid-June. The subsequent sharp decrease in testis size and the absence of spermatozoa suggest that the majority of males copulate during June and early July. The gradual increase in overall testis size (Fig. 1), as well as the presence and proliferation of early germinal stages (spermatogonia and primary spermatocytes; Fig. 2), from August to October show that spermatogenesis resumes shortly after copulation. Ovarian follicles undergoing vitellogenic growth (≥ 2 mm diameter) first appear in mid-April. Between April and July all females (but two) of adult size contained vitellogenic follicles (generally two per ovary) in both ovaries, indicating that virtually all adult females reproduce each season.

Moroccan and Spanish male *A. erythrurus* show similar cycles of reproductive maturation, evacuation of testes, and spermatogenesis. Based on changes in the mean number of spermatids and spermatozoa in the seminiferous tubules of individuals collected during a single breeding season, Bons (1969) suggested that individual males in Morocco experience two waves of sperm production per season. Histological examination does not confirm a similar phenomenon in males from Spain. Testes from a few individuals in our study did contain, in addition to cellular debris indicative of recent evacuation of spermatozoa, numerous primary spermatocytes in the seminiferous tubules. Whether these spermatocytes continue to develop into a second "wave" of spermatozoa in the same season, or simply degenerate, is unknown. The most notable difference between these two populations concerns the period during which reproductively mature males are present. This period is apparently shorter, but is followed by earlier resumption of spermatogenesis, in Spain.

Vitellogenesis and ovulation likewise occur over the same timespan in the two populations. Bons (1972) suggests two phases of vitellogenic growth during a single breeding season for larger female *A. erythrurus* in Morocco. Older (i.e. larger) females produce two clutches each year and younger individuals produce only one. Evidence for the production of two clutches during a single season can be inferred by: (1) simultaneous presence in an ovary of vitellogenic follicles of two distinct size classes, (2) asymmetrical vitellogenic growth of follicles between ovaries (as in allochronic ovulation [Jones, 1978]), or (3) the presence of young corpora lutea in addition to vitellogenic follicles. None of these conditions was observed during our study. While we cannot rule out the possibility that second

clutches are deposited by Spanish females, our data do not favor this hypothesis over one which infers reproduction over an extended period.

Mature females in Morocco lay between two and six eggs in early clutches, but second clutches contain only one to three eggs (Bons, 1962). In Spain, mature females may produce as many as five eggs, the average clutch size is three eggs, and there is no direct evidence for the production of multiple clutches. Thus the Moroccan population produces more and (sometimes) larger clutches per female than the Spanish population.

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