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Cyclic variation of chiasma frequency and distribution in *Podarcis sicula* (Reptilia: Lacertidae)

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Abstract

The seasonal variations of chiasma frequency and distribution have been studied in the lizard *Podarcis sicula*. In this species, as in *Phyllodactylus* (King & Hayman, Chromosoma 69: 131–154, 1978), chiasma frequencies vary following a definite annual cycle, and clearly different trends are shown by interstitial and terminal chiasmata.

A comparison between these seasonal chiasma frequency variations and those of environmental temperature shows the existence of a clear correlation between these two parameters. However, this correlation is different in the two types of chiasmata, and may be different within the same type of chiasma depending on the period of the year.

A more significant correlation is observed between chiasma cycles and annual variations of the haematic levels of sexual steroid hormones. In particular we observe a highly significant correlation between interstitial chiasma frequencies and testosterone concentration. A less precise correlation between terminal chiasma frequencies and estradiol concentration is also observed.

In *Podarcis*, as in *Phyllodactylus*, the sperm that will be used for fertilization derive from the spermatocytes showing the highest rate of interstitial chiasmata. This supports the hypothesis that the cyclic variations in interstitial chiasma frequencies represent a mechanism to ensure an adequate level of variability in a given population. The above mentioned correlation between chiasma frequencies and steroid hormone concentrations suggests that the seasonal chiasma cycles are controlled by the same environmental and hormonal factors regulating the spermatogenetic cycle.

Introduction

The influence of environmental factors, and in particular of temperature, on the frequency and distribution of chiasmata has been known for long (Elliott, 1955; Shaw, 1971; King & Hayman, 1978), but the meaning of this phenomenon and the mechanism controlling it are still unclear.

The situation described by King and Hayman (1978) in the male gekkonid *Phyllodactylus marmoratus* is particularly interesting. This species shows an annual cyclic variation in chiasma frequencies, which may play an important role in the reproductive strategy of this species. In fact, this species shows a delayed fecundation, which takes place in autumn, but the sperm remain in the oviduct until the next spring, when amphimixis occurs. The sperms produced in spring are not used for fertilization and presumably are lost or degenerate (King, 1977). King and Hayman (1978) have examined terminal and interstitial chiasmata in autumn and spring spermatocytes-I and have found that the highest interstitial chiasma frequency is observed in autumn; hence they suggest that 'only sperms with the greatest range of interstitial chiasma frequencies are used for fertilization' by this species.

It was considered interesting to investigate annual variations in chiasma frequencies of another saurian, the lacertid *Podarcis sicula*, which belongs to a lineage which is considered to be quite distant from the gekkonids (Estes, 1983) and shows a trend in the spermatogenetic cycle completely different from that of *Phyllodactylus*. In fact, in *Podarcis* insemination takes place in spring and is immediately followed by amphimixis. Therefore, in this species it is spring sperms which are used for reproduction, whereas the autumn sperms degenerate (Angelini *et al.*, 1980; Angelini & Ghiara, 1984).

The main purpose of this investigation was to find out to what extent the correlation reported by King and Hayman (1978) is present in other saurians. Moreover, we also tried to collect indications on the mechanisms which may control possible cyclic variations in chiasma frequency. This has been attempted by comparing firstly the annual trend of the latter with the annual trend of environmental temperature; and secondly by reviewing the data so far collected by some of us on the spermatogenetic trend in *P. sicula*, on its correlations with environmental factors, as well as on the variations in hormone levels in the blood associated with it (Angelini *et al.*, 1980; Ciarcia *et al.*, 1986).

Material and methods

A total of 5 males of *Podarcis sicula* were collected at the end of each month, except August, from November 1982 to October 1983 in the neigbourhoods of Scafati (Salerno). They were sacrificed the day after their capture after being anaesthetized with ether or tricainemetasulphonate.

Male meiotic preparations were made from testes by the squashing method and were stained with acetolactic orcein. Every month a total of at least 20 diplotenes or diakineses from different animals were examined. Only complete meiotic plates (n = 19, Gorman, 1973) were taken in consideration. In each meiotic preparation we determined both interstitial and terminal chiasma frequency, which we expressed as number of chiasmata per bivalent (Ch/b).

A fragment of testes was used for evaluating the stage of the spermatogenesis. It was fixed with Stieve, included in paraffin-celloidine, and the sections of 7 μm were stained with Galgano's trichrome stain (Fig. 1).

Monthly average temperature was calculated starting from the daily values which were obtained from the 'Istituto Sperimentale dei Tabacchi, Scafati-Salerno'. The data on the status of the gonad during the various months of the year and on androgen and estrogen levels in the blood were taken from the investigations of Angelini and coworkers, some of which are still in progress (see Ciarcia *et al.*, 1986, for the methods used).

Results and discussion

Cyclic variations in chiasma frequency and their control

From the results obtained (see Table 1) it appears that in *Podarcis sicula*, as in *Phyllodactylus* (King & Hayman, 1978), the chiasma frequency varies following a definite annual cycle (Fig. 2A). The reliability of this cycle appears to be confirmed by statistical analysis of variability carried out by means of Snedecor's F test. This analysis shows that the differences in the frequency of total, terminal and interstitial chiasmata observed in the various months are significant and greater than the differences found among the animals investigated each month (see Table 2).

The difference in cyclic variation between interstitial and terminal chiasmata is more evident than in *Phyllodactylus*.

The interstitial chiasma frequency is minimal (0.8 Ch/b) in late November, then slowly increases until the end of February and very rapidly increases reaching its maximum value of 1.29 Ch/b in March. Afterwards, it decreases slowly until the end of June, when it shows a similar value to that of November; it shows a new but more limited increase in July and then decreases slowly in the following months.

In the species examined terminal chiasmata show a markedly lower average frequency than do interstitial chiasmata, and a trend contrasting with them. Their frequency shows a minimum of 0.42 Ch/b in February, increasing very slowly until late May and rapidly in June, when it reaches the maximum value of 0.72 Ch/b at the same time as interstitial chiasma frequency reaches one of the minimum values.

At the end of July the terminal chiasma frequen-

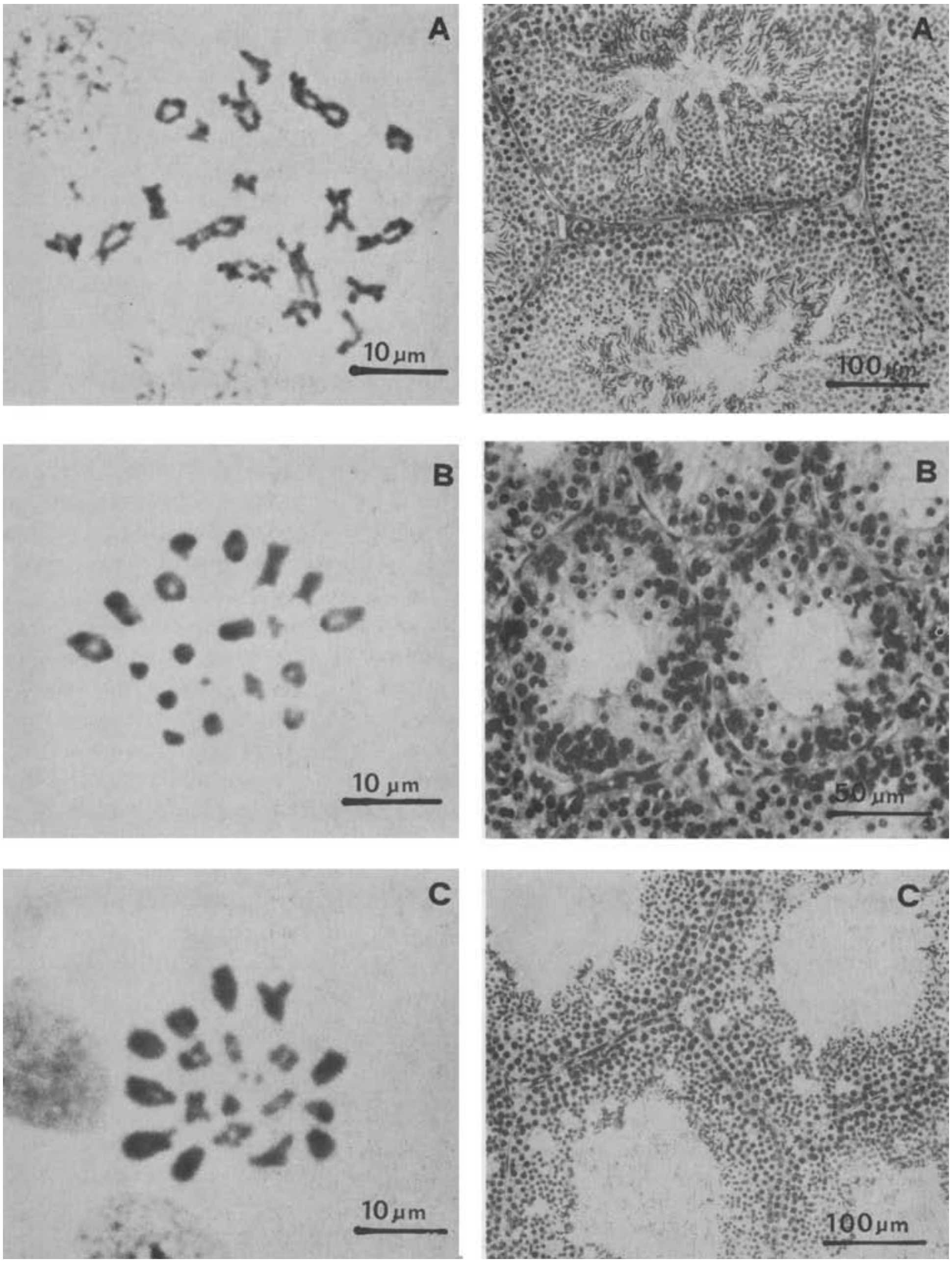

Fig. 1. Variations in chiasma frequencies (left) and in the stage of spermatogenesis (right) in *Podarcis sicula* in different periods of the year: (A) beginning of the reproductive period (late March), diplotene bivalents show a high level of interstitial chiasmata, in testis tubules all spermatogenetic stages, including many sperm, are present; – (B) shortly before the refractory period (late June), diplotene bivalents show a very low level of interstitial chiasmata; in the testis tubules only the early stages of spermatogenesis (spermatogonia and spermatocytes I) are present; – (C) autumn spermatogenesis (late September), in diplotene bivalents a low level of interstitial chiasmata, in the testis tubules only a few sperm are present, which will not be used for fertilization.

Table 1. Annual cyclic variations of total (Tot.ch.), terminal (Term.ch.) and interstitial (Int.ch.) chiasma frequencies and of environmental temperature (Temp.). S.E. = Standard error.

Month	Temp. °C	Tot. Ch. Ch/b	S.E.	Term. Ch. Ch/b	S.E.	Int. Ch. Ch/b	S.E.
November '82	12.5	1.33	±0.02	0.53	±0.04	0.80	±0.03
December '82	10.2	1.42	±0.03	0.57	±0.04	0.85	±0.04
January '83	8.1	1.42	±0.02	0.52	±0.02	0.90	±0.02
February '83	6.8	1.33	±0.04	0.42	±0.02	0.91	±0.04
March '83	10.4	1.77	±0.04	0.48	±0.05	1.29	±0.07
April '83	13.8	1.48	±0.03	0.52	±0.05	0.96	±0.05
May '83	17.1	1.48	±0.06	0.50	±0.04	0.98	±0.06
June '83	21.0	1.52	±0.03	0.72	±0.04	0.80	±0.04
July '83	25.9	1.47	±0.03	0.52	±0.04	0.95	±0.04
September '83	20.9	1.40	±0.03	0.53	±0.03	0.87	±0.04
October '83	16.2	1.42	±0.04	0.53	±0.03	0.89	±0.06

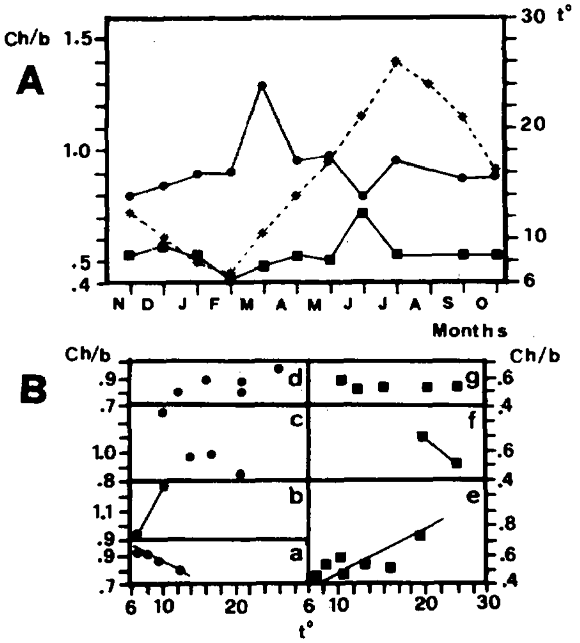

Fig. 2. (A) Annual variations in interstitial (dots) and terminal (squares) chiasma frequencies and in the monthly average temperature (asterisks). Abscissa: months; left ordinate: number of chiasmata per bivalent; right ordinate: temperature in °C; – (B) Comparison between temperature level (abscissa) and interstitial (left ordinate) and terminal (right ordinate) chiasma frequencies during different periods of the year: (a) November–February; (b) February–March; (c) March–June; (d) June–November; (e) December–June; (f) June–July; (g) July–December.

cy decreases rapidly, then it tends to become stable around the value of 0.5 Ch/b until December to decrease again until the minimum of late February.

These clearly different and sometimes contrasting trends shown by the two types of chiasmata strongly support King and Hayman's (1978) hypothesis that interstitial and terminal chiasmata are influenced by two different control mechanisms.

In order to get information on this problem we compared the trends of interstitial and terminal chiasma frequency with two factors whose influence on the various spermatogenetic stages in *P. sicula* is well known (Angelini *et al.*, 1980): temperature, which appears to be correlated with the level of chiasma frequencies in several species, and the level of the steroid hormones, testosterone and estradiol, in the blood.

The correlation between annual variations in temperature and variations in chiasma frequency appears to be different for the two types of chiasmata, and may be different within the same type of chiasma according to the period of the year (Fig. 2B). Terminal chiasma frequency shows a positive and statistically significant correlation (see statistical analysis of the correlation coefficient 'R' in Table 3) with temperature in the period December–June; a significant negative correlation in June–July, and does not show any correlation in the period July–December. The correlation between interstitial chiasma frequency and temperature is less evident. There seems to be a statistically significant correlation between these two parameters only when interstitial chiasma frequency increases. Such a correlation is negative from November to February, i.e. during the period of slow increase, and is positive in the period of fast increase in March. A negative but statistically not

Table 2. Statistical analysis of the significance of the differences among the mean values of terminal, interstitial and total chiasma frequencies for the various months of the year. These analyses are based on Snedecor's F test variance analysis. df = degrees of freedom; SS = sum of squares; MS = mean square; F = calculated F test values; P = probability level.

Item	Source of variation	df	SS	MS	F	P
Terminal chiasma frequency	Total	219	6.76			
	Between times	10	1.03	0.103	3.76	0.01
	Within times (Between animals)	209	5.73	0.027		
Interstitial chiasma frequency	Total	219	13.16			
	Between times	10	3.65	0.365	8.04	0.01
	Within times (Between animals)	209	9.50	0.045		
Total chiasma frequency	Total	219	8.60			
	Between times	10	2.87	0.287	10.63	0.01
	Within times (Between animals)	209	5.70	0.027		

significant correlation (Table 3, Fig. 2B) seems to exist also from March to June, whereas no correlation is evident in the other months (from June to October).

Different effects of temperature on chiasma frequency in different organisms and even in the same organism are already known (Elliott, 1955; Lin, 1982) and have been described by King and Hayman (1978) also in *Phyllodactylus.*

This suggests that the variations in environmental temperature and perhaps in other climatic parameters do not directly control chiasma frequency and distribution, but their influence is mediated by other, presumably endogenous, factors. In this regard, interesting information is provided by a comparison between cyclic variations in *P. sicula* chiasmata and testosterone and estradiol levels in blood, which are known to influence *P. sicula* spermatogenesis (Licht *et al.*, 1969; Botte & Angelini, 1980). This comparison (Figs. 3 and 4) shows a significant correlation (Fig. 4a, Table 4;

Table 3. Statistical analysis of the significance of the correlation coefficients regarding the linear relationships between interstitial or terminal chiasma frequency and temperature in different periods of the year. N = number of observations; Rvalue = value of correlation coefficient; P = Probability level.

Item	Time interval	N	R value	P
Terminal chiasma frequency	Dec – June	8	+0.810	5%
	Jun – July	2	–1.000	1%
	Jul – Dec	6	+0.677	NS
Interstitial chiasma frequency	Nov – Feb	4	–1.000	1%
	Feb – March	2	+1.000	1%
	Mar – June	4	–0.910	NS
	Jun – Nov	6	+0.753	NS

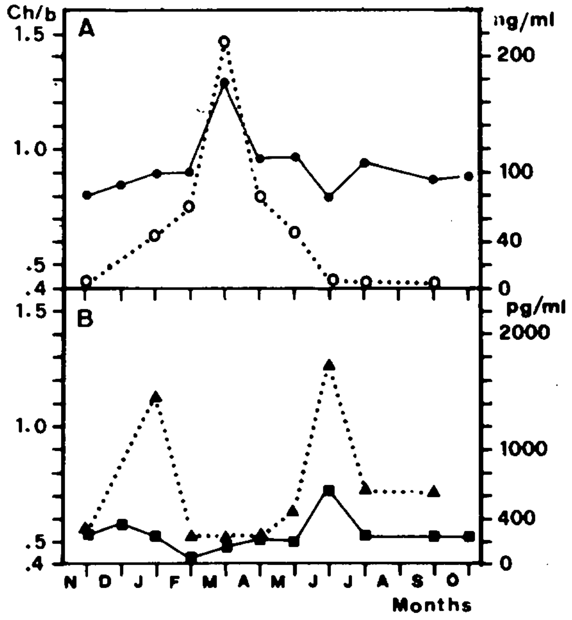

Fig. 3. Annual trends of chiasma frequency (symbols as in Fig. 2) and steroid hormone concentration: (A) interstitial chiasma frequency and testosterone concentration (ng/ml); – (B) terminal chiasma frequency and estradiol concentration (pg/ml).

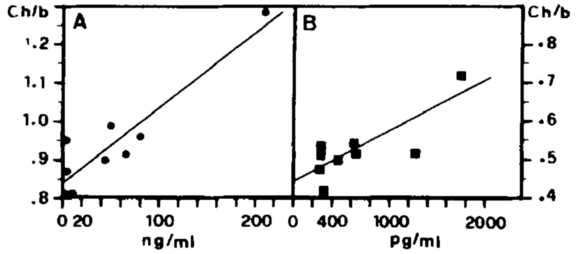

Fig. 4. (A) Comparison between testosterone concentration (abscissa in ng/ml) and interstitial chiasma frequency (ordinate in Ch/b); – (B) Comparison between estradiol concentration (abscissa in pg/ml) and terminal chiasma frequency (ordinate in Ch/b). Symbols as in Fig. 2.

correlation coefficient R significant at 1% probability level) between interstitial chiasma frequency and testosterone concentration. In addition the testosterone level shows an annual trend very similar to that of interstitial chiasmata (Fig. 3a). It also shows a minimum in late November to progressively increase, more rapidly from February, and reaches its maximum level in late March in correspondence with the highest level of interstitial chiasmata. Then it decreases to reach very low levels in late June and is more or less stable in the following months.

Conversely, terminal chiasmata show a correlation with the estradiol level in blood. This correlation too is statistically significant (Fig. 4b, Table 4), but it is not so precise during the year as the one between testosterone and interstitial chiasmata. The correlation between estradiol and terminal chiasmata is quite clear in the period February–September, mainly as regards the increase shown by these two parameters in late June (Fig. 4b). The situation is much less clear from November to January, when the estradiol level shows a new remarkable increase and chiasma frequency does not display significant variations.

Table 4. Statistical analysis of the significance of the correlation coefficients regarding the linear relationships between interstitial chiasma frequency and testosterone concentration and between terminal chiasma frequency and estradiol concentration.

Item	N	R value	P
Testosterone/Int. Ch. Freq.	9	+0.925	1%
Estradiol/Term. Ch. Freq.	9	+0.806	1%

Considering also the influence of the above mentioned hormones on the various spermatogenetic stages (Licht *et al.*, 1969; Angelini *et al.*, 1980), these results seem to suggest that testosterone and estradiol are possibly responsible for cyclic variations in the frequency of the two chiasma types, and more precisely testosterone for interstitial chiasmata and estradiol, in part at least, for terminal ones. This hypothesis finds further support in the constant frequency observed in the two types of chiasmata from July to November, when spermatogenesis, secondary sex characters and all of the steroid-dependent structures are not sensitive to hormonal influence ('refractory period') (Botte & Angelini, 1980).

Chiasma cycle and reproductive strategy

The cyclic trends of chiasma frequency observed in *Podarcis* and *Phyllodactylus* are markedly different; however they show some interesting analogies both in the correlation between the two chiasma frequencies and in that between chiasma frequency and spermatogenetic trend. In both species either type of chiasma reaches its maximum level in the period when the other shows its minimum (Fig. 2A).

Moreover, there is a particularly interesting correlation between interstitial chiasma frequencies and reproductive strategies in these species. King and Hayman (1978) have observed that in *Phyllodactylus* the sperms that will be used for fertilization derive from the spermatocytes-I that during their maturation show the highest rate of interstitial chiasmata. Likewise, in *Podarcis* the large increase of interstitial chiasmata in spermatocytes-I is found around the end of March, and, after about a month, the sperms of the spring spermatogenetic wave which will be used for fertilization, are obtained from these cells (Fig. 1a). Vice versa, a second reduced sperm production which is observed in late autumn and does not involve fertilization derives from the spermatocytes with the lowest rates of interstitial chiasmata and a moderate increase in the terminal ones (Figs. 1c, 2).

Several authors believe that interstitial chiasmata have a greater recombinational significance, unlike terminal chiasmata that would have instead a little recombinational role (Zarchi *et al.*, 1972; Hillel *et al.*, 1973). On this hypothesis King and Hayman (1978) have suggested that in *Phyllodactylus* the high interstitial chiasma frequency in sperm used for fertilization is a mechanism to ensure litters contain a higher level of genetic recombination and hence to maintain an adequate level of variability within a given population. The presence of such a correlation in another unrelated saurian with a different reproductive strategy, such as *Podarcis*, further supports this hypothesis and suggests that the use of cyclic variations in interstitial chiasma frequency, as a mechanism to ensure an adequate level of variability in a given population, is a quite widespread phenomenon among saurians.

In addition, the correlation between chiasma frequency and temperature, and especially that observed in *Podarcis* between interstitial chiasma frequency and testosterone level suggests that not only is the above mechanism closely linked to the saurian reproductive cycle, but it is presumably controlled, at least in part, by the same environmental and hormonal factors regulating this cycle.

However, further investigations are needed to confirm this hypothesis, and in particular, the extension of the study of the seasonal variations in chiasma frequency to other saurians with reproductive cycles different from the ones investigated, such as the saurians with continuous spermatogenesis and multiple mating in different periods of the year (Angelini & Ghiara, 1984). Experimental investigations, are at present in progress in our laboratory, on the influence of the various steroid hormones on the frequencies of the different chiasma types.

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