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Assessment of sexual maturation in the Moroccan locust *Dociostaurus maroccanus* (Thunberg)

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Abstract

The reproductive system and sexual maturation in *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae) were studied by dissecting males and females. The number of ovarioles per ovary ranged between 31 and 36: the closer a female is to the gregarious phase, as indicated by the ratio between the length of tegmen and the hind femur, the larger the number of ovarioles. Changes in the length of terminal oocyte and of body weight are associated with maturation of females, as revealed by the high correlation between these two parameters until the first oviposition in both field-collected and laboratory-reared females. Using these criteria, field samples of females were classified into nine categories, which correspond to the first three gonadotrophic cycles. Among field-collected *D. maroccanus* females, the number of *corpora lutea* per ovariole provides a good estimate of the number of egg pods laid per female. None of the field-collected females showed more than 2 *corpora lutea*.

The male genitalia are composed of a pair of testes, enclosed within a common sheath, with about 60 follicles, 15 pairs of accessory glands and one pair of seminal vesicles. Spermatogenic activity within the testicular follicles was studied by histological analysis. Spermatogonia divide mitotically throughout nymphal and adult life. Meiosis I is first observed in the third instar and spermiogenesis begins late in the fifth instar, being finished at adult emergence, at which time fully formed spermatozoa are observed. The number of sperm ultimately produced by a cyst was 64. Increasing body weight of males is proposed as a "marker" of sexual maturation since the presence of sperm in the seminal vesicle was not detected in any (>120) dissected males before they reached their basic weight, 7-10 days after emergence. This work provides simple criteria for assessing the maturation of *D. maroccanus* females and males and the realised reproductive potential of the species under field conditions.

Resumen

El sistema reproductor y la maduración sexual de *Dociostaurus maroccanus* (Thunberg) han sido estudiados mediante la disección secuencial de machos y hembras. En la hembra, el número total de ovariolos oscila entre 31 y 36, es mayor a medida que aumenta el grado de gregarización determinado mediante el cociente entre la longitud del élitro y el femur posterior. La maduración de las hembras está asociada a cambios en la longitud del oocito terminal y en el peso corporal, revelada por la gran correlación existente entre estos dos parámetros hasta la primera oviposición tanto en hembras de campo como de laboratorio. De acuerdo con estos criterios, las muestras de hembras de campo fueron clasificadas en nueve categorías, que corresponden con los tres primeros ciclos gonadotrópicos. En las hembras de campo, el número de *corpora lutea* por ovariolo proporciona una buena estimación del número de ootecas que pone cada hembra. Ninguna de las hembras recogidas en campo mostró más de 2 *corpora lutea*. La genitalia del macho está compuesta por un par de testículos, encerrados en una membrana común, con un número total de 60 folículos testiculares, 15 pares de glándulas accesorias y un par de vesículas seminales. La actividad espermatogénica en los folículos testiculares fue estudiada mediante histología. Las espermatogonias se dividen mitóticamente a lo largo de todos los estadios ninfales y el estado adulto, la meiosis I se observa por primera vez en el tercer estadio y la espermiogénesis, que comienza al final del quinto estadio ninfal, está terminada al emerger el adulto, momento en el que se observan espermatozoides completamente formados. El número de espermatozoides producidos en cada cisto fue de 64. Se propone el incremento del peso del macho como un marcador de maduración sexual puesto que la presencia de semen en la vesícula seminal no se detectó en ninguno de los más de 120 machos diseccionados antes de que se alcanzara el peso básico, 7-10 días después de la emergencia. Este trabajo proporciona criterios sencillos para evaluar la maduración sexual de hembras y machos de *D. maroccanus* así como su potencial reproductor en condiciones de campo.

Key words

Dociostaurus, Acrididae, locust, reproduction, development, ovariole, spermatogonia, sexual maturation.

Introduction

The Mediterranean or Moroccan locust *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae) is a major pest of agriculture in the Iberian Peninsula (Vázquez-Lesmes & Santiago-Alvarez 1993) and throughout much of the Mediterranean region (Uvarov 1977, Latchininsky & Launois-Luong 1992). In recent years, emphasis in the preventive control of this locust has changed from the use of broad-spectrum pesticides (Latchininsky & Launois-Luong 1992) towards more specific and environmentally sound products such as microbial pesticides (Hernández-Crespo & Santiago-Álvarez 1997) and Insect Growth Regulators (IGR) (Bouaichi *et al*. 1994). Furthermore, it has been pointed out that sub-lethal effects, such as interference in the reproduction of the species, could aid microbial and IGR acceptability and broaden their use, especially in an integrated pest manage-

ment approach. In fact, it has been reported that both entomopathogens and IGR can interfere in reproduction of adults of different locust species (Lockwood & Debrey 1990, Quesada-Moraga *et al*. 2000). Nevertheless, the implementation and evaluation of any control strategy toward *D. maroccanus* to impair its reproduction requires a comprehensive understanding of this process.

Although there is precise information on the external and internal genitalia of *D. maroccanus* females and males (Paoli 1937, Jannone 1940, Latchininsky & Launois-Luong 1992), to our knowledge, little work has been done on the sexual maturation of this species (Jannone 1940, Merton 1959). Chromatic changes associated with the hind tibiae of both sexes have been proposed as an indicator of sexual maturation (Merton 1959, Latchininsky & Launois-Luong 1992), yet these indicators seem to be inappropriate because they occur irregularly and only in part of the population (Latchininsky & Launois-Luong 1992). Moreover, the developmental differentiation of the male reproductive system and the patterns of spermatogenesis are still unknown. There are precise data on the biotic potential of this species under laboratory conditions (Santiago-Alvarez & Quesada-Moraga 1999), but this parameter is difficult to study under field conditions, *i.e.* without confining insects. The aim of the present work is to fill these gaps in our knowledge of *D. maroccanus* and to describe procedures for assessment of sexual maturation and oviposition.

Materials and Methods

The insects used in this study came from the field and from a stock colony maintained at 26 ± 2°C; 60 ± 4 % RH; 13L: 11D photoperiod, and reared by the method developed in our laboratory (Santiago-Alvarez & Quesada-Moraga 1999). Adult females were collected (1995-1998) at la Serena (Badajoz, Spain), the main permanent breeding area of *D. maroccanus* in the Iberian Peninsula. A population located in an area ~2000 m^2, at the 10 ha farm "La Servilleta" within the breeding area of la Serena, was selected to monitor the maturation of adult females. Collections of adults were made over the full interval of adult field occurrence, from the first-detected adult, 29 April, 1997, to last, 10 June, 1997. We measured the mean weight of all the female locusts from each sample, and afterwards, calculated the relative percentage of females per sexual maturation stage and date of collection.

Nymphs and adult males were collected at la Serena from 1995-1997. Samples of males collected in 1998 were accidentally destroyed.

The degree of crowding of the locust population was assessed by the ratio (T / F) between the length of the tegmen (T) and the metathoracic femur (F), which provides a good index for this species (Uvarov 1966). Measurements of the length of the tegmina and hind femur were made in accordance with the method of Moreno-Márquez (1942). The indicative values of solitary males and females are 1.49 and 1.57 respectively, and 1.74 and 1.72 of gregarious males and females respectively (Moreno-Márquez 1942). The smaller the value, the greater the solitariousness, the higher the value, the greater the gregariousness.

In 1997, field-collected females were analysed by a standard technique for assessing ovarian status (Launois-Luong 1978). Females were collected weekly in order to obtain individuals at all developmental stages between the imaginal moult and the end of life. In 1997, the oviposition period lasted about 1 month. The criteria used to monitor progress of sexual maturation and to analyze models of ovarian activity were: number of ovarioles per ovary, change over time in the length of terminal oocyte (this for the oocyte nearest to the lumen of the oviduct, and hence the first to develop with the onset of maturation), change over time in the mean body weight of adult females and presence and number of *corpora lutea* detected in the pedicels of the ovarioles. Among laboratory-reared females, we measured change over time in the length of the terminal oocyte and body weight. The number of egg pods and the number of *corpora lutea* in each female were obtained to make a correlation test (Pearson). The relationship between body weight and the length of the terminal oocyte was predicted by least-squares regression (Statistics 4.1 for Windows, Analytical Software, 1986).

In 1997 using these criteria, field samples of adult females were collected at intervals, from the imaginal moult to the end of life, and grouped into nine categories referred to as stages I to IX. Stages I to III represent the first gonadotropic cycle. Females at stage I increase their weight about 50%, reaching the intermediate stage II, at which time they are ready to copulate. At stage III, the oocytes complete their development and females reach their maximum weight, which is then maintained with small fluctuations throughout their life. Stages IV-VI and VII-IX correspond to stages I-III for the second and third gonadotrophic cycles, respectively.

Precise observations and measurements were made of the male reproductive system to assess: the change over time in the volume of the testes, the number of testicular follicles and accessory glands, the change over time in the coloration of the glands and the content of the seminal vesicle. Volume was calculated for each gonad by using the formula: Volume = $\pi/6$ (length x width), assuming that the adult testes are an ellipsoid (Holt & North 1970).

For study of spermatogenesis, the testes of 10 males per nymphal instar and 25 adult males (5 males each: 0, 2, 5, 10 and 15 days old), were fixed in Bouin's solution (Pantin 1968) and embedded in paraffin. Counts of the number of generations of secondary spermatogonia and primary spermatocytes were done in serial sections cut at 7 µm, and stained with Iron Hematoxylin (Sigma C.I. 75290) (Pantin 1968). The number of sperm per bundle was calculated from the number of primary spermatocytes.

Results

The ovaries of newly moulted females are elongate, 7.4 ± 1.6 mm (n = 10) in length, and reach a maximum length of more than 30 mm in gravid adults, which occurs around 15 days after adult emergence. Ovariole number by ovary, considering both ovaries in the calculation, is characterized by individual variation (range 24-41). The distribution within this range was similar not only within each year, but also

Table 1. Morphological parameters of the gonads of *D. maroccanus* females.

Year	Number of ovarioles				Number of testicular follicles			
	N	Total Mean ± SD	Range	T / F Females Mean ± SD	N	Total Mean ± SD	Range	T / F Males Mean ± SD
1995	29	31.3 ± 3.8 **a**	24-40	1.48 ± 0.08 **a**	29	61.6 ± 4.7 **b**	50-71	1.44 ± 0.08 **a**
1996	22	34.0 ± 3.2 **b**	28-41	1.55 ± 0.07 **b**	27	56.9 ± 4.7 **a**	50-64	1.49 ± 0.07 **b**
1997	62	36.5 ± 2.6 **c**	30-41	1.61 ± 0.06 **c**	66	67.8 ± 3.8 **c**	60-72	1.59 ± 0.10 **c**
1998	10	34.7 ± 4.4 **b**	32-38	1.58 ± 0.05 **c**	-	-	-	-

N = Number of gonads; E / F = length of tegmen/length of hind femur; means within columns with the same letter are not significantly different (least significant difference (LSD) at the 5% level).

Table 2. Relationship between length of the terminal oocyte and body weight of adult *D. maroccanus* females.

Origin of females	N	Regression formula	Slope error	r^2
Field	61	Y = 1.47X - 0.73	0.07	0.88
Laboratory-reared	52	Y = 1.28X - 0.63	0.09	0.80

N = number of dissected females; Y = Log (length of terminal oocyte in mm); X = body weight in g; r = regression coefficient.

between the 3 years of the study. This value was directly related to the T / F index (Table 1).

The length of the terminal oocyte, which is the largest and most mature one, reached a maximum value of 4-5 mm before being laid. The readiness of a female to oviposit is indicated by a 75% body weight increase, from 0.51 ± 0.20 g (n = 10) at adult emergence, to 0.90 ± 0.09 g (n = 20), 2 weeks later, at the time of first oviposition. Field-collected and laboratory-reared females show a high correlation between length of terminal oocyte and body weight until first ovulation, which can be expressed by the regression formula shown in Table 2.

Fig. 1 indicates that maturation does not occur synchronously under field conditions. At adult emergence, all the females were immature (stage I) but 1 week later, around 45% of females were at stage II and 20% of females were at the onset of vitellogenesis (stage III). Two weeks after emergence, at the moment of first oviposition, 80% of females were mature and 15% had begun the second gonadotrophic cycle (Fig. 1). Analysis of ovarian activity in *D. maroccanus* indicates the presence of *corpora lutea* within each ovarian pedicel. No collected female showed more than 2 *corpora lutea* per ovariole; 13 females displayed 2, 29 displayed 1 and the rest had no *corpora lutea* (Table 3). Correlation (Pearson) between the number of egg pods and the number of *corpora lutea* obtained from 53 laboratory-reared females was 0.8.

We analysed the number of eggs per egg pod from egg pods collected in three years of the study (in 1996 egg pods were destroyed by a predator) in the same area where females were sampled (Table 4). We obtained an average of 30-31 eggs per egg pod and consequently, the mean percentage of functional ovarioles (number of eggs per oviposition / number of ovarioles) ranged between 87% and 100% (Table 4).

In newly moulted males, the total volume of the two testes, enclosed within the common sheath, is 64-65 mm^3 as a mean (n = 54), which remains unchanged across adult male life. The testes are accompanied by 15 pairs of narrow accessory glands (10 hyaline pairs, 4 white pairs and one opalescent pair) and one pair of seminal vesicles. On the other hand, the number of testicular follicles is characterized by individual variation (range 51-72). The distribution within this range was similar for the 3 years of the study and the mean number is not clearly related to the T/F index, as indicated by the data of 1995 (Table 1).

Spermatogenic activity within the testicular follicles is only apparent by histological analysis (Fig. 2 A, B). Spermatogonia divide mitotically throughout nymphal and adult life (Table 5). Meiosis I, in which primary spermatocytes are produced, occurs early in the third instar. The number of generations of secondary spermatogonia was 4; the number of primary spermatocytes per cyst, 16; therefore, the number of sperm ultimately produced by a cyst is 64 (Fig. 2 A, B). Fifth-instar nymphs contain spermatogonia, spermatocytes in all stages of division and spermatids, although spermiogenesis begins late in this instar (Fig. 2 B), being just finished at adult emergence; thus, newly emerged adult

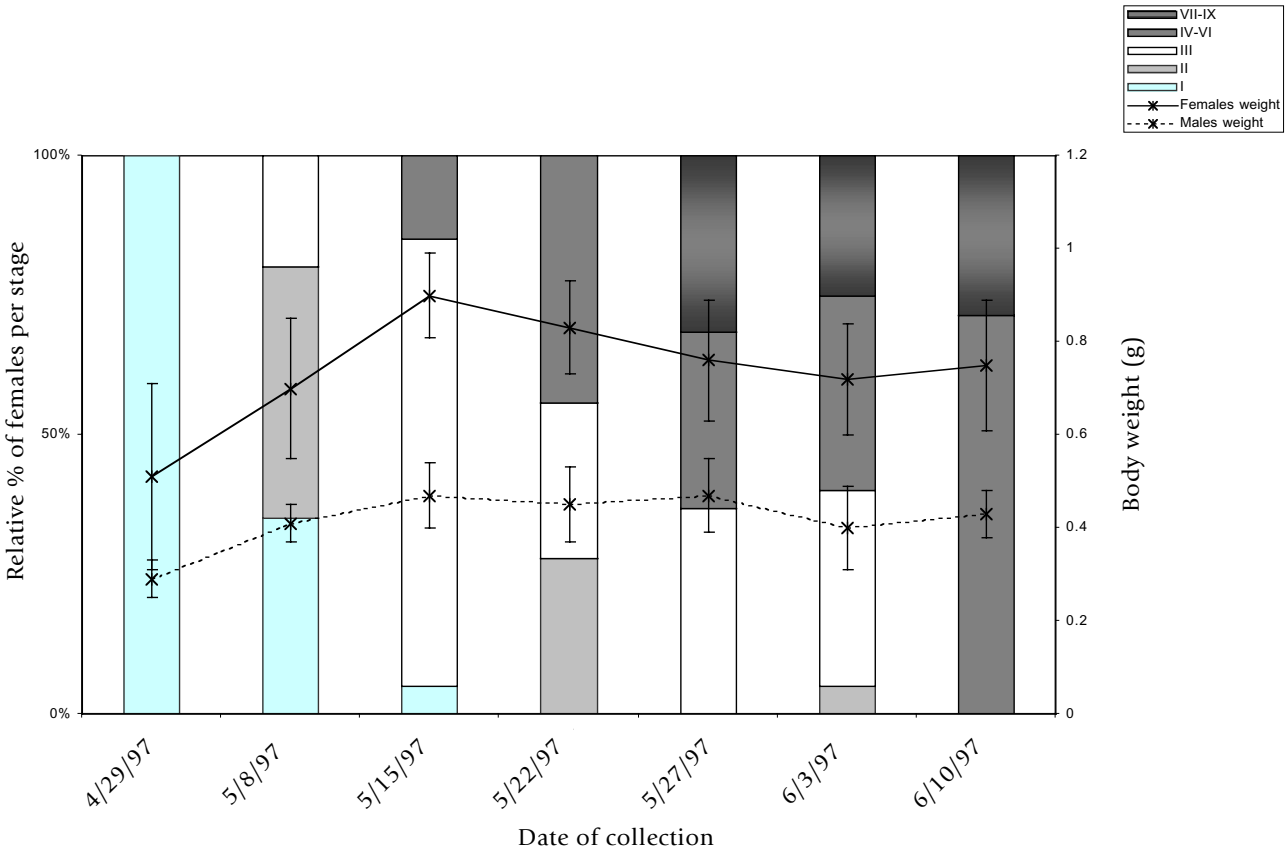

Fig. 1. Development of maturation stages of females and average weight (means ± SD) of males and females throughout adult life.

Table 3. Maturation stages of *D. maroccanus* adult females collected at the permanent breeding area of la Serena in 1997.

Adult Stage	N	L.T.O (mm)	Weight	% copulated females	Number of *corpora lutea*
		Mean ± SD	Mean ± SD		
I	18	0.86 ± 0.17	0.51 ± 0.20	0	0
II	15	2.37 ± 0.48	0.75 ± 0.12	66.6	0
III	39	4.70 ± 0.40	0.88 ± 0.09	100.0	0
IV	8	1.87 ± 0.23	0.71 ± 0.09	100.0	1
V	3	2.75 ± 0.43	0.76 ± 0.10	100.0	1
VI	18	4.70 ± 0.40	0.81 ± 0.10	100.0	1
VII	11	1.70 ± 0.30	0.64 ± 0.08	100.0	2
VIII	2	2.55 ± 0.60	0.56 ± 0.01	100.0	2
IX	0	-	-	-	-

N = Number of females; L.T.O. = Length of terminal oocyte.

Table 4. Mean number of eggs per egg pod in field-collected pods.

Year	N	Egg / egg pod	
		Mean ± SD	Range
1995	21	31.6 ± 5.0 **a**	23-43
1997	40	31.9 ± 4.3 **a**	20-43
1998	40	30.6 ± 4.7 **a**	20-39

N = Number of egg pods. Means within columns with the same letter are not significantly different (least significant difference (LSD) at the 5% level).

Table 5. Developmental timetable of spermatogenesis in *D. maroccanus* males. The most frequent cell stage (++) and other observed stages (+) are indicated.

Stages	n	SPG	SPC1	SPC2	SPD		SPZ
					NE	E	
N1 and N2	10	++					
N3	8	++	+				
N4	8	+	++	+			
N5	9	+	+	++	+	+	
Adult (0 days)	6	+	+	+	+	++	++
Adult (2 days)	7	+	+	+	+	++	++
Adult (5 days)	6	+	+	+	+	++	++
Adult (10 days)	5	+	+	+	+	++	++
Adult (15 days)	5	+	+	+	+	++	++

N1-N5 are the nymph stages. n = Number of dissected insects. SPG = Spermatogonia, SPC1 = Primary spermatocytes, SPC2 = Secondary spermatocytes, SPD = Spermatids (NE = no elongation, E = elongation), SPZ = Sperm bundles.

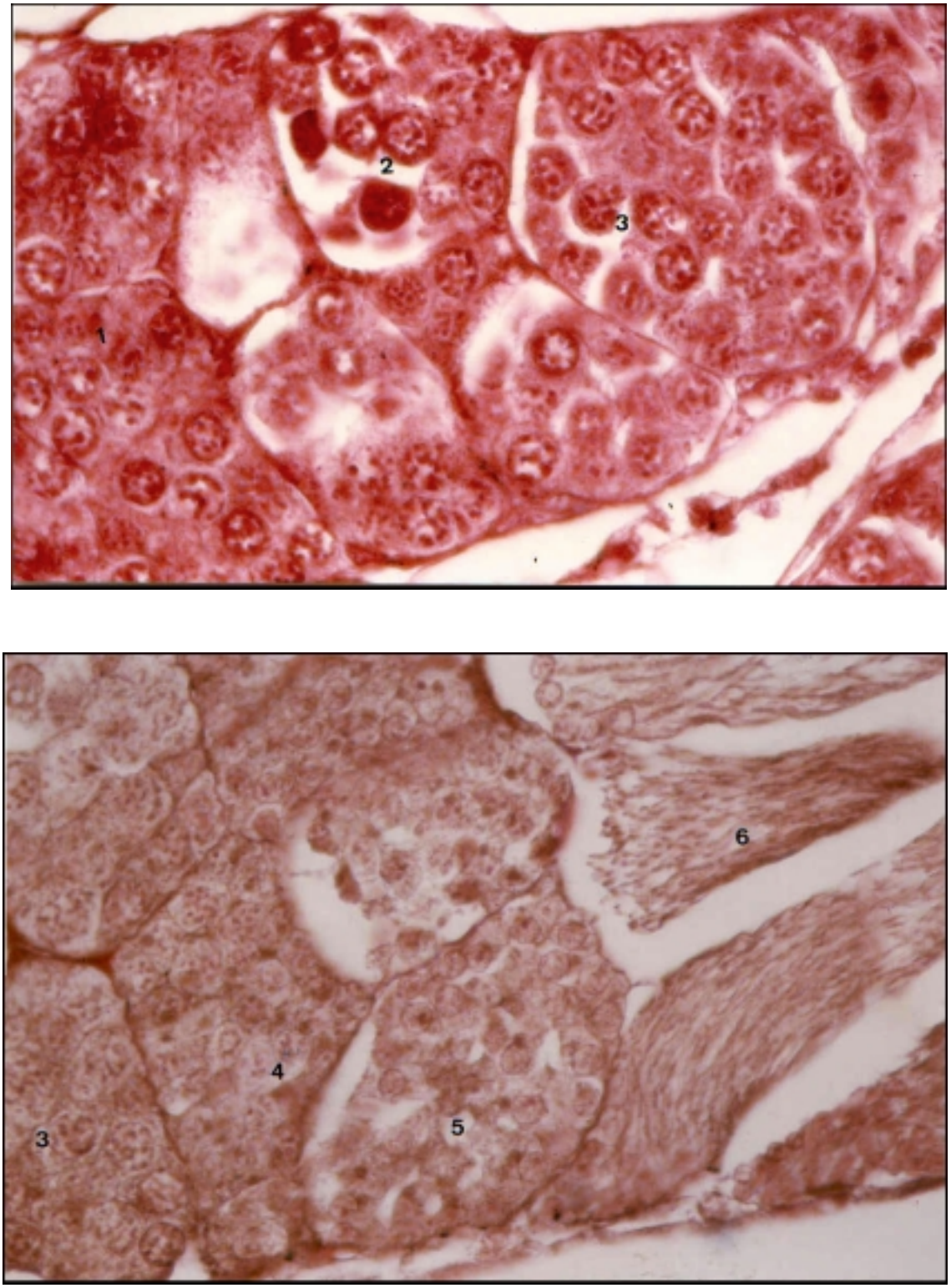

Fig. 2. Hematoxylin stained 7-µm sections of testicular follicles showing spermatogenic activity. **A** Transverse follicular section of a fourth instar nymph (400X). **B** Longitudinal follicular section of a fifth instar nymph (400X). 1 = cyst containing eight spermatogonia; 2 = cyst containing 16 primary spermatocytes; 3 = cyst containing 32 secondary spermatocytes; 4 = cyst containing 64 spermatids; 5 = early stage of spermiogenesis; 6 = last stage of spermiogenesis showing fully formed spermatozoa.

males, 0 days old, have fully formed spermatozoa.

The seminal vesicles of more than 120 newly emerged males contained no spermatozoa and the accessory glands were narrow and transparent, containing no secretion. Quantities of sperm appeared in the seminal vesicles 7-10 days after emergence, at which time the presence of white secretion in at least two of the four white pairs of accessory glands was observed, indicating that the male had acquired readiness to copulate. The only external sign associated with readiness to copulate was a 50 % body weight increase from 0.29 ± 0.04 g at adult emergence, to 0.41 ± 0.04 g (n = 10), 7-10 days later, which is maintained with small fluctuations throughout life (Fig. 1).

Discussion

It is known that solitarious-phase females of *D. maroccanus* are smaller and lay smaller clutches than do gregarious phase locusts (Moreno-Márquez 1942). Moreover, our work indicates that the number of ovarioles in *D. maroccanus* is positively correlated with the degree of crowding, as in *Locustana pardalina* (Walker) (Albrecht 1967). This response to crowding is opposite to the one observed in more "typical" locusts such as *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forskål) (Uvarov 1966, Pener 1991).

Variation in the length of terminal oocytes has been used to define maturation stages in *L. migratoria* (Phipps 1950) and *D. maroccanus* (Merton 1959). Our work includes an additional parameter, body weight, to define these stages in *D. maroccanus*. In the Mediterranean or Moroccan locust, body weight is highly correlated with length of terminal oocyte as in the Australian plague locust, *Chortoicetes terminifera* (Walker) (Clark 1965). This correlation provides a useful tool to determine the possible gonadotrophic effect of different control measures on females up to first oviposition without dissecting them. The validity of this tool requires knowledge of the age of the females at the moment of being treated. It is necessary to point out that only 3 females were found at stage V and only 2 individuals at stage VIII, so that the statistical value of these data is very low; however they can indicate the evolution of the size of the terminal oocyte and body weight over each gonadotropic cycle.

The asynchronous pattern of female maturation observed in our work could be expected under a full range of field conditions, because it seems that it is due to the natural variation of the locust population, more than to variation in environmental conditions. The average weight of collections at each date has been obtained using females belonging to different maturation stages, but it is easy to calculate by land managers and could be useful to detect the proximity of the first oviposition. This date indicates the period before the regression lines obtained by us can be applied, and when treatments against adults could be effective to interfere with their reproduction.

Assessment of realised fecundity of some locust females can be achieved by analyzing the number of *corpora lutea* in each ovariole and those superimposed in the same ovariole (Launois 1972, Launois-Luong 1978). Sometimes *corpora lutea* and oocyte resorption bodies have similar shapes and color, which could create confusion in estimating the number of eggs produced per female but not in estimating the number of egg pods per female. Our work indicates little oocyte resorption: 87-100% of functional ovarioles. This indicates that the number of *corpora lutea* is a good estimation of the number of egg pods per female, though very difficult to calculate under field conditions (*i.e.* if females are not confined). This result is also supported by the high correlation we have obtained between these two variables from laboratory-reared females.

Our results clearly show that *D. maroccanus* females do not oviposit more than 2 pods under field conditions, which is in agreement with the studies of Merton (1959) in Cyprus, who found that no females, out of total of more than 2500 dissected, had more than two *corpora lutea* per ovariole (Merton 1959). Among the females dissected by Merton, only 20 were at stage IX, and we did not find any females at this stage. If a third oviposition occurred following our study, it could only have involved a small percentage of the population.

The pattern of testicular maturation of *D. maroccanus* indicates that male fertility could be successfully impaired at any time from the nymphal to adult stage, using any sterilising treatments. It is necessary to point out that orthopterans are radiosensitive insects (Willard & Cherry 1975). Nevertheless, pest control by releasing sterile irradiated males is more successful in monogamous species (Reinhardt *et al*. 1999). (*D. maroccanus* females mate more than once, so that irradiation could be used to investigate the degree of sperm removal of the second mating rather than to control this locust (Reinhardt *et al*. 1999).) The number of sperm per bundle in *D. maroccanus* seems to be very low if compared with the range of 128-512 observed in most acridids (White 1955).

In general, males of less specialised species of Acridoidea seem to produce more sperm than those of derived species (White 1955); Gomphocerinae belong to the latter group (Vickery 1997). Nevertheless, the number of sperm per bundle is not a direct index of sperm production since the number of sperm bundles produced by an individual male certainly varies from species to species (White 1955). Our observations on the number of accessory glands do not agree with those of Jannone (1940), who proposed that *D. maroccanus* males have 14 pairs of accessory glands, while we have observed 15 pairs.

The patterns of weight gain throughout male life are similar to those observed in *L. migratoria* (Phipps 1950) and *S. gregaria* (Forskål) (Norris 1954), but in contrast to the latter species, we have not observed mature males of *D. maroccanus* below the basic weight of 0.40-0.45 g. This clearly indicates that the increasing body weight of males could be proposed as a "marker" of sexual maturation in *D. maroccanus*. Furthermore, the presence of sperm in the seminal vesicle was not detected in any of the dissected males before reaching the basic weight.

This work provides simple criteria for assessing the maturation of *D. maroccanus* females and males and for evaluating the realised reproductive potential of the species under field conditions.

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