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Cytological and developmental analysis of tychoparthenogenesis in *Locusta migratoria*

M. C. PARDO, M. D. LÓPEZ-LEÓN, J. CABRERO & J. P. M. CAMACHO*
Departamento de Genética, Facultad de Ciencias, Universidad de Granada, E-18071 Granada, Spain

A number of females of *Locusta migratoria* have shown tychoparthenogenetic reproduction (a kind of accidental thelytoky) characterized by: (i) a female-biased primary sex ratio; (ii) the production of embryos with abnormal ploidy levels, mainly haplodiploid mosaics; (iii) a longer time for embryo development; and (iv) the capability of producing female offspring that reproduced in the absence of males. Perfect diploidization is not essential for parthenogenetic embryos to reach the adult stage but a great majority of embryo cells must be diploid to complete properly embryogenesis and hatch. In addition, diploidy is apparently necessary to the ovary of parthenogenetic females so that eggs laid without mating can hatch. Cytological analyses of embryos at different developmental ages have shown that parthenogenetic embryos begin haploid and gradually become diploid, thus passing through a haplodiploid mosaic stage. The most likely mechanism for ploidy restoration is the restitution of the sister products of cleavage mitoses, although our results show that cell fusion could be another mechanism, the relative importance of which remains to be tested in future work. Although parthenogenetic females showed a fecundity comparable to that of sexual females, their reproductive output was significantly lower because of a decrease in the number of embryos per pod and a consequent decrease in the rate of embryo production.

Keywords: *Locusta migratoria*, parthenogenesis, ploidy restoration, thelytoky, tychoparthenogenesis.

Introduction

Tychoparthenogenesis is a kind of occasional thelytoky characterized by the spontaneous hatching of a small proportion of eggs laid by virgin females. This process has been reported in a high number of orthopteran species, mainly in Tettigonioidea, Tetrigoidea and Acridoidea, including several locust species (see Hewitt, 1979, for a review). In *Locusta migratoria* it was firstly reported by Plotnikov (1915) and was studied in detail by Bergerard and Seugé (1959). These authors showed the presence of haploid and diploid cells in the parthenogenetic embryos and, with the aid of a B chromosome as a marker, they deduced that these diploid cells were derived from restitution of the sister products of early cleavage mitoses. Parthenogenetic females, however, were almost completely unproductive, most of them being weak and incapable of laying eggs. This contrasts with the cases of *Apotettix eurycephalus* (Nabours & Foster, 1929) and *Schistocerca gregaria* (Hamilton, 1955) where seven and six successive parthenogenetic generations, respectively, were reared in the laboratory.

The present paper reports on three independent parthenogenetic lines in *L. migratoria* that were bred in our laboratory during three consecutive generations. A comparative analysis of reproductive output of parthenogenetic females in relation to sexually reproduced females was performed, as well as a combined cytological and developmental analysis of the process of ploidy restoration, which has shown that cell fusion is another mechanism involved, in addition to restitution, in this phenomenon.

Materials and methods

Adult males and females (and last instar nymph females) of the locust *L. migratoria* were collected in field populations at Armilla, Gabias and Padul (Granada) during September and October from 1990 to 1992. Female nymphs were isolated from

*Correspondence.

males to preserve their virginity and perform controlled crosses. Adult males and females were bred to obtain new virgin females for additional crosses. Furthermore, several egg-pods from a laboratory culture from the Barcelona Zoo were incubated and the specimens obtained were used for some of the crosses.

In the process of isolating females to preserve their virginity we noted that some of these virgin females laid eggs that after incubation yielded some hatchlings. These females were the start of the parthenogenetic lines. In addition, some other females, which had been crossed with a male, laid eggs that contained haplodiploid mosaic embryos, thus showing signs of parthenogenesis. For details on the types of crosses see Results and Table 1.

Adults were reared at 27°C under a 12L:12D photoperiod, whereas the eggs were incubated at 28°C in the dark. Fixation of embryo and adult tissues and their cytological analyses were performed as described in Viseras *et al.* (1990), Camacho *et al.* (1991) and Pardo *et al.* (1994b, 1995b).

Flow cytometry was used for the determination of ploidy level in some parthenogenetic females, following a technique similar to those employed by Daunay *et al.* (1993). Ovarioles were chopped in PBS and the cell suspension was washed several times with new PBS after centrifugation. Then 100 μL of the cell suspension was diluted in 2 mL PBS, filtered through nylon nets with 40 μm meshes and stained with ethidium bromide. Nuclei were analysed in an ORTHO CYTORON flow cytometer.

For statistical analyses, the Shapiro–Wilk's test was first applied to each variable to test normality. As most failed to fit a normal distribution, and it was impossible to normalize them with the usual data transformations, nonparametric tests were employed. The only exception was the frequency of polyploid cells in haplodiploid mosaic embryos, in which case a Student's *t*-test was used.

Parthenogenetic strains

A number of *L. migratoria* females, most from the Barcelona Zoo laboratory culture and a few from the natural populations, have shown parthenogenetic reproduction characterized by: (i) a female-biased primary sex ratio (measured in 6-day-old embryos); (ii) the production of some embryos with abnormal ploidy levels — haploids, polyploids, polysomics and, the most frequent, haplodiploid mosaics (Figs 1 and 2); (iii) a longer time for embryo development; and (iv) the capability shown by some virgin females to produce female offspring that reproduced in the absence of males for three consecutive generations (in three different experiments with Barcelona Zoo females).

The first parthenogenetic strain was obtained in 1990 from a female that had been placed together with a male (although we do not know whether they actually mated). This female yielded all-female offspring, 11 of which reached the adult stage (F_1). These 11 females were isolated in individual cages and part of their eggs were dissected to analyse embryos, the remainder were incubated to term to obtain adults. All females laid eggs even though they were virgin, but the eggs from only four of the 11 females contained embryos, and in only three of these four females did some eggs hatch producing F_2 nymphs (11 eggs from one female, eight from another and two from the remainder). In all cases the nymphs were females and died at different stages of development, none reaching maturity.

In some egg-pods incubated to term, the eggs failed to hatch. The dissection of these eggs showed that some contained embryos with interrupted development and with conspicuous size differences (even within the same egg-pod), indicating that development was interrupted at different stages.

Cytological analyses of ovarioles and gastric caecae in eight of the 11 F_1 parthenogenetic females showed that, whereas all gastric caecae cells analysed were diploid in all the females, the ovariole cells analysed were diploid in five females but with a mosaicism for the ploidy level in three of these females, although diploid cells were predominant. This indicates that parthenogenetically developed embryos can reach the adult stage even though diploidization is not perfect. Remarkably, the F_1 females that were perfectly diploid in their ovarioles produced F_2 nymph offspring whereas the three that were mosaic failed to yield nymph offspring. This suggests that perfect gonadal diploidization may be a necessary condition for hatching of the eggs produced by parthenogenesis.

The second and third parthenogenetic strains started in 1993. One of these progressed to the F_3, and showed the same characteristics mentioned above for the first strain, except that in this case some F_1 females were larger than normal. The third strain progressed only two generations and was noteworthy for the large size of the three F_1 females which produced only three nymphs that died before reaching adulthood. The ovarioles of two of the three F_1 females were analysed by flow cytometry to estimate ploidy level in comparison with a female raised by sexual reproduction. The DNA index (i.e. the DNA amount in cells at G_0 and G_1 interphase periods

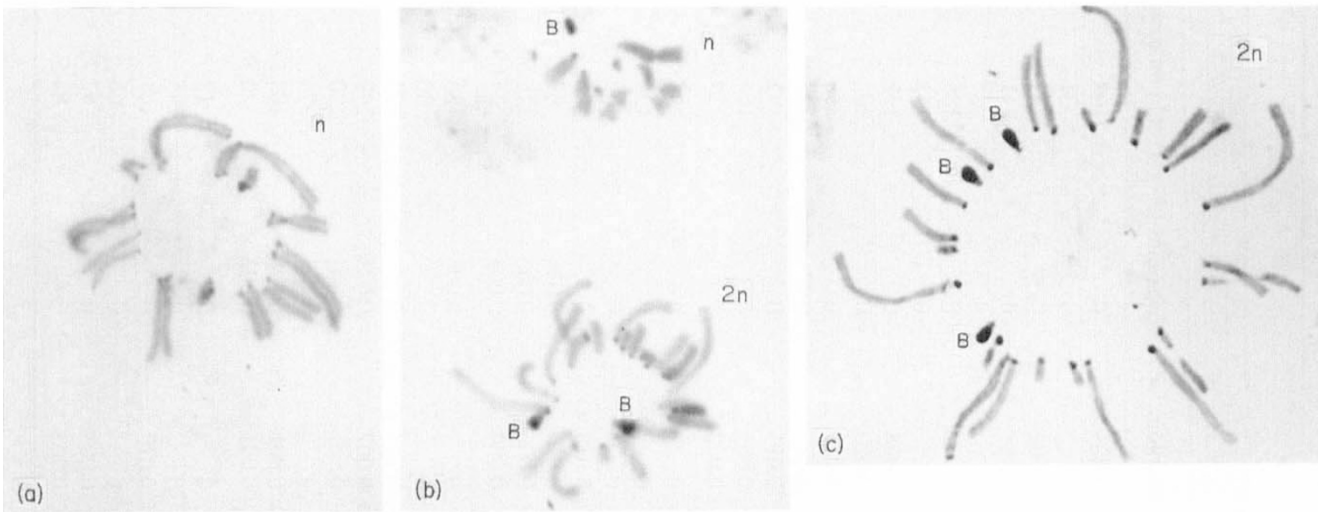

Fig. 1 Parthenogenetic embryos with different ploidy levels: haploid (a), haplodiploid mosaic (b) and diploid (c).

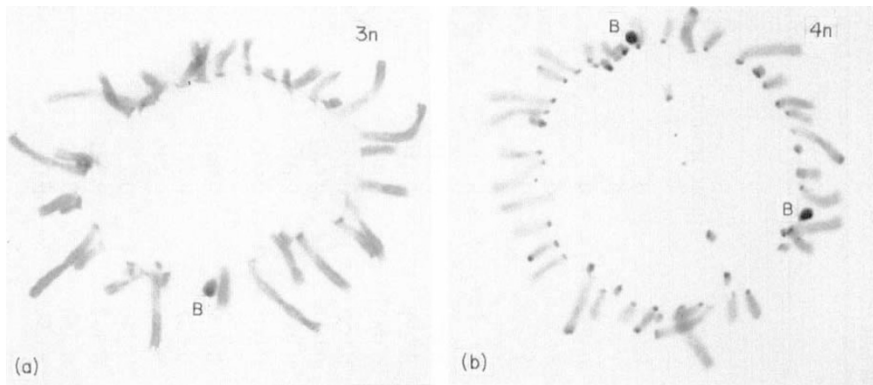

Fig. 2 Polyploid parthenogenetic embryos of *Locusta migratoria*: triploid (a) and tetraploid (b).

from the parthenogenetic females relative to that of cells from the sexual female) of the predominant cells in one of the parthenogenetic females was 1.44, indicating that these cells were triploid. In the second female, however, the main peak corresponded to diploid cells (DNA index = 0.87) but there was a small population of triploid cells (DNA index = 1.39). As a consequence, some females which developed through parthenogenesis were larger than normal because they contained a proportion of triploid cells.

Results

Female-biased primary sex ratio

The following data were obtained in a total of 27 controlled crosses that differed in the conditions under which the crosses were performed and in the resulting primary sex ratio, measured in embryos from 6 to 12 days of development. The sex ratio was measured from the proportion of male embryos ($2n = 23 + X0$ chromosomes) among all analysed embryos in a cross.

Four types of cross may be distinguished on the basis of the primary sex ratio and male availability (Table 1): crosses showing sexual reproduction exclusively, with primary sex ratio not significantly different from 0.5 (Type I crosses, nos 1–9, 11, 13 and 16); those showing a mixture of sexual and parthenogenetic reproduction, with primary sex ratio significantly lower than 0.5 but still producing some males (Type II crosses, nos 10, 12, 14, 15 and 17); crosses showing parthenogenesis exclusively, with the production of all-female progeny despite the female parent being with a male throughout the experiment (Type III crosses, nos 18–23); finally, forced parthenogenesis in virgin females that were never with a male, with the obvious production of all-female progeny (Type IV crosses, nos 24–27).

Table 1 Relative frequency of the different types of embryos found in each of the 27 crosses of *Locusta migratoria* analysed

Type of cross	Cross no.	Parental population ♀	Parental population ♂	Embryo offspring: Diploid ♂♂	Diploid ♀♀	Diploid Total	Haplodiploid	Haploid	Polyploid	Polysomic	Total embryos
I	1	G	A	0.523	0.472	0.995	0	0	0.005	0	193
	2	G	A	0.484	0.516	1	0	0	0	0	31
	3	G	A	0.496	0.500	0.996	0	0	0.004	0	244
	4	G	A	0.488	0.512	1	0	0	0	0	211
	5	G	G	0.500	0.500	1	0	0	0	0	28
	6	A	G	0.508	0.492	1	0	0	0	0	185
	7	B	A	0.467	0.533	1	0	0	0	0	152
	8	B	A	0.417	0.561	0.978	0.015	0	0.008	0	132
	9	B	A	0.520	0.451	0.971	0.010	0	0.020	0	102
	11	A	A	0.535	0.447	0.982	0.008	0	0.010	0	396
	13	G	A	0.442	0.514	0.956	0.043	0	0	0	208
	16	B	G	0.421	0.526	0.947	0.042	0	0.011	0	95
	Mean			0.483	0.502	0.985	0.010	0	0.005	0	164.75
	SE			0.011	0.010	0.005	0.005	—	0.002	—	29.08
II	10	B	G	0.387	0.552	0.939	0.061	0	0	0	181
	12	A	B	0.272	0.449	0.721	0.257	0.022	0	0	136
	14	G	A	0.184	0.461	0.645	0.349	0.007	0	0	152
	15	G	P	0.143	0.603	0.746	0.238	0	0.016	0	63
	17	B	G	0.194	0.403	0.597	0.371	0	0.016	0.016	62
	Mean			0.236	0.494	0.730	0.255	0.006	0.006	0.003	118.80
	SE			0.043	0.036	0.059	0.055	0.004	0.004	0.003	24.09
III	18	A	A	0	0.459	0.459	0.536	0	0	0.005	183
	19	B	A	0	0.500	0.500	0.500	0	0	0	50
	20	B	A	0	0.750	0.750	0.250	0	0	0	72
	21	B	G	0	0.729	0.729	0.243	0	0.007	0.021	140
	22	B	G	0	0.585	0.585	0.408	0	0	0.008	130
	23	B	G	0	0.527	0.527	0.459	0	0	0.014	74
	Mean			0	0.592	0.592	0.399	0	0.001	0.008	108.17
	SE			—	0.050	0.050	0.051	—	0.001	0.003	20.78
IV	24	B	—	0	0.824	0.824	0.176	0	0	0	17
	25	B	—	0	0.386	0.386	0.614	0	0	0	145
	26	B	—	0	0.287	0.287	0.703	0	0.010	0	101
	27	B	—	0	0.265	0.265	0.735	0	0	0	68
	Mean			0	0.441	0.441	0.557	0	0.003	0	82.75
	SE			—	0.131	0.131	0.130	—	0.003	—	84.50

Population codes: G: Gabias; A: Armilla; B: Barcelona; P: Padul.

Frequency of embryos with abnormal ploidy levels

Table 1 shows the frequencies of the different types of embryos (diploid, haploid, haplodiploid, polyploid and polysomic) observed in the 27 crosses analysed. Haploid embryos were extremely rare: only three of the 136 embryos analysed in the cross no. 12, and one of the 152 embryos examined in the cross no. 14, were haploid. The lack of haploid embryos in the Types III and IV crosses, which reproduced exclusively by parthenogenesis, suggests that in these crosses the onset of the diploidization process functioned better than in crosses with sexual reproduction. Comparisons between the four types of cross, by means of the Kruskal–Wallis nonparametric ANOVA, showed that the frequency of haplodiploid embryos differed significantly between types of cross ($H = 20.95$, d.f. = 3, $P < 0.001$), its frequency being low in Type I crosses but increasing gradually in Types II–IV in parallel with a decrease in the frequency of male diploid embryos (Table 1). A similar result was obtained for polysomic embryos ($H = 11.8$, d.f. = 3, $P = 0.008$), suggesting that the appearance of polysomic embryos may be in some way linked to parthenogenesis, presumably by imperfections in the diploidization process. Most polyploid embryos were triploid (Fig. 2a) or tetraploid (Fig. 2b), but their frequency did not differ significantly among cross types ($H = 2.31$, d.f. = 3, $P = 0.511$). This suggests that the appearance of polyploid embryos is independent of the mode of reproduction and might simply be the result of a kind of developmental noise.

Diploidization during embryo development

We have analysed the diploidization process of the haploid eggs developed through parthenogenesis by means of the cytological analysis of embryos fixed at different days of incubation in each of the crosses nos 25–27. As a measure of the diploidization stage of each embryo, we have used the diploidization index (DI), i.e. the proportion of diploid cells found among the total cells analysed (20 or more per embryo). DI is equal to 0 in haploid embryos, 1 in embryos completely diploidized, and will show intermediate values in haplodiploid embryos.

As Table 2 shows, DI differed significantly among the three groups of embryos fixed at different incubation times (6, 8 and 10 days) in the cross no. 25, as well as among the five groups of embryos fixed at 6, 7, 8, 10 and 12 days of incubation in the cross no. 26. However, there were no significant differences among embryos of 6, 7 and 8 days analysed in the cross no. 27, although in this case the time span was shorter.

One egg-pod from each cross, which had been incubated to term to obtain parthenogenetic adults, failed to hatch. Dissection of the eggs revealed embryos with interrupted development. Cytological analysis showed a DI of about 0.6 in these embryos

Table 2 Comparison of the diploidization index (DI) between embryos of *Locusta migratoria* of different development ages in the crosses nos 25–27

					Kruskal–Wallis test†		
Cross no.	Incubation	DI	SE	*N*	*H*	d.f.	*P*
25	6 days	0.54	0.06	24	11.52	2	0.003
	8 days	0.75	0.03	98			
	10 days	0.74	0.07	23			
	to term	0.63	0.07	7			
26	6 days	0.49	0.07	11	42.56	4	<0.001
	7 days	0.80	0.03	40			
	8 days	0.50	0.05	18			
	10 days	0.79	0.05	20			
	12 days	1.00	0	12			
	to term	0.60	0.09	3			
27	6 days	0.42	0.06	8	3.49	2	0.170
	7 days	0.64	0.05	45			
	8 days	0.61	0.08	15			
	to term	0.55	0.07	13			

†Excluding the embryos incubated to term.

(Table 2), indicating that they had failed in the diploidization process. Hence, it is clear that a great majority of cells must be diploid to finish embryogenesis properly and to hatch.

In four crosses that produced haplodiploid embryos (nos 12–15) the mother possessed B chromosomes, which provided the possibility of analysing the diploidization process by comparing the number of Bs between haploid and diploid cells from haplodiploid embryos. As the B chromosome of *L. migratoria* is mitotically unstable from early embryogenesis (Pardo *et al.*, 1995b), the comparison has to be made statistically and independently for each cross. As Table 3 shows, the mean number of Bs was significantly greater in diploid than haploid cells from haplodiploid embryos in the four crosses. No significant differences were found, however, if the mean number of Bs in haploid cells was doubled (simulating the diploidization process). Hence, it is clear that the diploidization of the haploid eggs occurs by a doubling of the whole genome.

Parthenogenesis, fecundity and fertility

Fecundity in insects is usually measured in terms of egg production and fertility in terms of viable offspring (Chapman, 1976; Ridley, 1988). Table 4 shows the mean number of eggs and embryos per pod observed in each of the 27 crosses performed. The nonparametric Kruskal–Wallis ANOVA comparing the four types of crosses classified according to their primary sex ratio showed the absence of significant differences between cross types for the number of eggs per pod ($H = 4.68$, d.f. = 3, $P = 0.20$) and for the number of embryos per pod ($H = 6.30$, d.f. = 3, $P = 0.10$). This indicates that in absolute terms the females that reproduced by parthenogenesis were as fecund as those reproducing sexually. Given that a high proportion of the embryos produced by parthenogenesis did not complete embryogenesis or failed to hatch, and that many of those reaching the nymph stage died before becoming adult, it is clear that the number of viable offspring should be conspicuously lower than that measured by the total number of embryos because many are unviable. A possible approach to the number of viable offspring measured at embryo stage could be the number of embryos that were diploid in all analysed cells. As Table 1 shows, the production of diploid embryos was higher in Type I crosses and decreased progressively in Type II–IV crosses, and the differences were significant ($H = 19.70$, d.f. = 3, $P < 0.001$). Consequently, parthenogenesis was associated with a significant decrease in fertility. In fact, the proportion

Table 3 Comparison of the mean number of B chromosomes ($\overline{M}_B$) between haploid and diploid cells from haploidiploid embryos of *Locusta migratoria* by means of the Wilcoxon signed rank test

Type of cross	Cross no.	Bs in the mother	Diploid cells		Haploid cells		Wilcoxon ($2n$ vs. n)		Haploid cells		Wilcoxon ($2n$ vs. $n \times 2$)	
			$\overline{M}_B$	SE	$\overline{M}_B$	SE	T^+	P	$\overline{M}_B \times 2$	SE	T^+	P
I	13	3B	2.78	0.36	1.61	0.20	0	0.016	3.22	0.40	5.5	0.406
II	12	1B	1.11	0.18	0.60	0.09	0	<0.001	1.20	0.17	9	0.273
	14	3B	2.51	0.16	1.38	0.09	0	<0.001	2.75	0.18	48.5	0.102
	15	1B	1.33	0.25	0.73	0.12	6	0.007	1.47	0.24	0	1

of eggs containing an embryo was significantly higher in Type I crosses than in the remainder (Table 4) ($H = 8.68$, d.f. = 3, $P = 0.034$).

An analysis of egg and embryo productivity per day was performed in 26 crosses (excepting the cross no. 5, in which these data were not registered). The Kruskal–Wallis ANOVA showed no significant differences between the four types of crosses for either egg or embryo productivity. However, a grouping of the two types of crosses showing exclusively parthenogenetic reproduction (III and IV) and those showing reproduction exclusively or predominantly sexual (I and II) indicated that the differences in egg productivity were close to significant and those in embryo productivity were significant (Table 5), the number of embryos produced per day sexually being about double that of parthenogenetic crosses. This result corroborates that parthenogenesis decreases female fertility.

Discussion

Tychoparthenogenesis by gradual diploidy restoration

The *L. migratoria* females analysed in the present investigation have shown two modes of reproduction: most showed sexual reproduction exclusively, some produced all female offspring through parthenogenesis and still others yielded progeny by

Table 4 Average production of eggs and embryos per egg-pod by *Locusta migratoria* in 26 of the 27 crosses analysed (cross no. 5 is not included because these data are not known)

Type of cross	Cross no.	No. of pods	Eggs		Embryos		Embryos/eggs	
			$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
I	1	4	67.50	6.45	53.75	9.98	0.773	0.089
	2	4	48.50	3.33	10.00	4.24	0.196	0.078
	3	7	61.00	6.04	36.43	7.33	0.581	0.096
	4	4	59.75	2.25	55.50	2.90	0.927	0.016
	6	4	58.50	11.81	47.75	17.20	0.687	0.230
	7	3	72.67	5.49	53.33	13.68	0.715	0.153
	8	4	46.25	10.44	34.50	14.91	0.644	0.174
	9	5	44.00	3.90	21.20	7.97	0.433	0.151
	11	8	69.38	4.20	52.25	4.74	0.759	0.063
	13	5	68.80	3.34	42.20	11.18	0.596	0.158
	16	7	24.14	2.77	14.14	3.44	0.572	0.122
	Total	—	56.41	4.38	38.28	5.00	0.626	0.058
II	10	4	65.25	10.40	47.00	4.88	0.751	0.072
	12	6	75.17	4.01	27.83	9.08	0.368	0.112
	14	6	70.50	6.03	32.50	6.18	0.448	0.064
	15	4	55.75	8.61	18.50	5.07	0.319	0.059
	17	3	43.33	11.39	23.33	8.01	0.530	0.152
	Total	—	62.00	5.67	29.83	4.88	0.484	0.076
III	18	8	40.38	5.22	23.25	5.78	0.509	0.096
	19	5	32.00	9.38	11.00	5.04	0.246	0.106
	20	10	31.40	5.67	7.70	2.24	0.202	0.044
	21	6	43.50	5.71	24.00	6.32	0.492	0.121
	22	4	79.00	26.35	35.50	12.79	0.449	0.110
	23	4	36.75	4.15	17.75	3.25	0.494	0.092
	Total	—	43.84	7.29	19.87	4.10	0.399	0.056
IV	24	3	46.00	5.51	6.33	6.33	0.115	0.115
	25	4	63.75	8.29	40.50	13.05	0.570	0.178
	26	4	58.75	1.93	28.75	4.96	0.491	0.084
	27	5	59.00	4.64	12.40	8.80	0.183	0.117
	Total	—	56.88	3.80	22.00	7.78	0.340	0.112

The figures include some egg-pods in which all eggs lacked an embryo.

Table 5 Comparison of egg and embryo productivity per day between the four types of crosses differing in the degree of parthenogenetic reproduction

Type of cross	No. of crosses	Egg productivity per day		Embryo productivity per day	
		$\bar{x}$	SE	$\bar{x}$	SE
I	11	17.16	1.56	11.95	1.68
II	5	15.06	2.08	7.80	2.16
III	6	13.25	2.71	6.04	1.42
IV	4	13.41	1.41	5.27	1.88
Kruskal–Wallis test		$H = 3.76$, $d.f. = 3$, $P = 0.288$		$H = 6.04$, d.f. = 3, $P = 0.110$	
I+II	16	16.51	1.24	10.65	1.39
III+IV	10	13.32	1.65	5.73	1.08
Mann–Whitney test		$U = 115$, $P = 0.065$		$U = 119$, $P = 0.040$	

means of both processes. The observed parthenogenesis is a kind of thelytoky in that it leads to the production of females. Because it occurs only occasionally, this is a case of tychoparthenogenesis. The way in which diploidy is restored indicates that this is also a case of postmeiotic multiple conversions (according to the terminology introduced by Lamb & Willey, 1987). This kind of parthenogenesis has been reported in several species of Orthoptera (see Hewitt, 1979, for a review), and had formerly been reported in *L. migratoria* by Bergerard & Seugé (1959). Whereas these authors observed that the parthenogenetic females were always weak and generally incapable of laying eggs, those in the present report laid almost the same number of eggs as the sexual females.

The capability for parthenogenetic reproduction presumably depends on the accuracy in the diploidization process. Diploidy restoration in *L. migratoria* is a gradual process, as shown by: (i) the predominance of haplodiploid embryos among the offspring of unfertilized females; and (ii) the progressive decrease in the frequency of haplodiploid embryos over development parallelled by the increase in the frequency of diploid female embryos. Bergerard & Seugé (1959) showed that developmentally inhibited regions of parthenogenetic embryos contained a proportion of haploid cells higher than regions showing normal development. Consistent with this, we have observed that parthenogenetic embryos that failed to hatch, after incubation to term, were mainly haplodiploid and showed DI comparable to embryos of only 6 days of development. This suggests an intimate dependence of normal development on diploidy restoration. However, some parthenogenetically developed females were haplo-diplo-polyploid mosaics in their ovarioles, indicating that some haploid eggs may become adult even though diploidization is not perfect. Diploidy, nevertheless, seems to be necessary for parthenogenetic reproduction because none of these mosaic females laid eggs, whereas all females that did were perfect diploids in the ovarioles. Even the exceptional females from the third parthenogenetic strain, which laid eggs even though they were triploid or diplotriploid mosaic in their ovarioles (analysed by flow cytometry), yielded only three nymphs that died at an early stage.

DI has proven to be a good measure of the diploidization process along embryonary development. It increases progressively in embryos in parallel with developmental age. The B chromosome is also a good marker of the diploidization process because its frequency in diploid cells of haplodiploid embryos is about double that of haploid cells in the same embryos. Bergerard & Seugé (1959) made a similar observation, but they did not consider the mitotic instability of *L. migratoria* Bs and hence the intraindividual variation in embryos (Viseras *et al.*, 1990; Pardo *et al.*, 1995b).

These observations suggest that the most likely cytological mechanism for diploidization is the restitution of the sister products of early cleavage mitosis, as was proposed by Hewitt (1979). This phenomenon, however, does not explain the presence of triploid cells in 4.94 per cent of the 628 haplodiploid embryos analysed, but of tetraploid cells only in 0.96 per cent of them. Restitution should always lead to the production of cells with even numbers of genomic sets. In the 31 haplodiploid embryos that showed triploid cells, however, the frequency of $3n$ cells ($\bar{x} = 0.166$, SE = 0.031) was more than three times that of $4n$ cells in the six embryos that showed tetraploid cells ($\bar{x} = 0.049$, SE = 0.0005) ($t_{35} = 3.75$, $P = 0.0006$). Hence pheno-

mena other than restitution might also be involved, the most likely being cell fusion of haploid and diploid cells to give rise to triploid cells, although the fusion of haploid cells to become diploid, or else of diploid cells to form tetraploid cells, cannot be ruled out. In fact, a high tendency to cell fusion by haploid cells, compared with diploid cells, would explain the predominance of diploid and triploid cells in the haplodiploid mosaic parthenogenetic embryos. Cytological evidence of such cell-fusion phenomena is shown in Fig. 3.

Tychoparthenogenesis and reproductive output

The low rate of hatching and development to adulthood in tychoparthenogenetic grasshoppers is well known (Hamilton, 1955; Bergerard & Seugé, 1959; Smith, 1969), and our present results are consistent with this idea. In *L. migratoria*, this is not a result of decreased fecundity as parthenogenetic females showed about the same egg-clutch size and egg productivity per day as sexual females. Fertility of parthenogenetic females (measured as embryo productivity per day), however, was about half that of sexual females because of a significant decrease in the number of embryos per pod. Fertility and total reproductive output decreased as a consequence of parthenogenetic reproduction. Hamilton (1953) reported a similar result in *Schistocerca gregaria* parthenogenetic females, i.e. similar numbers of eggs per pod but lower numbers of nymphs emerging from the eggs.

Since males of *L. migratoria* transfer, with the ejaculate, nutrients that are used by the females for egg production (Pardo *et al.*, 1994a), it is surprising that the rate of egg production did not decrease significantly in the parthenogenetic females even though some of them never mated (e.g. Type IV females). A direct relationship between the rate of copulation, ejaculate nutrient transfer and female fitness, has been recently shown in the grasshopper *Eyprepocnemis plorans* (Pardo *et al.*, 1995a). In *L. migratoria*, perhaps ejaculate nutrient contribution to eggs relative to that of the female was not very high because the females were allowed feed *ad libitum* and under these circumstances male investment loses importance (Gwynne, 1984; Butlin *et al.*, 1987; Simmons & Bailey, 1990; Mullins *et al.*, 1992).

Although one of the advantages of parthenogenesis, with respect to sexual reproduction, is an increase in the reproductive output as a result of the exclusive production of females (White, 1973; Williams, 1975; Cuellar, 1977; Maynard Smith, 1977, 1978; Nur, 1989), the low fertility showed by the parthenogenetic females in *L. migratoria* makes this kind of reproduction clearly disadvantageous with respect to sexual reproduction in this species. This low fertility is reflected in a decreased number of embryos developing from unfertilized eggs and is most likely the result of the imperfections of this parthenogenetic system, i.e. the gradual diploidy restoration, and/or to an imprecise induction system, which could be some of the reasons why regular parthenogenesis has not evolved in this species. A possibility remaining to be tested is that parthenogenesis in *L. migratoria* is induced by endosymbiont bacteria similar to those inducing parthenogenesis in some hymenopterans (Stouthamer, 1989; Breeuwer *et al.*, 1992; Rousset *et al.*, 1992; Hurst, 1993; Louis *et al.*, 1993; Stouthamer *et al.*, 1993)

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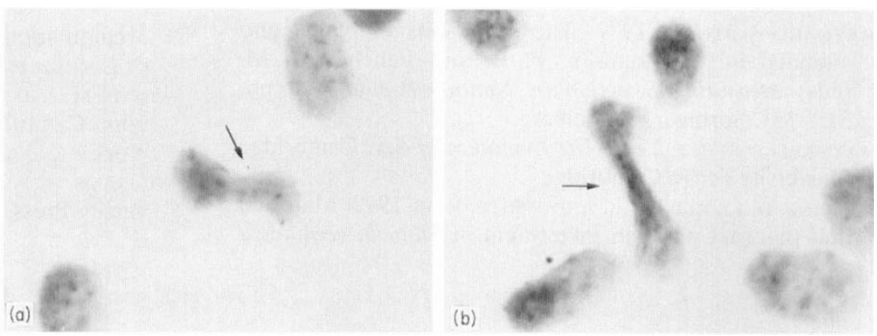

Fig. 3 Cell fusion in haplodiploid mosaic embryos of *Locusta migratoria*. In both cases the nuclei fusing (arrow) appear to be haploid whereas some of the surrounding nuclei seem to be diploid.

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