Genet Sel Evol (1994) 26, 413-430

Original article

The genetic control of ovariole number in *Sitophilus oryzae* L (Coleoptera, Curculionidae) is temperature sensitive

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(Received 3 May 1993; accepted 29 April 1994)

Summary – The influence of genetical and epigenetical factors on the determinism of the ovariole number was studied in *Sitophilus oryzae*. This weevil normally possesses 4 ovarioles with apical bacteriomes harbouring symbiotes. By selecting females with a reduced number of ovarioles, the genetic control of this trait was demonstrated with regard to the easy selection of this character and its similar transmission to the progeny by females and males. Several genes could be implicated and a weak maternal effect cannot be rejected. The character is temperature sensitive: at 30°C, abnormal females with 2 or 3 ovarioles prevail, and conversely, normal females appear at 20°C. The reduction of the number of ovarioles is correlated with a decrease in fitness (reduced progeny and lighter body weight of females). Only ovary formation seems to be affected, because a higher progeny per ovariole in abnormal females can be indicative of a normal yolk production in the fat body. Counting bacteria in ovaries shows that the regulation of symbiote number per female occurs at the ovariole itself and not within the whole insect.

***Sitophilus oryzae* / ovariole number / selection / genetic control / temperature-sensitive character**

Résumé – Le contrôle génétique du nombre des ovarioles chez *Sitophilus oryzae* L (Coleoptera, Curculionidae) est sensible à la température. *L'influence de facteurs génétiques et épigénétiques sur le déterminisme du nombre des ovarioles a été étudiée chez* Sitophilus oryzae. *Ce charançon possède normalement 4 ovarioles terminés par un bactériome apical hébergeant des symbiotes. En sélectionnant les femelles ayant un nombre réduit d'ovarioles, nous avons pu démontrer le contrôle génétique de ce caractère : sélection aisée et transmission à la descendance aussi bien par les femelles que par les mâles. Plusieurs gènes pourraient être impliqués et un léger effet maternel se manifeste par ailleurs. Le caractère est thermo-sensible : à 30° C, les femelles anormales à 2 ou 3 ovarioles sont majoritaires, alors que, à 20° C, le phénotype normal prédomine. La réduction du nombre des ovarioles est corrélée avec une diminution de la fitness*

(descendance réduite et poids corporel des femelles plus faible). Seule la formation de l'ovaire semble être affectée, car une fertilité plus grande par ovariole chez les femelles anormales indiquerait une production normale de vitellus dans le corps adipeux. Les dénombrements de bactéries dans les ovaires ont montré que la régulation du nombre de symbiotes par femelle intervient au niveau de l'ovariole lui-même, plutôt qu'au niveau de l'insecte entier.

***Sitophilus oryzae* / nombre d'ovarioles / sélection / contrôle génétique / caractère thermosensible**

INTRODUCTION

Sitophilus oryzae, the rice weevil, is a major cereal pest. The factors affecting the biotic potential have been studied by several authors (Birch, 1945; Segrove, 1951; Nardon, 1978a), but little attention was focused on genetic factors, particularly those affecting reproduction. The present study completes previous observations concerning the variability of the structure of the female genital tract and the influence of various factors (Nardon and Grenier, 1983).

S oryzae females have 2 ovaries, each divided into 2 ovarioles. This structure is typical of the *Sitophilus* genus, as well as of the majority of Rhynchophorinae (Murray and Tiegs, 1935; Vernier, 1970; Ganesalingam, 1974). At the anterior tip of each ovariole, a bacteriome containing intracellular bacteria is formed (Mansour, 1930; Nardon, 1971). This apical bacteriome disappears in the absence of symbiotes (Nardon, 1973).

On account of their economic importance, these weevils have been extensively studied and seem to possess great genetic stability with regard to the few morphological anomalies which have been observed. In *S granarius*, a wing malformation was noticed by Strong (1959), and in *S oryzae* 2 mutations have been described: one affecting antenna and called 'fused antennae' (Campbell-Brown and Champ, 1971) and the other modifying rostrum and elytra morphology (Nardon and Nardon, 1983). In another Curculionidae, *Hypera postica*, some females with 5 ovarioles were observed in a natural population by Hower (1971) but with a very low frequency (0.1–0.2%). The same observation has been made for *S oryzae* (Nardon and Grenier, 1983).

However, after irradiation, different kinds of ovarial anomalies could be obtained: either of functional (absence of oogenesis) or structural origin (variable ovariole number) (Nardon, 1978b; Nardon and Grenier, 1983). Structural anomalies were the most frequent when young larvae were irradiated during ovary formation since irradiation acts directly on the differentiation process. The persistence of these anomalies in the next generations, as well as the appearance of abnormal females in the progeny of irradiated fathers suggested a genetic determinism.

In *Drosophila*, genetic studies have shown that the ovariole number varies from 1 strain to another often with a geographical pattern and seems to be under the control of a polygenic system (De Scheemaeker-Louis, 1970, 1971; Thomas-Orillard, 1975; Capy *et al*, 1993). In some Scarabaeinae, Pluot (1979) observed reduction of the number of ovarioles during the larval development, from 6 primitive ovarioles

to only 1 definitively formed ovariole. This phenomenon is genetically controlled and is the result of a regressive phylogenic evolution.

In *S oryzae*, a newly introduced laboratory strain was found to possess some females with only 2 or 3 ovarioles. We thought that this would be a good opportunity to start selection on the reduction of the ovariole number with this strain, in order to study the genetic control (chromosomal and epigenetical) in the determinism of the ovariole number in *Sitophilus*. With such a strain, which normally presents a reduced number of ovarioles, we had the advantage, contrarily to structural anomalies previously artificially obtained in the laboratory, of rejecting additional effects of irradiation treatment. It was also interesting to study the influence of the reduction of ovariole number on fertility and female body weight, as well as on the regulation of the number of ovarial symbiotes. During experiments on selection, we found that a decrease in temperature modified ovariole distributions, so we studied the effect of temperature on this character more carefully.

MATERIALS AND METHODS

Breeding and test for temperature influence

The *S oryzae* strain used for selection (called the W strain) was found on wheat in a grocery in Lyon and breeding was conducted on wheat, in latticed plastic boxes kept in ventilated incubators at 27.5°C and 75% RH (Laviolette and Nardon, 1963).

For temperature effects on the ovariole number, 4 temperatures were tested: 20, 23.5, 27.5 and 30°C. In order to limit the 'female effect', we tested homogeneity of 3 series of females which we first allowed to lay eggs at 27.5°C for 4 d, before each was transferred to another temperature for a 1-week egg-laying period.

Selection procedure and crossings

At its arrival in the laboratory, the W strain presented 5 females out of 197 with structural ovarial anomalies: 4 females with 3 ovarioles and 1 with 2 ovarioles. The selection was conducted from the progeny of these abnormal females. At the beginning of the selection, only females with 1, 2 and 3 ovarioles were allowed to give progeny for the next generation (G_0 to G_8). After this time, selection was only applied in G_{12}, G_{35} and G_{36}, when the percentage of abnormal females was decreasing (see table I).

For genetic experiments, virgin adults were obtained by isolating oviposited wheat kernels in cavities bored in a polyurethane plate and covered with a glass sheet. Egg-laying density was chosen so as to have only 1 imago emerging from a kernel. After sex determination and before crossings, weevils were kept separated on wheat until sexual maturity.

Crosses were effected between the abnormal line selected for a reduced number of ovarioles (called A line) and the original normal line with 4 ovarioles (called N control line and bred without selection). These crosses were conducted at 27.5°C with generation G_6, at the beginning of the selection, and with G_{50}, when the character was well established in the population. For each experiment we studied parental F1 and F2 hybrid generations as well as the reciprocal crosses in G_{50}. The

Table I. Selection of the 'reduced ovariole number' character and distribution of ovariole number in *S oryzae* females over 50 generations.

Generation		*Percentage of females*[a]					*No of females dissected*	*No of ovarioles (mean)*
		4 ov	*3 ov*	*2 ov*	*1 ov*	*0 ov*		
0	*	97.46	2.03	0.51			197	3.97
2	*	66.67	26.39	6.94			72	3.60
3	*	67.52	26.75	5.73			157	3.62
4	*	59.04	28.51	12.45			249	3.46
5	*	36.84	32.46	30.70			114	3.06
6	*	38.81	34.23	26.00	0.76	0.19	523	3.11
7	*	22.65	33.39	42.93	0.95	0.07	4 507	2.78
8	*	3.16	17.89	78.42	0.53		190	2.24
9		13.10	28.43	55.94	2.44	0.10	985	2.52
10		9.84	21.62	67.05	1.99		953	2.38
11		13.30	27.85	57.17	1.68		955	2.53
12	*	8.86	21.14	68.00	2.00		355	2.34
13		5.91	17.28	74.51	2.29		1 134	2.27
14		5.91	19.09	73.03	1.97		508	2.29
16		3.45	14.32	80.36	1.88		957	2.19
17		12.90	20.97	63.71	2.42		124	2.44
18		5.71	19.43	72.57	1.71	0.57	175	2.28
19		6.93	22.77	67.66	2.64		303	2.34
20		5.00	12.00	83.00			100	2.22
22		7.44	22.73	68.18	1.65		242	2.36
23		2.02	15.24	82.27	0.47		643	2.19
25		2.33	30.23	67.44			43	2.35
35	*	13.39	33.39	52.28	0.94		635	2.59
36	*	13.09	30.76	55.16	0.99		1 115	2.56
40		7.99	25.13	66.45	0.44		1 365	2.41
50		3.21	15.03	81.04	0.73		965	2.21

[a] Percentage of females having the various numbers of ovarioles (ov). * Selection was applied at this generation (only females with 1, 2 and 3 ovarioles were allowed to give progeny for the next generation).

phenotype could be determined easily in females by dissecting ovaries, but in males, we named the brothers of females of N line with 4 ovarioles 'normal' males, and those of females with 1–3 ovarioles 'abnormal' males .

Determination of biological parameters

Fertility

The whole progeny of a female was difficult to measure because the total egg-laying time was longer than 30 weeks. Therefore, the fertility was estimated by the mean number of progeny per female per day for a 1-week egg-laying period, at the time of maximal fertility (2 or 3 weeks after adult emergence from the grain).

Sex ratio

Total female and male numbers were noted throughout the experiments, but the sex ratio was not taken into account in the results, because it remained stable (close to 1).

Weight

Adult females were individually weighed on an electromagnetic balance (Setaram $\gamma 21$) with a 10^{-2} mg precision.

Ovariole number

After weighing, females were dissected in a Yeager solution (pH 6.8, pO 400 mosm; Nardon and Grenier, 1983). The last tergite was lifted with small forceps to extract the genital apparatus *in toto*. In the 'abnormal' line, ovaries could show from 0 to 4 ovarioles.

Determination of symbiote number in ovaries

Symbiotes are rod-shaped and more or less flexuous bacteria (Nardon, 1971). They are located in a bacteriome at ovariole tips in trophocytes and oocytes. Their number was estimated by counting in a Thoma's cell under a phase-contrast microscope. For each female, ovaries were crushed in 0.1 ml Yeager solution. At the same time, the morphology of the ovariole tip (single or double) was also recorded.

RESULTS

Selection of the 'reduced number of ovarioles' character at 27.5°C

Selection of the abnormal A line was studied on 50 successive generations. Dissections of females were effected on each generation at the beginning of the selection, but only on some of them at the end. Results are reported in table I, while the relative evolutions of ovariole mean numbers in both A line and N control line appear in figure 1. Heterogeneous numbers of dissected females in successive generations were due to 2 different strategies: genetic experiments with numerous dissections and simple records of selection with few females.

At G_0, only 4 females with 3 ovarioles and 1 with 2 ovarioles were observed in the total population of 197 females (ovariole mean number $\overline{x} = 3.97$). By selecting abnormal females for egg laying, we obtained 33% of abnormal females in G_2 ($\overline{x} = 3.60$) and 6 generations after (G_8), we got a line with nearly 97% of females having a reduced ovariole number ($\overline{x} = 2.24$). After this time, every female of the progeny contributed to the next generation without selection of females with a low number of ovarioles and percentages of abnormal females remained around 87%. With just a new selection of abnormal females at G_{12}, the character stabilized near 95% anomalies without selection until G_{34}. At G_{35} and G_{36}, selection was practised because the percentage of abnormal females was under 87%. After this

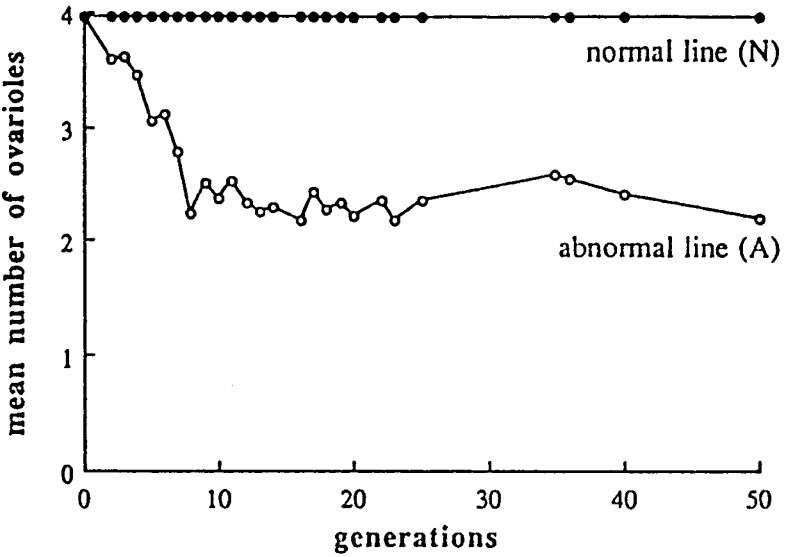

Fig 1. Evolution of the mean ovariole number in the selected (A) line and in the control (N) line during 50 successive generations of selection for a reduced number of ovarioles.

Table II. Ovariole distribution in progeny of females with 4 ovarioles, but from abnormal lines, and in crossings between these females (G_{50}) and normal males from control line.

Generation[a]	*Percentage of females*[b]					*No of females dissected*	*No of ovarioles (mean)*
	4 ov	*3 ov*	*2 ov*	*1 ov*	*0 ov*		
G_0	97.46	2.03	0.51			197	3.97
G_6	36.67	36.67	23.33	3.33		60	3.07
G_7	25.05	35.49	38.69	0.7	0.06	1 561	2.85
G_{12}	17.86	33.93	47.32	0.89		112	2.69
G_{30}	18.82	34.12	45.88	1.18		85	2.71
G_{50}	1.92	21.15	76.92			52	2.25
♀ A × ♂N (G_{50})	56.30	25.19	18.52			135	3.38

[a] ♀ with 4 ov; [b] percentage of females having the various numbers of ovarioles (ov).

time no more selection was applied, and at the end of the experiment (G_{50}) a well-established character was obtained with 96.8% ovarial anomalies and an ovariole mean number of 2.21. A minimum of 2 ovarioles seemed to be an asymptotic value.

The effect of selection could also be observed on the progeny of females showing a normal phenotype with 4 ovarioles (table II). The mean number of ovarioles decreased according to the generation from 3.97 (G_0) to 2.25 (G_{50}). At each generation, matings were effected between brothers and sisters, but only progenies

of females with 4 ovarioles were analyzed in this case. We found that mothers with 4 ovarioles gave abnormal daughters and anomaly percentages increased throughout selection. It was evident that the male genotype has been modified by selection; nevertheless, in G_{50}, the high percentage of abnormal daughters cannot be explained by genotypic modification of males only. At this generation, crosses between females with 4 ovarioles and abnormal males would give progeny similar to F1 hybrids (see AN cross in table IV below) with 54% anomalies, but we obtained 98% anomalies, like in crosses between abnormal females and males. Moreover, we could verify with hybrids between abnormal selected females in G_{50} and males from the normal line, that at this time, the anomaly percentage is intermediate between parents (44%). Therefore, the genotype of these females was modified, even if their phenotype was normal.

At each generation, we could also observe a relationship between the proportion of females with 3 ovarioles and the proportion of females with 4 and 2 ovarioles. This relation could be expressed by the following formula:

$$f(3\,\mathrm{ov}) = \sqrt{f(4\,\mathrm{ov}) \times f(2\,\mathrm{ov})}$$

where the frequency of 3 ovariole females is about the square root of the product of frequencies of females with 2 and 4 ovarioles (geometrical mean).

These results proceeded from observed ratios. At the beginning of the selection, females with 4, 3 and 2 ovarioles were in the ratio 9:3:1 in G_3 ($\chi^2 = 1.77$, 2 *df*), 4:2:1 in G_4 ($\chi^2 = 0.74$, 2 *df*), 1:1:1 in G_5 ($\chi^2 = 0.68$, 2 *df*) and after this time, the proportions were inverted. For example, they were 1:2:4 ($\chi^2 = 0.61$, 2 *df*) in G_9, 1:3:9 ($\chi^2 = 1.20$, 2 *df*) in G_{12}, 1:4:16 ($\chi^2 = 0.57$, 2 *df*) in G_{18} and 1:5:25 ($\chi^2 = 0.36$, 2 *df*) in G_{50}. The χ^2 tests calculated on 23 generations (observations on 15 562 females) showed that the experimental ratios were compatible with the theoretical ones: with 2 *df*, $\chi^2 = 5.99$ at the 5% significance level.

Crosses between normal and reduced ovariole number lines at 27.5°C

Crosses at the G_6 generation

Crosses between N and A lines were conducted at 27.5°C, and results appear in table III. At the 6th generation, the anomaly percentage was only about 61% in the parental A line ($\bar{x} = 3.11$). In F1 hybrids, distributions of ovarioles in both reciprocal crosses were not significantly different at the 5% level ($\chi^2 = 4.09$; 2 *df*) but they were very different from the parental A population: $\chi^2 = 187.7$ for the ♀ A × ♂ N (AN) cross and $\chi^2 = 346.7$ for the ♀ N × ♂ A (NA) cross, with 2 *df*. Therefore, the ovariole mean numbers were both very close (3.75 in AN and 3.80 in NA) but higher than arithmetical ($\bar{x} = 3.54$) or geometrical ($\bar{g} = 3.51$) parental means. Therefore, from this experiment, it could be thought that neither sex-linked effects nor epigenetical factors were involved.

As egg-laying F1 females had heterogeneous phenotypes (4, 3 or 2 ovarioles), the F2 obtained was difficult to analyse. We have thus reported in table III only the composition of the F2 produced by F1 females with 4 ovarioles in order to appreciate the 'disjunction' of the character. It seemed more important in AN (hybrids which inherited the abnormal mother cytoplasm) than in NA (hybrids with a normal

Table III. Crosses between abnormal (A) and normal (N) lines at the 6th generation of selection.

Generation	*Crosses*	*Couple number*	*Percentage of females*[a]					*No of females*	*No of ovarioles (mean)*
			4 ov	*3 ov*	*2 ov*	*1 ov*	*0 ov*		
P	♀ A × ♂ A	(41)	38.81	34.23	26.0	0.76	0.19	523	3.11
	♀ N × ♂ N	(?)	97.46	2.03	0.51			197	3.97
F1	♀ A × ♂ N	(41)	79.22	16.14	4.64			539	3.75
	♀ N × ♂ A	(45)	83.30	13.07	3.63			1 102	3.80
F2	♀ AN × ♂ AN	(20)	60.60	24.53	14.18	0.69		3 779	3.45
	♀ NA × ♂ NA	(9)	84.14	12.77	3.09			517	3.81

P = parental generation; F1 and F2 = 1st and 2nd generations. In F2, only progeny of F1 females with 4 ovarioles are reported. [a] Percentage of females with the various numbers of ovarioles (ov).

cytoplasm). Therefore, the smaller number of ovarioles in AN ($\overline{x} = 3.45$) than in NA ($\overline{x} = 3.81$) may suggest a weak maternal effect.

Crosses at the G_{50} generation

These results appear in table IV. At the 50th generation of selection, the abnormal line (A) reached 95% anomalies and the normal line (simultaneously bred but without selection) had only 2.8% anomalies. It could be noted that the original line N did not vary, because the ovariole mean number stayed unchanged between G_0 and G_{50} (G_0 $\overline{x} = 3.97$ and G_{50} $\overline{x} = 3.96$).

Table IV. Crosses between the abnormal line (A) and the normal line (N) at the 50th generation of selection.

Generation	*Crosses*	*Parental couple number*	*Percentage of females*[a]					*No of females*	*No of ovarioles (mean)*
			4 ov	*3 ov*	*2 ov*	*1 ov*	*0 ov*		
P	♀ A × ♂ A	(23)	5.06	20.68	73.84	0.42		237	2.30
	♀ N × ♂ N	(9)	97.20	1.87	0.93			214	3.96
F1	♀ A × ♂ N	(64)	45.94	27.31	26.75			714	3.19
	♀ N × ♂ A	(46)	53.49	23.03	22.93	0.33	0.22	916	3.29
BC	♀ AN × ♂ N	(30)	72.15	17.72	10.13			474	3.62
	♀ NA × ♂ A	(35)	23.79	22.58	53.23	0.40		496	2.70
F2	♀ AN × ♂ AN	(13)	53.57	22.14	23.57	0.71		140	3.29

P = parental generation; F1 and F2 = 1st and 2nd generation of crosses and BC = backcrosses between F1 hybrids and parents. [a] Percentage of females having the various numbers of ovarioles (ov).

In F1, AN and NA reciprocal hybrids showed anomaly percentages and ovariole mean numbers which were intermediate between those of parents (arithmetical mean $\bar{x} = 3.13$ and geometrical mean $\bar{g} = 3.02$). It is noteworthy that the 2 distributions were significantly different ($\chi^2 = 9.87$; 2 *df*, $p < 0.01$), as well as their mean numbers of ovarioles ($\varepsilon = 2.47$). The F1 hybrids have abnormal cytoplasm (AN) and showed more anomalies than the reciprocal (perhaps due to a maternal effect?). The mean number of ovarioles of ♀ AN × ♂ AN F2 progeny (obtained from a random sample of F1 females with 4, 3 or 2 ovarioles) was not significantly different from F1 values ($\varepsilon = 1.20$ for AN F1 and $\varepsilon = 0.12$ for NA F1).

By comparing every ovariole mean number in the different crosses, we got significant differences as follows: $\bar{x}$ P AA < $\bar{x}$ $BC_{NA\times A}$ < $\bar{x}$ ($F1_{AN}$ = F2 = $F1_{NA}$) < $\bar{x}$ $BC_{AN\times N}$ < $\bar{x}$ P NN.

This relation is typical of a polygenic character, but nevertheless back-crosses had ovarial anomaly percentages which were intermediate between those of the F1 hybrid and those of the parent used in the cross (27.85% anomalies in ♀ AN × ♂ N and 76.21% in ♀ NA × ♂ A). Theoretical ovariole mean numbers in back-crosses could be estimated by the means between F1 hybrids and parents and were very close to the observed values:

- in ♀ AN × ♂ NN: $\bar{x} = 3.61$ or $\bar{g} = 3.59$ *versus* 3.62 observed;
- in ♀ NA × ♂ AA: $\bar{x} = 2.78$ or $\bar{g} = 2.74$ *versus* 2.70 observed.

In the same analysis, the F2 distribution can be treated as a mixture with 1/4 N genotype, 1/4 A genotype and 1/2 F1 genotype.

Temperature influence on ovariole number

The effect of temperature on the 'reduced ovariole number' character was studied at 2 different times of selection: in the 12th and 23rd generations.

In G_{12}, the selected character was well established (anomaly percentage higher than 90%) and the ovariole distribution was stable for several generations (see table I). After egg laying, grains were kept at 2 different temperatures: 27.5°C (normal temperature for laboratory weevil breeding) and 23°C. These results appear in table V.

When the temperature decreased from 27.5 to 23°C, the distribution of ovarioles in the progeny was modified: percentage of normal females increased from 5.9 to 24.7% while the percentage of 2 ovarioles was halved (74.5 to 35.6%) and that of 3 ovarioles doubled (17.3 to 39.6%). The mean ovariole number increased from 2.27 to 2.89 ovarioles per female. This study was carried out on 4 successive generations by selecting progeny of females with 2 and 3 ovarioles. Ovariole mean numbers observed in F1 stayed nearly unchanged in F2 and F4, with only the same decrease in ovariole mean numbers at the 2 temperatures, due to the selection pressure.

In G_{23}, for a better study of the effects of temperature on the character expression, egg laying and larval development were performed at 20, 23.5, 27.5 and 30°C (temperatures compatible with insect development). To avoid 'female effects', progenies of the same females were compared at 27.5°C and at another temperature. The 3 series were homogeneous at 27.5°C ($\chi^2 = 4.26$ with 4 *df*), and so the results were grouped. These results are reported in table VI.

Distributions were clearly different as a function of temperature ($\chi^2 = 540$ with 6 *df*; $p < 0.001$). The increase of temperature enhanced the expression of the

Table V. Ovariole distribution in females as a function of temperature (27.5 and 23°C) at the 12th generation of selection (observations were made on 4 generations F1 to F4).

Temperature (°C)	*Generation*	*Percentage of females*[a]				*No of females*	*No of ovarioles (mean)*
		4 ov	*3 ov*	*2 ov*	*1 ov*		
27.5	F1	5.91	17.28	74.51	2.29	1 134	2.27
	F2	5.91	19.09	73.03	1.97	508	2.29
	F4	3.45	14.32	80.36	1.88	957	2.19
23	F1	24.70	39.58	35.57	0.15	672	2.89
	F2	19.35	32.66	46.77	1.21	248	2.70
	F4	16.79	32.99	49.34	0.88	685	2.66

[a] Percentage of females having the various numbers of ovarioles (ov).

Table VI. Distribution of the number of ovarioles in females as a function of temperature (23rd generation of selection).

Temperature (°C)	*Percentage of females*[a]				*No of females*	*No of ovarioles (mean)*
	4 ov	*3 ov*	*2 ov*	*1 ov*		
20	44.07	29.15	26.78	0	295	3.17
23.5	17.31	34.17	47.84	0.68	439	2.68
27.5	2.02	15.24	82.27	0.47	643	2.19
30	0.72	3.24	94.96	1.08	278	2.04

[a] Percentage of females having the various numbers of ovarioles (ov).

character (55.93% ovarial anomalies at 20°C *versus* 99.28% at 30°C) and modified the ovariole distribution (26.78% females with 2 ovarioles at 20°C *versus* 94.96% at 30°C, for example). There was a negative linear correlation between ovariole mean number and temperature ($r = -0.992$).

Relationships between reduced number of ovarioles and biological parameters

Fertility

For both A and N lines, we represented the mean number of the progeny obtained per female and per d, at the time of maximal fertility, as a function of the ovariole number in females. Different selection times were considered, G_{12}, G_{29} and G_{50} (table VII).

Selection on the A line also seemed to reduce the fertility because the observed values in the A line were always much lower than in the unselected N line. Nevertheless, although weaker, the fertilities obtained with the selected line could be compared with each other because females are the same age at egg-laying time

Table VII. Female fertility as a function of the ovariole number.

No of ovarioles	*Progeny (per female per d)*[a]			
	Normal line (N)	*Abnormal line (A)*		
		G_{12}	G_{29}	G_{50}
4	6.19 ± 0.51 (46)	2.84 ± 0.54 (11)	2.85 ± 0.98 (4)	3.60 ± 0.66 (16)
3	5.49 ± 1.75 (5)	2.35 ± 0.36 (23)	2.46 ± 0.29 (14)	3.36 ± 0.35 (26)
2	5.00 ± 0.67 (4)	1.86 ± 0.27 (57)	2.02 ± 0.29 (29)	2.91 ± 0.27 (41)
1	–	–	1.42 (2)	2.43 (2)

N = normal line; A = abnormal line at different generations (G_{12}, G_{29} and G_{50}); egg-laying female number in parentheses. [a] At maximum fertility.

and experiments were conducted in the same abiotic conditions. We noted a linear correlation between the progeny number per female and the number of ovarioles of the mother (all correlation coefficients were close to 1). In the abnormal line, we also observed that for the same ovariole number, there was a tendency to a fertility restoration across generations which might be due to natural selection.

Female body weight

To investigate the effect of ovariole number on female weight, in the G_{42} generation, we weighed and then dissected 2 female samples (A and N lines). The results appear in table VIII.

In the A line, mean weights of the 3 series (4, 3 and 2 ovarioles) were not significantly different from each other at the 5% level ($t < 0.58$), but were very different from the N line (t from 4.6 to 12.10). In the selected A line, the ovariole number of females did not appear to have any influence on weight, but the body weights of the females were significantly lower than those of N line females.

Table VIII. Female weights in normal (N) and abnormal (A) lines as a function of the ovariole number.

Line	*No of ovarioles*	*Female weight ± tS/VN** *(mg)*	*No of females*	*Student's test*
Normal (N)	4	1.664 ± 0.422	74	a
Abnormal (A)	4	1.367 ± 0.119	9	b
	3	1.351 ± 0.369	38	b
	2	1.337 ± 0.326	82	b

a, b = results of Student t test. Means with the same letter were not different at the 5% significance level. $P < 0.05$.

Ovarial symbiote number per female

The ovarial bacteriome, which is located at the tip of each ovariole, is filled with symbiotes. This bacteriome generally appears as a single structure. In the A line, when the ovary could not differentiate into 2 entire ovarioles, the apical bacteriome could sometimes be divided, and the unique ovariole showed 2 apical more or less well-separated bacteriomes. Morphological studies of these phenomena were made by Nardon and Grenier (1983).

The symbiote density per female, as well as the apical bacteriome aspect, were studied as a function of ovariole number at the G_{50} generation, and the results are reported in table IX.

Table IX. Symbiote number per female as a function of ovariole number and apical bacteriome structure at the G_{50} generation.

No of ovarioles	*Bacteriome*	*No of symbiotes per female*	*No of females*	*No of repetitions*
4	single	82 365 ± 6 380	14	224
3	single	63 957 ± 5 370	15	240
2	single	41 587 ± 3 970	26	416
2	double	79 785 ± 7 082	16	256
1	single	17 188 ± 7 035	2	32

When apical bacteriomes were normal (single), the female had a symbiote number nearly proportional to the ovariole number. The correlation coefficient was 0.998 and the regression line of symbiote number per female (y) as a function of the ovariole number (x) was of the form:

$$y = 21\,790\,x - 3\,201$$

In females with 2 ovarioles, but when the bacteriome was divided, even partially, the symbiote number per ovariole was twice as high.

DISCUSSION

Genetic control of the ovariole number

In *Drosophila*, the study of the influence of irradiation on genesis of the female genital apparatus (Geigy, 1931; Aboïm, 1945) showed that its differentiation is largely independent of the germ cells. Nevertheless, in the absence of germ cells, the ovarioles never develop. Therefore, according to Aboïm, the complete morphogenesis of ovaries results from an interaction between germ cells and mesodermal cells.

In *S oryzae*, the genesis of ovaries was described by Murray and Tiegs (1935) and Tiegs and Murray (1938). In the early embryo, the germ cells arise by migration of cleavage cells into the periplasm, at the posterior pole of the egg, and become

associated with symbiotic bacteria. The future bacteriocytes of ovaries also develop very early and, when the germ band is formed, they become associated with the germ cells. At the end of the 2nd day, the germ cells come in close contact with the adjacent coelomic sacs and their mass divides into right and left halves. The investing sheath of the gonad arises from the splanchnic wall of the posterior coelomic sac. The genital ducts develop on the 4th day from the mesodermal cells that ensheath the gonad. Shortly before the larva emerges, a pair of solid stalks are formed at the base of the 9th segment where they impinge on the epidermis. Therefore, in the young larva, rudimentary ovaries appear as 2 spherical bodies embedded in the fat body. These ovaries grow and divide into 2 pear-shaped bodies which rapidly expand during the larval stage. In the early prepupa, the development proceeds more rapidly, the stalks lengthen and a lumen is formed. The imaginal disk differentiates into vagina and oviduct. During pupation, the ovarial tubules elongate and bacteriomes containing intracellular symbiotes are formed at the tip of each ovariole (Mansour, 1930; Nardon, 1971). They disappear in the absence of symbiotes (Nardon, 1973). The ovary divides from the apex to the oviduct, and all intermediate structures can be found from 4 to 2 typical ovarioles (Nardon and Grenier, 1983). This suggests a factor of division acting at the apex of ovaries, perhaps on the terminal filament, as proposed by King *et al* (1968) and Eiche (1972) in *Drosophila*, where, during ovarial morphogenesis, the terminal filament seems to determine of the ovariole number. Such a phenomenon probably occurs in *Sitophilus*.

From this description, it appears that the formation of ovaries results from 3 main events. In the absence of germ cells, it is probable that the rudiment of ovaries is not formed in the egg, and this would explain the fact that some females are completely devoid of ovarioles (table I). This is the first level of control. The second level is the division of the mass of germ cells. In the absence of this division, we obtain females with only 1 ovariole (table I). The third level, and the more frequently affected, is in the larva and the young pupa, where the single ovaries may divide. When the 2 rudimental ovaries divide, 4 ovarioles are obtained, when only one divides, 3 ovarioles are formed, and only 2 ovarioles occur in the absence of division.

In the *S oryzae* female, our results clearly show, as previously expected (Nardon and Grenier, 1983), the presence of a genetic determinism of the number of ovarioles. This character is easily affected by selection and, at 27.5°C, only 8 generations were necessary to decrease the mean number of ovarioles from 3.97 to 2.24, with no more reliable progress afterwards. The genetic control can be demonstrated by crosses in the G_6 and G_{50} generations, since males are as able as females to transmit the genetic factor(s) to their daughters. No sex-linked effect is detectable. The question arises as to the nature of the genetic system of control involved.

In G_{50}, the F1 values are significantly different from each other at the 5% significance level, and therefore, it is possible that a maternal effect also occurs. If we consider the complexity of the morphogenesis, this suggests the probable

intervention of several genes, which could be reinforced by the observation of the following relationship between means, evoking a polygenic system:

$$P_1 < BC_{P1} < (F1 = F2) < BC_{P2} < P2$$

In *Drosophila melanogaster*, numerous studies have dealt with the influence of the genome on the ovariole number (Robertson, 1957; Teissier, 1958; Melou, 1961). David (1961) showed that the ovariole number character was rapidly and completely transmitted by males and depended on nuclear polygenic hereditary factors. De Scheemaeker-Louis (1970) described a polygenic system where the estimated number of genes varied between 4 and 12, 12 being the most probable value. Thomas-Orillard (1975) located the genes on chromosomes II and III. These genes have additive effects with interactions of dominance type, as shown by a positive correlation between high number of ovarioles and numerous dominant genes (Thomas-Orillard, 1982). A maternal effect associated with the presence of a picornavirus also occurs (Thomas-Orillard, 1984; Thomas-Orillard and Jeune, 1985) which increases the number of ovarioles, and at the same time reduces the mean development time and gives heavier females.

Evidence for temperature sensitivity of the character

In *Sitophilus*, the 'reduced ovariole number' character was enhanced by a temperature increase (remaining in biological conditions), leading to a negative linear correlation between the mean of ovariole number and temperature. Penetrance and expressivity of the character varied as a function of temperature: at 30°C, the penetrance was almost complete (95%) with most of the females with 2 ovarioles, while at 20°C the penetrance only reached 56% with females having 2 or 3 ovarioles in nearly equal quantity. The different expressivity (2 or 3 ovarioles) depends on the moment when the division is elicited or suppressed.

This temperature-sensitive effect can be compared with Wool and Mendlinger's observations (1973) on a spontaneous morphological mutation in *Tribolium castaneum* larva and pupa: 3 or 4 urogomphi could be seen on the terminal abdominal segments in mutants instead of 2 in normal insects. The penetrance of the gene was variable: incomplete at 30°C and full at 25°C.

Many temperature-sensitive mutations have been described in *Drosophila*: in mutations affecting pteridine concentration (lethal mutation at 29°C and viable mutation at 22°C, Grigliatti and Suzuki, 1970); in eye facet arrangement (heterozygous females are of wild type at 29°C and mutant type at 21°C, Foster and Suzuki, 1970); and in a homeotic mutation affecting imaginal discs, giving normal arista segment of the antennal complex at 29°C and a tarsus at 17°C, with all the intermediate forms (Grigliatti and Suzuki, 1971).

The variation in thoracic bristle number described by Louis *et al* (1988) in *D simulans* is also temperature sensitive. The expressivity of this trait was mediated by a hereditary virus (DSV). At extreme temperatures (17 and 28°C) wild phenotypes were observed, whereas the abnormal character was expressed between these limits (Garcia-Vazquez and Comendador, 1989). In *Sitophilus*, it would be interesting to search for the presence of such a virus or another microorganism,

using antibiotics or infestation experiments. It must be recalled that the symbiotic bacteria of *S oryzae* seem to enhance the rate of ovarial anomalies, which are significantly reduced in aposymbiotic weevils (Nardon and Grenier, 1983).

Relationships with other biological characters

Fertility and female body weight

In *S oryzae*, female fertility is correlated with the ovariole number, both in normal (N) and in abnormal (A) lines, but selection reduced fertility of A lines by more than 50%. The same phenomenon was also observed in *Drosophila* (De Scheemaeker-Louis, 1970). It might be thought that whatever the genetical determinism is, it acts essentially (or exclusively) on ovary formation and does not modify yolk production in the fat body. A compensation can take place during vitellogenesis. As a matter of fact, regulation of vitellogenesis seems to occur at the individual level, because a female with 2 ovarioles gives more progeny per ovariole than a female with 4 ovarioles:

- in the N line: 2.5 progeny/ovariole with 2 ov mothers *versus* 1.55 with 4 ov mothers;
- in the A line: 1.46 progeny/ovariole with 2 ov mothers *versus* 0.90 with 4 ov mothers.

Conversely, female body weight is not correlated with ovariole number (females of the abnormal line have similar body weights), but abnormal line females were significantly lighter than those of the normal line. Several hypotheses may explain these decreases in fitness (fertility and weight).

1) The decreasing number of ovarioles by itself should have diminished the female body weight, but this hypothesis can be rejected because a variation from 2 to 4 ovarioles in the abnormal line has no effect on the body weight.

2) A consanguinity effect may have occurred because selection started with a small female number and selection pressure is high. Therefore, an increase in homozygosity might be directly responsible for the reduction in the reproductive fitness of selected strains (Lerner, 1954, in Pyle, 1976).

3) Selection can lead to the mortality of a part of the population by the expression of lethal genes when homozygous.

4) An epigenetical factor may interfere, such as the maternally inherited DSV virus (Louis *et al*, 1988) in *D simulans*, which can modify the thoracic bristle number but also decreases fertility and viability in its host (Comendador *et al*, 1986).

5) The lower weights are possibly not the result of selection, but rather the consequence of a faster stabilization of body weight in the laboratory. Indeed, we used to observe that laboratory breeding in optimal conditions and without selection always induces an increase in body weight. When the W strain was introduced in the laboratory, adult body weights were very light (♀ = 1.23 ± 0.07 mg and ♂ = 1.16 ± 0.07 mg). Forty-two generations later, female weights (1.66 ± 0.04 mg) were a third higher than the weights at arrival. On the other hand, when selection was applied, the body weight became stable faster but at a lower level. This phenomenon can be explained by the coselection of genes influencing the body weight, by a faster selection of recessive homozygotes or perhaps more simply by consanguinity effects.

6) A positive genetical correlation between ovariole number and fertility on one hand, and ovariole number and body weight on the other might explain the selection response observed, when selecting on the 'ovariole number' criterion.

Ovarial symbiote number per female

Despite the fact that in *Sitophilus* ovaries symbiotes are harboured in not only apical bacteriomes but also oocytes and trophocytes, when the apical bacteriomes are normal (single) the symbiote number is nearly proportional to the ovariole number. On the contrary, when the ovariole tip is divided (even partially), the symbiote number per ovariole is doubled. Therefore, the regulation of the symbiote number seems to happen at the ovary itself and not in the whole organism.

The observation of a double apical bacteriome could suggest an effect on the division of the terminal filament of the ovariole, as described above.

CONCLUSIONS

To conclude, our results suggest that the number of ovarioles in the *S oryzae* weevil can be controlled by several genes acting in the larva and young pupa, to allow the division of the rudimental ovaries. Epistatic interactions are also acting with the embryo morphogenesis. The expression of this morphogenetic system is highly sensitive to temperature. The selection for a lower number of ovarioles affects the fitness of the weevil and particularly reduces the fertility (despite a compensating effect) and the female body weight.

REFERENCES

Aboïm AN (1945) Développement embryonnaire et post-embryonnaire des gonades normales et agamétiques de *Drosophila melanogaster*. *Rev Suisse Zool* 52, 53-154

Birch LC (1945) The biotic potential of the small strain of *Calandra oryzae* and *Rhizopertha dominica*. *J Anim Ecol* 14, 125-127

Campbell-Brown M, Champ BR (1971) A fused-antenna mutant of *Sitophilus oryzae* L. *J Stored Prod Res* 7, 217-220

Capy P, Pla E, David JR (1993) Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *Drosophila simulans*. I. Geographic variations. *Genet Sel Evol* 25, 517-536

Comendador MA, Plus N, Louis C, Lopez-Ferber M (1986) Endemic microorganisms of a *Drosophila simulans* strain and their relationships with the non-mendelian transmission of a character. *Genet Sel Evol* 18, 131-144

David J (1961) Etude quantitative du fonctionnement ovarien chez *Drosophila melanogaster*. *Bull Biol Fr Belg* 95, 521-535

De Scheemaeker-Louis M (1970) Variation génétique estimée d'après la réponse du nombre d'ovarioles à la sélection dans deux populations expérimentales de *Drosophila melanogaster* Meigen. *Arch Biol (Liège)* 31, 495-631

De Scheemaeker-Louis M (1971) Sur une tendance à la régulation du nombre d'ovarioles chez *Drosophila melanogaster*. *Ann Genet* 14, 219-223

Eiche A (1972) Effects of sublethal X-ray doses on the number of ovarioles in *Drosophila melanogaster* populations. *Hereditas* 71, 253-258

Foster GG, Suzuki DT (1970) Temperature-sensitive mutations in *Drosophila melanogaster*. IV. A mutation affecting eye facet arrangement in a polarized manner. *Proc Nat Acad Sci USA* 67, 738-745

Ganesalingam VK (1974) Morphological studies on the differentiation in the ovary of the adult *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). *Ceylon J Sci* 11, 1-8

Garcia-Vazquez E, Comendador MA (1989) Temperature effect on the morphological S character related to DSV hereditary reovirus in *Drosophila simulans*. *J Invert Pathol* 54, 1-9

Geity R (1931) Action de l'ultra-violet sur le pôle germinal dans l'œuf de *Drosophila melanogaster* (castration et mutabilité). *Rev Suisse Zool* 38, 187-288

Grigliatti T, Suzuki DT (1970) Temperature-sensitive mutations in *Drosophila melanogaster*. V.A mutation affecting concentrations of pteridines. *Proc Nat Acad Sci USA* 67, 1101-1108

Grigliatti T, Suzuki DT (1971) Temperature-sensitive mutations in *Drosophila melanogaster*. VIII. The homeotic mutant, ss^{a40a}. *Proc Nat Acad Sci USA* 68, 1307-1311

Hower AA Jr (1971) An abnormality in the reproductive system of field-collected alfalfa weevils, *Hypera postica*. *Annals Ent Soc Amer* 64, 951-952

King RC, Aggarwal SK, Aggarwal U (1968) The development of the female *Drosophila* reproductive system. *J Morphol* 124, 143-166

Laviolette P, Nardon P (1963) Action des rayons γ du cobalt 60 sur la mortalité et la fertilité des adultes d'un charançon du riz. *Bull Biol Fr Belg* 97, 305-333

Louis C, Lopez-Ferber M, Comendador M, Plus N, Kuhl G, Baker S (1988) *Drosophila* S virus, a hereditary reolike virus, probable agent of the morphological S character in *Drosophila simulans*. *J Virol* 62, 1266-1270

Mansour K (1930) Preliminary studies on the bacterial cell mass (accessory cell-mass) of *Calandra oryzae* the rice weevil. *Q J Microsc Sci* 73, 421-436

Melou JP (1961) Etude du nombre d'ovarioles chez diverses souches françaises et japonaises de *Drosophila melanogaster*. *Ann Gen* 3, 25-28

Murray FV, Tiegs OW (1935) The metamorphosis of *Calandra oryzae*. *Q J Microsc Sci* 77, 404-495

Nardon C, Nardon P (1983) Etude morphologique et génétique de certaines anomalies du rostre et des élytres chez le charançon *Sitophilus granarius* L (Col Curculionidae). Isolement d'une variété à rostre court. *Bull Soc Ent Fr* 88, 284-292

Nardon P (1971) Contribution à l'étude des symbiotes ovariens de *Sitophilus sasakii*: localisation, histochimie et ultrastructure chez la femelle adulte. *C R Acad Sci* 272D, 2975-2978

Nardon P (1973) Obtention d'une souche aposymbiotique chez le charançon *Sitophilus sasakii* Tak: différentes méthodes et comparaison avec la souche symbiotique d'origine. *C R Acad Sci* 277D, 981-984

Nardon P (1978a) Étude des interactions physiologiques et génétiques entre l'hôte et les symbiotes chez le Coléoptère Curculionide *Sitophilus sasakii* (= S *oryzae*). Thèse de doctorat, INSA-Université Lyon I, IDE 78003, 2 vol

Nardon P (1978b) Etude de l'action des rayons X sur les symbiotes ovariens et l'ovogénèse chez *Sitophilus oryzae* L (Col Curculionide). *Bull Soc Zool Fr* 103, 295-300

Nardon P, Grenier AM (1983) Etude des divers types d'anomalies ovariennes rencontrées chez le charançon *Sitophilus oryzae* L (Col Curculionidae). Action de divers facteurs expérimentaux: sélection, antimétaboliques, irradiation, absence de symbiotes. *Bull Soc Ent Fr* 88, 292-300

Pyle DV (1976) Effects of artificial selection on reproductive fitness in *Drosophila*. *Nature* (*Lond*) 263, 317-319

Pluot D (1979) Evolution régressive des ovarioles chez les Coléoptères Scarabaeinae. *Ann Soc Ent Fr* 15, 575-588

Robertson FW (1957) Studies in quantitative inheritance. X. Genetic variation of ovary size in *Drosophila*. *J Gen* 55, 410-427

Segrove F (1951) Oviposition behaviour in the two strains of the rice weevil, *Calandra oryzae*. *J Exp Biol* 28, 281-297

Strong RG (1959) To the teratology of the granary weevil, *Sitophilus granarius* (Linnaeus) (Coleoptera: Curculionidae). *Wasmann J Biol* 17, 63-68

Teissier G (1958) Distinction biométrique des *Drosophila melanogaster* françaises et japonaises. *Ann Genet* 1, 2-10

Thomas-Orillard M (1975) Tentative de localisation des gènes qui président au déterminisme du nombre d'ovarioles chez la drosophile. *Archiv für Genetik* 48, 116-127

Thomas-Orillard M (1982) Structure d'effets du système multifactoriel qui modèle la morphogénèse ovarienne de la Drosophile. *Arch Zool Exp Gén* 122, 455-465

Thomas-Orillard M (1984) Modifications of mean ovariole number, fresh weight of adult females and developmental time in *Drosophila melanogaster* induced by *Drosophila* C virus. *Genetics* 107, 635-644

Thomas-Orillard M, Jeune B (1985) Gene actions involved in determining the number of ovarioles and sternite chaetae in freshly collected strains of *Drosophila melanogaster*. *Genetics* 111, 819-829

Tiegs OW, Murray FV (1938) Embryonic development of *Calandra oryzae*. *Q J Microsc Sci* 80, 159-284

Vernier JM (1970) Anatomie et histologie des ovaires et de l'appareil génital de *Sitophilus granarius* (Col Curculionidae). *Ann Soc Ent Fr* 6, 243-265

Wool D, Mendlinger S (1973) The eu mutant of the flour beetle, *Tribolium castaneum* Herbst. Environmental and genetic effects on penetrance. *Genetica* 44, 496-504