

Secondary sex ratio adjustment in a pseudo-arrhenotokous insect, *Hypothenemus hampei* (Coleoptera: Scolytidae)

*L'ajustement du sex-ratio secondaire
chez un insecte pseudo-arrhénotoque,
Hypothenemus hampei (Coleoptera: Scolytidae)*

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RÉSUMÉ

La théorie de la sélection naturelle par compétition locale pour les partenaires sexuels (LMC) prédit que les espèces endogames effectuant la totalité de leur développement, ainsi que leur accouplement, en habitat extrêmement fragmenté, présentent des sex-ratios biaisés en faveur des femelles. Chez de telles espèces, les femelles contrôlent le sex-ratio de leur progéniture en produisant des œufs fécondés, donnant des femelles diploïdes, et des œufs non fécondés, donnant des mâles haploïdes (arrhénotoque). Le scolyte du café, *Hypothenemus hampei*, présente de telles caractéristiques, à la différence près que ses mâles sont fonctionnellement haploïdes, mais caryologiquement diploïdes (pseudo-arrhénotoque). Néanmoins, nous montrons ici qu'en conditions expérimentales, les femelles *H. hampei* contrôlent avec précision le sex-ratio de leur progéniture. Cependant, l'ajustement du sex-ratio en fonction du nombre de fondatrices en compétition au moment de la ponte pourrait être simplement dû à la conjonction de 2 facteurs : la précocité de l'éclosion des mâles et la limitation de la taille de la fratrie adulte imposée par la capacité portante du milieu. Ainsi, bien qu'en accord qualitatif avec les prédictions théoriques, la réponse aux conditions locales pourrait dans ce cas s'avérer purement fortuite en ce sens qu'elle ne résulterait pas d'une adaptation du comportement de la mère au moment de la ponte. Nous remarquons que les taux d'endogamie estimés dans les populations naturelles de *H. hampei*, bien qu'étant inférieurs à l'attendu théorique chez une espèce où les croisements entre frères et sœurs seraient la règle, restent compatibles avec les conditions de la LMC. ▲

Mots clés : scolyte du café, *Hypothenemus hampei*, Scolytidae, endogamie, pseudo-arrhénotoque, compétition locale pour la reproduction, sex-ratio, capacité du milieu, taille de ponte.

ABSTRACT

The natural selection theory of local mate competition (LMC) predicts that inbreeding species undergoing complete development and reproduction within small patches of habitat have female-biased sex ratios. In such species, mothers control the sex ratio of their brood by laying either fertilized eggs that develop into diploid females or unfertilized eggs that develop into haploid males (arrhenotoky). The coffee berry borer, *Hypothenemus hampei*, has some of the features of a typical LMC species. However, males are functionally haploid but karyologically diploid (pseudo-

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arrhenotoky). Here, we showed experimentally that female *H. hampei* nevertheless precisely control the sex ratio of their brood. Also, the proportion of males increases with the number of competing foundresses, a result qualitatively in accordance with the theory. However, such a response could be the fortuitous result of the earlier recruitment of males versus females, together with the presumption that the carrying capacity of an experimental plug of rearing medium imposed a limit to brood size at maturity. In addition, we noted that the estimates of the inbreeding coefficient in natural *H. hampei* populations are substantially lower than the theoretical expectations for a species undergoing regular sib-breeding, although they remain consistent with partial inbreeding resulting from the colonization of habitat patches by a few (1-3) mothers. This situation, although not being "extreme" inbreeding, still meets the conditions for LMC. ▲

Key words: coffee berry borer, *Hypothenemus hampei*, Scolytidae, inbreeding, pseudo-arrhenotoky, local mate competition, sex ratio, carrying capacity, brood size.

VERSION ABRÉGÉE

L'existence de sex-ratios déséquilibrés chez un certain nombre d'espèces d'insectes a conduit au développement de la théorie de la compétition locale pour les partenaires sexuels (LMC). L'habitat caractéristique de telles espèces est extrêmement fragmenté, les croisements y ont lieu entre frères et sœurs, et les femelles fécondées s'en échappent pour coloniser de nouveaux fragments d'habitat. Celles-ci y déposent leurs œufs, qui s'y développent jusqu'à la maturité. Dans ce contexte, la sélection naturelle tend à minimiser la part de l'effort reproductif consacrée aux descendants mâles, et à favoriser ainsi l'apparition d'un sex-ratio biaisé en faveur des femelles. La théorie de la LMC prédit également que les femelles colonisatrices sont capables d'ajuster le sex-ratio de leur descendance en fonction de la présence ou non d'autres femelles, car le sex-ratio optimal de leur progéniture dépendra de la présence, ou non, de familles non apparentées partageant le même fragment d'habitat.

Ainsi que le prédit la théorie, les espèces endogames montrent des sex-ratios biaisés en faveur des femelles; les mâles sont en général sédentaires et de taille réduite, et les femelles sont capables de moduler le sex-ratio de leur progéniture en fonction des conditions locales (taille de l'habitat, compétition avec d'autres femelles pour le site de ponte). L'arrhenotokie, système de reproduction où les mâles, haploïdes, sont issus d'œufs non fécondés et les femelles, diploïdes, d'œufs fécondés, permet aux femelles de contrôler et d'ajuster le sex-ratio de leur progéniture au moment de la ponte. La situation d'endogamie est supposée avoir facilité l'évolution de l'haplo-diploïdie, mais toutes les espèces haplo-diploïdes ne sont pas nécessairement endogames.

Le scolyte de la cerise du café, *Hypothenemus hampei*, présente la plupart des caractéristiques des espèces soumises à la LMC, dont un sex-ratio fortement biaisé en faveur des femelles, mais son mode de reproduction est de type pseudo-arrhenotokie: cette espèce est caryologiquement diplo-diploïde, et fonctionnellement haplo-diploïde. Bien que de par son impact économique, *H. hampei* soit un des Scolytidae les mieux étudiés à ce jour, nous n'avons relevé aucune donnée dans la littérature sur la distribution des sex-ratios en fonction du niveau de compétition entre mères pour le site de ponte, ni sur la variance du nombre de mâles par fratrie. De telles données nous permettent ici de tester l'adaptativité du sex-ratio biaisé chez cette espèce pseudo-arrhenotokie. Nous avons réalisé l'infestation expérimentale de pastilles de milieu semi-artificiel par des femelles *H. hampei* matures disposées selon un plan aléatoire, soit isolément, soit par groupes de 2 ou 3, dans les puits de plaques à microtitration. A la fin de l'expérience (qui a duré 2 mois à une température de $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$), nous avons mesuré les proportions des sexes dans chaque famille, estimé la variance interfamilles du sex-ratio secondaire (ici défini

comme la proportion de mâles chez les descendants parvenus à l'état adulte) et comparé celle-ci à la variance binomiale afin de déterminer si la mère est capable de contrôler avec précision le sex-ratio de sa progéniture, et de l'ajuster en fonction du nombre d'autres mères simultanément présentes sur le site au moment de la ponte.

L'information fournie par 183 familles incomplètes (en ce sens qu'une partie des individus étaient encore immatures à la fin de l'expérience) a été utilisée pour établir la dynamique d'apparition des mâles, à l'aide de la courbe du nombre de mâles en fonction de la taille de la partie mature de la fratrie. Nous avons observé que le sex-ratio moyen par fratrie décroissait d'une valeur approximative de 0,2 lorsque peu d'individus étaient parvenus au stade pupe ou imago, vers la valeur asymptotique de 0,08 lorsque le sexe de tous les descendants était connu. Ainsi, le sex-ratio secondaire est indépendant de la taille de la fratrie, sauf pour les fratrices de petite taille, où les mâles sont en surproportion par rapport à la valeur asymptotique. Lorsqu'il était possible de déterminer quel individu avait, le premier, atteint la maturité, celui-ci était un mâle dans 20 % des cas. La proportion de mâles dans 74 fratrices complètement matures était de 0,082, le nombre de mâles dans une fratrie étant étroitement corrélé à celui des femelles (test de corrélation de rangs; $p < 0,01$). Les fratrices de taille supérieure à 16 possédaient pour la plupart au moins 2 mâles. Plus d'un mâle par fratrie est probablement nécessaire pour assurer la fécondation des femelles dans les délais les plus brefs – et, ainsi, la colonisation de nouvelles cerises de café – car un mâle ne peut féconder en moyenne que 2 ou 3 femelles par jour. Par ailleurs, nos observations permettent d'envisager qu'un certain taux de mortalité aléatoire affecte la fratrie. La production de mâles surnuméraires (par rapport à un seul mâle par fratrie) serait alors adaptative, puisque les fratrices constituées uniquement de femelles seraient perdues. En effet, les femelles vierges ne quittent pas leur cerise d'origine, et leurs œufs n'éclosent pas.

La variance du sex-ratio secondaire, estimée pour chaque taille de fratrie adulte sur l'ensemble des fratrices, complètes ou incomplètes, fondées par une seule femelle ($N = 257$) est en général inférieure à la variance binomiale (comparaison des rangs sur les différences entre observations par paires; test unilatéral; $p < 0,05$). Ce résultat, ajouté à l'observation d'un sex-ratio asymptotique, donc indépendant de la taille de la fratrie, renforce l'idée selon laquelle les femelles *H. hampei* sont capables de contrôler avec précision le sex-ratio de leur progéniture, cela selon un mécanisme qui reste incompris. Sont-elles pour autant capables de modifier l'ajustement du sex-ratio en fonction du nombre de femelles en compétition pour le site de ponte? A première vue, nos résultats sont qualitativement en accord avec les prédictions de la théorie de la LMC, en ce sens que le sex-ratio

augmente de façon significative avec le nombre de femelles fondatrices (test de Friedman ; $p < 0,02$). Cependant, une analyse approfondie de l'ensemble des résultats montre qu'un tel ajustement du sex-ratio secondaire peut résulter non pas d'un ajustement du sex-ratio primaire, mais d'une mortalité supérieure des femelles liée à la séquence de ponte. En effet, la capacité du milieu est limitée à un nombre fixe d'adultes (environ 13 à 15), quel que soit le traitement expérimental (c'est-à-dire 1, 2 ou 3 femelles placées à pondre simultanément sur la même pastille de milieu). Comme la proportion de mâles augmente au fur et à mesure que la taille de la fratrie diminue, on s'attend à retrouver, fortuitement, un sex-ratio secondaire augmentant avec le nombre de fondatrices, puisqu'alors la taille moyenne d'une fratrie individuelle diminue. De fait, selon ce modèle, les valeurs du sex-ratio secondaire prédites empiriquement à partir de la courbe de la proportion de mâles en fonction de la taille de la fratrie (données obtenues sur 183 familles incomplètes fondées par une seule femelle) permettent de prédire que les familles complètes fondées par 1, 2 et 3 femelles présenteront des sex-ratios secondaires respectivement égaux à 0,105, 0,145 et 0,170. Ces chiffres, en particulier les 2 derniers, ne sont pas significativement différents des chiffres observés en réalité. Ainsi, l'ajustement du sex-ratio secon-

daire en fonction du niveau de compétition entre femelles pour le site de ponte pourrait tout simplement s'expliquer par la conjonction de 2 facteurs : la précocité de la maturité chez les mâles par rapport aux femelles et la limitation de la capacité du milieu, c'est-à-dire de la taille de la fratrie parvenant à l'âge adulte. Dans ce contexte, la précocité des mâles pourrait être perçue, là encore, comme un caractère adaptatif.

Le scolyte du café présente donc l'ensemble des caractéristiques reconnues chez les espèces fortement endogames soumises aux conditions de LMC, à la différence près qu'il s'agit ici, pour la première fois, d'un insecte pseudo-arrhénotoque. Les valeurs des taux d'endogamie estimés chez *H. hampei* à l'aide de marqueurs enzymatiques ou de marqueurs phénotypiques (résistance aux insecticides) révèlent qu'une proportion substantielle des croisements a lieu, dans la nature, entre individus non apparentés. Au premier abord, ces observations pourraient paraître contradictoires avec les taux d'endogamie extrêmes qu'on s'attend à observer chez une espèce où les croisements entre frères et sœurs seraient la règle, mais elles restent compatibles avec les taux d'appariement attendus sous l'hypothèse que chaque cerise du café est colonisée, dans la nature, par un petit nombre de femelles (1-3). Ces conditions restent bien caractéristiques d'une situation de LMC. ▲

The occurrence of extraordinary sex ratios in some species of insects has led to the development of the theory of local mate competition (LMC; [1-4]). Typical LMC species reproduce locally within patches of habitat, brothers mate their sisters, and fertilized females disperse to lay offspring in new patches of habitat. Restricting mating to such extremely divided populations means that a few males are sufficient to inseminate the more numerous females present in the patch. Natural selection for maximizing the colonizing efficiency of a mother's offspring in the long term results in sex ratios biased towards females. Selection for minimal allocation to males is similarly expected to result in the production of dwarfed and/or flightless males. Natural selection is also expected to favour some plasticity in offspring sex ratio as a response to the level of competition exerted by the presence of other foundresses in the same patch of habitat, because the optimal sex ratio in a brood depends on the presence or absence of other broods [3]. In accordance with the general expectations from the LMC theory, female-biased sex ratios occur in a number of inbreeding insects and mites [1, 4]; males are often smaller and less mobile than females [4]; females produce a greater proportion of sons in response to conspecific females in parasitoid wasps [2, 4], fig wasps [5, 6] and mites [4, 7]. The evolution of adaptive sex ratio adjustment in response to local conditions requires a sex determination mechanism allowing maternal control [4, 8, 9]. LMC species usually have diploid females and haploid males (arrhenotoky; [1]). This mode of sex determination enables females to control both the sex and the sex ratio of their offspring at oviposition, by regulating the sperm's access to the egg. The question of whether haplodiploidy has evolved in LMC species, or the LMC niche has been invaded by haplodiploid species is yet subject to debate [10]. Chronic inbreeding will facilitate the evolution of haplodiploidy, but for many haplodiploid species inbreeding is likely to be a derived condition.

The coffee berry borer, *Hypothenemus hampei*, a beetle of the family Scolytidae, has some of the features of a typical LMC species (reviews in [11] and [12]). Fertilized females tunnel into coffee berries where they lay their brood. The offspring undergo complete development inside the berry, from which mostly if not only [13] inseminated daughters escape to colonize new berries. As predicted by the LMC theory, males are outnumbered by females, and are smaller and flightless. Inbreeding species of the family Scolytidae often have an haplodiploid mode of sex determination [11], but pseudo-arrhenotoky [8, 14-16] has been inferred to be the mechanism of sex determination in *H. hampei* [17, 18]. In this case, both sons and daughters develop from fertilized eggs, and unfertilized eggs do not hatch [19]. *H. hampei* is karyologically diploid [17, 20, 21] but sons apparently neither express nor transmit to F2 the genes they inherit from their fathers [18]. One set of chromosomes, presumably the paternal chromosomes, is heterochromatinized [20, 21] and is presumed to be eliminated after meiosis I. Pseudo-arrhenotoky is thought to allow the mothers to control and manipulate the sex ratio of their broods, although the underlying mechanism is unknown [15, 16].

In the following and throughout this article we term "secondary sex ratio" the proportion of males observed among offspring at the adult (pupa or imago) stage, and we use the term "brood" without distinction for the offspring of either single females or groups of females put together to oviposit. Some data on the effect of competition among *Hypothenemus hampei* mothers on their offspring's secondary sex ratio and brood size have been reported by Ticheler [22] (summarized in [11]). The average proportion of sons apparently increased with the number (set as 1 and 2) of mothers infesting a coffee berry, but decreased again in berries experimentally colonized by 4 mothers. Also, the inferred average *per capita* brood size apparently decreased when the number of found-

dresses increased. However, distributions of sex ratios and brood sizes within experimental groups were not provided, thus allowing no estimation of sex ratio variance and no test of adaptive sex ratios. In a different context, Moore *et al.* [23] found that the inferred *per capita* brood size decreased with an increasing number (1-6) of females infesting a berry, a result in accordance with the hypothesis of competition among families for food resource. No data on sex ratios were available from this study. Apart from the incomplete results provided by Ticheler [22], we are not aware of any experimental setup designed to test the adaptiveness of sex ratio biases in inbreeding Scolytidae. This was the purpose of the present experiments. Here we tested whether *Hypothenemus hampei* females were able to manipulate the sex ratio of their broods under conditions of competition. Groups of females were induced to oviposit on artificial medium. The response, in terms of brood size and secondary sex ratio, was investigated. We report that the proportion of males in a brood had an asymptotic value and that its variance was less than binomial, thereby suggesting the presence of some mechanism for precise sex ratio control. The proportion of males significantly increased with the number (1, 2, or 3) of competing mothers, in accordance with the qualitative expectations of the LMC theory. However, the mechanism by which sex ratio was adjusted may simply consist of the truncation of brood size in conditions where resources are limited, together with an average earlier recruitment of males *versus* females.

Materials and methods

Emerging females (*i.e.* mature, attracted by light, hence presumed to be fertilized and ready to lay offspring; [13]) were randomly chosen from a laboratory population obtained from samples collected in New Caledonia and mass-reared for over 10 generations in the laboratory. These were allowed to oviposit in the U-shaped wells of 96-well microtitre plates containing rearing medium. Six-mm wide discs were bored off from a 5-mm thick layer of freshly prepared rearing medium using a cork-borer and plugged into each well. Each well thus contained on average \pm SD 0.137 ± 0.005 g freshly prepared rearing medium, corresponding to 0.046 ± 0.003 g dry weight, as measured on a sample of 30 plugs before and after 4 days in a stove at 65° C. The rearing medium was derived from a previous recipe [24]. It consisted of 4 g/100 ml (%) wheat germ, 1% casein, 2% sucrose, 15% green coffee berry powder, 0.175% methyl-P-hydroxybenzoate, 0.12% Na propionate, 0.03% sorbic acid, 0.025% penicillin, 0.025% streptomycin, 0.25% ascorbic acid and 0.1% van der Zant vitamins in 3.5% agar, poured as an even 5-mm thick layer into a mold and subsequently dried for 6 h in a stove at 50° C.

Groups of 1-3 emerging females were placed according to a random design in microtitre plate wells filled with rearing medium. The plates were covered with glass slides, placed into airtight plastic bags to avoid moisture loss and left in darkness at $28 \pm 1^\circ$ C. All mothers were removed 1 month after the start of the experiment, and their offspring were allowed to continue development for 1 more month, after which the experiment was stopped. We thereby prevented the occurrence of a second generation in

the experiment, because 2 months are the approximate duration for complete development [13]. Each plug of infested medium was then dissected under a dissecting microscope. The offspring in each plug were sorted according to their developmental stage (egg, larva, male or female pupa, male or female imago) and counted. Families in which at least 1 of the founding females died during the first month of the experiment were discarded. Cells in which females did not lay eggs (*i.e.* 9.7% of 1-mother, 14.3% of 2-mother and 13.4% of 3-mother groups) or in which none of the eggs laid hatched (24.1%, 13.6% and 13.8% of the remainder, respectively) were also excluded. Only data from broods ($N = 74, 36$ and 28 , respectively) where all individuals were pupae or imagos, hence could be sexed, were retained for the analysis of the relationship between the number of founding females and their brood size and secondary sex ratio. In all the other families, a proportion of eggs and/or larvae were still present at the end of the experiment. The information from these families hereafter designated "partially mature" was used independently for addressing the question of timing in the production of males and for estimating an asymptotic sex ratio. Among these, the 1-mother families ($N = 183$) were of particular interest to that purpose.

The null hypothesis of no correlation between the number of sons and the number of daughters in a brood was tested using Spearman's test of rank correlation. The null hypothesis of no effect of treatment (that is, 1-mother, 2-mother and 3-mother setups) on brood size was tested using 1-way Kruskal-Wallis non-parametric analysis of variance (ANOVA). The null hypothesis of no effect of treatment on brood sex ratio was tested, after grouping brood sizes into 6 classes (1-4, 5-8, 9-12, 13-16, 17-20 and 21-26), using Friedman's test for randomized blocks [25] in lieu of 2-way ANOVA. Ideally, grouping should be avoided, to remove a possible effect of brood size on sex ratio, especially in the lower range of brood size values; but grouping was necessary to avoid empty brood size classes. Both Kruskal-Wallis' statistic (H) and Friedman's statistic (χ^2) were compared to the χ^2 distribution with 2 degrees of freedom [25]. Spearman's and Kruskal-Wallis' statistics were computed using the package Biomeco [26]. To test whether the variance of secondary sex ratio in 1-mother broods was less than binomial, we compared the variance estimate of the proportion of males to the expected binomial variance for a given brood size by means of a 1-tailed non-parametric test for paired observations [27]. The binomial variance was estimated as $n^2pq/(n-1)$ with n = sample size for a given brood size and p (q) = average frequency of males (females) in that class.

Results

The information from partially mature 1-mother broods showed the following. The average sex ratio, plotted as a function of mature brood size, decreased slightly from an initial value of about 0.2 to an asymptotical value of approximately 0.08 (Fig. 1). The sex ratio was independent of brood size, except for small brood sizes. We observed a trend towards adult males being produced earlier than expected if their recruitment to a brood were random, as shown by the initial sex ratios being usually larger than the asymptotical value (Fig. 1). We also observed that the first

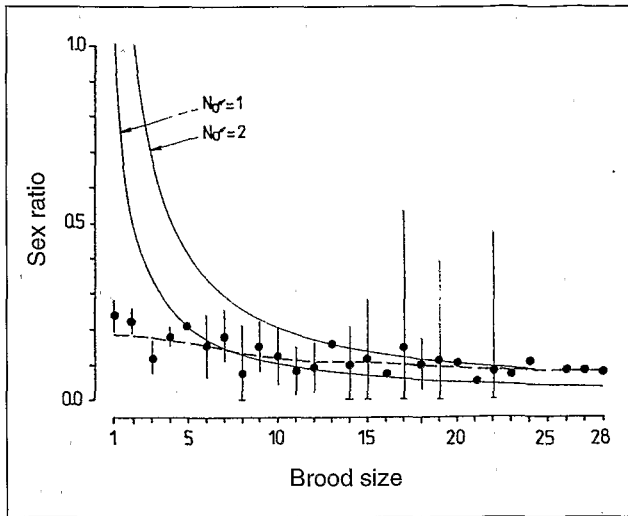


Figure 1. *Hypothenemus hampei*. Average sex ratio (defined as the proportion of sons in a brood) plotted as a function of mature brood size. Dotted curve represents the actual data, smoothed using 5-order mobile mean. Solid curves represent the modelled sex ratios for 1 male/brood and 2 males/brood. Vertical bars are the \pm SD confidence intervals around mean. Data are from 183 partially mature 1-foundress broods reared on semi-artificial medium for 2 months, with the mother allowed to oviposit for the first month.

individual to achieve development was a male in 20 % of 1-mother broods for which it was possible to determine which individual matured first. Random recruitment of males to a brood would have corresponded to a much lower value (8.9%). The model of 1 male per brood did not fit the actual data (Fig. 1). New males were recruited quite regularly as brood size increased (Fig. 2). There was no male-less brood of size ≥ 15 , at which stage about 40% broods already contained 2 males, and about 10% broods already contained 3 males (Fig. 2).

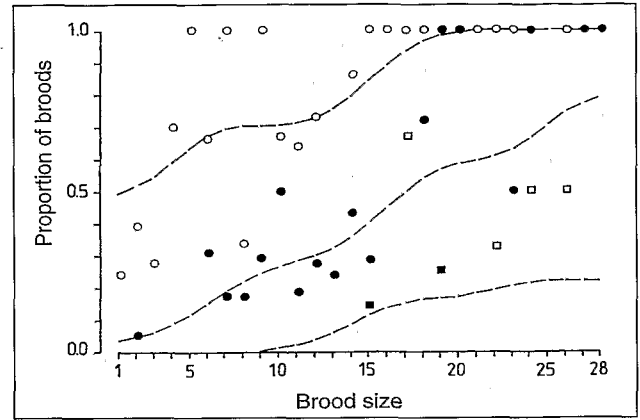


Figure 2. *Hypothenemus hampei*. Proportion of broods with at least 1 male (open circles), 2 males (full circles), 3 males (open squares) and 4 males (full squares), plotted as a function of mature brood size. Dotted curves represent 1-, 2- and 3-male data smoothed using 5-order mobile mean. Data are from 183 partially mature 1-foundress broods reared on semi-artificial medium for 2 months, with the mother allowed to oviposit for the first month.

Broods where all individuals could be sexed (that is, made up of adults or pupae only) had sizes ranging from 1 to 26, 1-23 and 1-18 in 1-mother, 2-mother and 3-mother batches, respectively (Table I). A significant trend towards more males with increasing number of females per brood was noted, independently of treatment (Table I). The average brood size decreased accordingly, and this variation was significant (Table II). Adding the number of mothers to brood size led to a total number of 14.4, 14.7 and 13.1 individuals in 1-mother, 2-mother and 3-mother batches, respectively, a result consistent with the hypothesis that the carrying capacity of an experimental cell (i.e. a plug of about 0.137 g of rearing medium in the well of a microtitre plate) was on the average limited to about 13-15 adult beetles. Finally, the proportion of males signifi-

Table I
Hypothenemus hampei. Number of sons as a function of the number of daughters in broods from groups of 1, 2 and 3 mothers

Treatment	Number of sons	Number of daughters																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1 (N = 74)	0																									
	1	4	2		1	3		4	1	2	1		1	1	1	1					4					
	2				2	1	1			3	1		1	1		1	2	1	3	1	3					1
	3								1		2	1	1		2	2	3		2	5		1				
2 (N = 36)	0		2				1		1					1	1					1						
	1		1		3			1	1					4	1					1						
	2						1	1				1	1	4	2	2	2	2		1			1			
3 (N = 28)	0	1	2	2	1		1							1			1									
	1					1	1				2	1		1			1									
	2						1	1							1											
	3			1							1	1				1										
	4									1						1	1									

Spearman's rank correlation coefficient between number of sons and number of daughters (r_s): $r_s = 0.371$, $p < 0.01$ in 1-mother batches; $r_s = 0.399$, $p < 0.05$ in 2-mother batches; $r_s = 0.386$, $p < 0.05$ in 3-mother batches. Data are from totally mature broods reared on semi-artificial medium for 2 months, with mothers allowed to oviposit for 1 month. N: sample size.

Table II
Hypothenemus hampei. Mean \pm SD brood size and sex ratio (SR) (proportion of males in brood) in clutches laid by 1, 2 and 3 mothers and predicted sex ratio under LMC theory [3]. Raw data in Table I

Treatment	N	Brood size	Observed SR	Predicted SR
1	74	13.38 \pm 6.62	0.082 \pm 0.077	—
2	36	12.70 \pm 5.78	0.128 \pm 0.107	0.214
3	28	10.08 \pm 5.94	0.167 \pm 0.204	0.303
Non-parametric ANOVA		1-way H = 6.01 p < 0.05	2-way χ^2 = 8.58 p < 0.02	

N: sample size; H: Kruskal-Wallis' statistic; χ^2 : Friedman's statistic. Both H and χ^2 were compared to χ^2 with 2 degrees of freedom [25].

cantly increased with the number of females induced to oviposit simultaneously in a cell (Table II). The average sex ratio was 1:11.2, 1:6.8 and 1:5.0 in 1-mother, 2-mother and 3-mother batches, respectively. The 2 latter values ranked below those predicted by the LMC theory [3] (Table II). The sex ratio variance was estimated at every brood size > 1 using all 1-mother brood data when sample size was ≥ 2 . To test whether the sex ratio was more precise than binomial, we compared each estimate to the corresponding estimate of binomial variance. Seventeen out of a total of 24 variance estimates were less than binomial; we rejected the binomial model (Wilcoxon's signed-ranks test for paired observations: 24 paired observations; sum of negative ranks = 87; 1-tailed; $p < 0.05$).

Additional, miscellaneous observations made under the stereoscopic microscope gave some information relevant to the interpretation of the foregoing results. First, competition between mothers for the access to the resource was evident. Single mothers usually dug an entrance hole within a few hours after the start of the experiment whereas mothers in groups of 2 or 3 usually first spent considerable time on the surface of the experimental cell, up to several days before each had dug her own hole. We regularly observed interactions between females. Those involved in digging activity allocated their time in alternately digging and fighting with the other females. Some of these fights ended up in the mutilation of the legs of the attacked. Second, we suspect the occurrence in the brood of some mortality caused by siblicide. Cephalic capsules of larvae, some of which were at an advanced stage of development, were found among debris at the end of the experiment. *H. hampei* adults and larvae have powerful mandibles that allow them to attack and devour their conspecifics. No direct observation of such behaviour was made though.

Since on average males were recruited to a brood earlier than females, and the carrying capacity of an experimental cell is limited, we empirically modelled the outcome of sex ratio for a limited brood size using the smoothed data of Figure 1, and assuming: (1) no manipulation by mothers of their offspring sex ratio *per se*; and (2) some control of adult offspring number either through the number of eggs laid by mothers or through mortality of juveniles once the carrying capacity is reached. We could then predict that broods of average sizes 13.38, 12.70/2 and 10.08/3 (Table II) should have sex ratios of about 0.105, 0.145 and

0.170, respectively. These values do not significantly depart from the secondary sex ratios that were actually observed (Table II; this data set is independent of that used for setting the values of the parameters of the empirical model).

Discussion

Beetles of the family Scolytidae offer an experimental system in which to study the ecology and evolution of inbreeding, sex determination and adaptive sex ratio biases ([1, 11]; J.H. Werren, *in litt.*). Inbreeding and outbreeding both occur in Scolytidae. Inbreeding species commonly show high sex ratio biases with dwarf males. While diplodiploidy appears to be the rule among outbreeding Scolytidae, inbreeding species have been found to be arrhenotokous (review in [11]) or pseudo-arrhenotokous (*Hypothenemus hampei*: [18]). Hamilton [1] pointed out the female-biased sex ratios in inbreeding Scolytidae as evidence in favour of the LMC theory, and predicted that other inbreeding species with sex ratio biases should also have an haplodiploid mode of sex determination. Arrhenotoky provides an easy means for a mother to control the sex ratio of her brood [8, 9]. In mites, pseudo-arrhenotoky has also been suspected to confer the same advantage [15, 16].

It has been claimed previously that *Hypothenemus hampei* broods usually contain only 1 male, and that sex ratio variation in this case is the reciprocal of brood size [11]. The present results showed that the number of sons in a brood is correlated with the number of daughters; more than 1 male is usually present in broods larger than 16; the sex ratio has an asymptotical value, and hence becomes independent of brood size. More than 1 male per brood is likely to be necessary for all females to be mated in a short time, because of the limited mating ability of individual males [13, 19]. The cost of producing several males (as opposed to only 1 male per brood) is likely to be balanced by the benefit, in terms of colonizing ability of females, of their earlier insemination, hence resulting in the optimization of the insemination rate. Also, the production of additional males may be an insurance in face of possible mortality affecting the brood, a phenomenon that was inferred here from the observation of larval remains among debris at the end of the experiment. Those broods losing their only male would be totally unable to spread outside the berry and found new colonies, since virgin females do not disperse [13] and eggs laid by virgin females do not hatch [19]. Besides, the occurrence of some mortality among early stages in a brood provides some explanatory mechanism for the observation that brood size appeared to be bounded by the carrying capacity of an experimental cell. Quantification of mortality rates at early stages under different LMC levels is desirable here, for such data would allow a definitive test of the hypothesis that carrying capacity determines adult brood size (as opposed to the control of brood size at oviposition).

The fact that males on the average mature earlier than females ([19]; present results) also appears to be adaptive in 2 aspects. First, it ensures that females are inseminated earlier than would occur if the timing of the recruitment of males to a brood were random, enabling the females to depart earlier from the berry, and subsequently to found another family in a newly colonized berry earlier. Since

berries constitute limited habitat that vanishes with time, in that the reproductive season of the host is limited [13], fitness is maximized when the duration of the life cycle is minimized. Second, it provides in itself a mechanism for sex ratio adjustment in the presence of conspecific mothers, provided the resource offers limited carrying capacity. The carrying capacity appeared to be limited in the present experimental conditions. Since a coffee berry also constitutes such a limited resource, with a carrying capacity ([13, 22, 23]; P. Cochereau, unpublished) only moderately larger than that of the experimental cell used in our experiment, one can reasonably assume that the fortuitous adjustment of sex ratio hypothesized here should also occur in the coffee berry. As shown by LMC models [3], the adjustment of sex ratio is indeed adaptive, hence also the early eclosure of males. To summarize, optimization of the insemination rate of females and multiple colonization by conspecific mothers are both possible selective forces favouring the earlier eclosure of males than of females (as opposed to random timing of recruitment). Furthermore, these 2 factors together with partial brood mortality are also possible selective forces favouring the occurrence of several males (as opposed to only one) per brood.

One can ask whether *Hypothenemus hampei* mothers effectively control their offspring's sex and sex ratio. On the one hand we showed that, except in very small broods, the proportion of males in 1-mother broods is independent of brood size: mothers regularly produce new males as brood size increases. It has been reported that mothers lay their eggs as clusters of about 10 [28], and presumed [11] that each of these may contain 1 male, given an approximately 1:10 sex ratio ([13, 22]; present results). Such an inference, together with the observation that sex ratio variance is lower than binomial (present results) lead to the assumption that mothers do control the sex ratio of their brood. On the other hand we are inclined to accept the idea of fortuitous sex ratio adjustment as a response to the simultaneous presence of several foundresses. If this proves to be the correct explanation for secondary sex ratio variation among experimental treatments, then the mother does not need to modify her strategy by adjusting sex ratio at the time of oviposition (primary sex ratio). Thus, while mothers control the sex and sex ratio of their offspring, thereby confirming that pseudo-arrhenotoky somehow does allow sex ratio control [15, 16], we had no indication that they also manipulate sex ratio in response to different LMC levels.

An examination of data from the literature together with the present data leads to the conclusion that the biological features of *Hypothenemus hampei* mostly conform to the biofacies of "extreme" inbreeding drawn by Hamilton [1]: (1) "The primary sex ratio is spanandrous - that is, females greatly preponderate". We observed a female-biased sex ratio whose average value in 1-mother broods was about 1 to 11; (2) "Reproduction is arrhenotokous". In *H. hampei*, reproduction was inferred to be pseudo-arrhe-

notokous [18], a cytological system also suspected to allow females to control their brood's sex ratio ([15, 16]; present work), although the underlying mechanism is unknown; (3) "There is at least 1 male in every batch of offspring". Taken together, all broods of size > 19, and already 95% of broods of size = 16 contained at least 1 male, with a shift towards males developing earlier than expected if the timing of their recruitment to a brood were random; (4) "There is gregarious development, as a group of siblings, from egg to adult". This is usually the case in *H. hampei* (see *Introduction*); (5) "Adult males eclose first and can mate many times". In *H. hampei*, adult males tend to be recruited earlier than adult females ([19]; present study). That they can mate many, or at least several times, derives from the observation that most emerging females are inseminated, for these are able to lay eggs that hatch [13]. Up to 128 females were reported to be inseminated by a single male under experimental conditions [13], with a rate of insemination of 2 to 3 females per day [13, 19]; (6) "Mating takes place immediately after (or even before) eclosure of adult females". Both Bergamin [19] and Giordanengo [13] have shown that males were able to fertilize their sisters within a few days after their eclosure; (7) "Males are disinclined, or unable, to emigrate from the batch". This point has been addressed through laboratory experiments [13]; males usually did not leave the berries containing adult offspring, although nearly 0.3% of them did so; (8) "Females can store sperm; one insemination serves to fertilize the whole egg production". To our knowledge, this last point has not been investigated yet. Genetic markers, such as insecticide resistance phenotypes [12, 18] and allozymes [29], could be useful for testing the possibility of multiple paternity.

Sib-mating was thought to be the rule in *Hypothenemus hampei* as coffee berries are reported to be generally infested by a single mated female [13, 30]. However, the observation of multiply infested berries at the end of the fructification cycle of the host [12, 13, 31], raises the possibility that matings between individuals from different female lines may occur in the wild. The inbreeding coefficient, measured at the scale of the coffee field using allozymic [29] and resistance phenotype [12] markers, varies from 0.087 to 0.679 depending on the marker and the season. Assuming that all inbreeding is by crossing between sibs, the proportion produced by outcrossing every generation can therefore be estimated as ranging from about 72% to about 10%, which in any case is large enough to depart from the extreme inbreeding assumed to be characteristic of LMC species, particularly those with highly biased sex ratios, as in *H. hampei*. Nevertheless, the above estimates of inbreeding rate in natural populations are somewhat consistent with those expected under the hypothesis that a small number of mothers (1-3) congregate in a coffee berry at the time of oviposition. This situation, although not being "extreme" inbreeding, still meets the conditions for LMC. ▼

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