Female dispersal and reproduction in the ambrosia beetle *Xyleborinus saxesenii* Ratzeburg (Coleoptera; Scolytinae)

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Zusammenfassung: Untersuchungen zur kooperativen Brutpflege des Ambrosiakäfers *Xyleborinus saxesenii* Ratzeburg (Coleoptera; Scolytinae)

Kooperative Brutpflege und überlappende Generationen bei Arthropoden sind vorwiegend in den Gruppen der Hautflügler und Termiten zu finden. Weniger bekannt ist, dass auch Ambrosiakäfer (Coleoptera; Scolytinae und Platypodinae) in sozialen Gruppen leben. Adulter Nachwuchs verbleibt vor dem Ausfliegen für einige Zeit im Nest, um sich der Brutpflege, der Nestverteidigung, sowie der Zucht von Pilzgärten zu widmen, die Ambrosiakäfer zu Nahrungszwecken an den Wänden ihrer Gangsysteme im Holz anlegen.

Mit Hilfe einer optimierten Bruttechnik in Glasröhrchen dokumentieren wir überlappende Generationen von Weibchen in Galerien des Ambrosiakäfers *Xyleborinus saxesenii* Ratzeburg (Coleoptera; Scolytinae). Nach Erlangung der Geschlechtsreife verzögerten adulte Töchter den Ausflug für durchschnittlich 23 Tage; nach dem Tod des Gründerweibchens übernahmen einige Töchter die Galerie. Da bei *X. saxesenii* die Weibchen im mütterlichen Nest durch ihre Brüder besamt werden, können sie ihre Eier auch dort ablegen. In unserer Studie haben sich jedoch nur 25% der Töchter im mütterlichen Nest fortgepflanzt, unabhängig von der Brutgrösse, was darauf hinweist, dass der verzögerte Ausflug vorwiegend indirekte Fitnessvorteile bietet. Diese Annahme wird durch frühere Studien unterstützt, die zeigen, dass der Verbleib der Töchter den Bruterfolg der Mutter verbessert. Dies ist vermutlich darauf zurückzuführen, dass Pilzzucht von sozialen Gruppen effektiver bewerkstelligt werden kann. Es ist daher anzunehmen, dass Sozialität und Pilzzucht bei Ambrosiakäfern in engem Zusammenhang entstanden sind.

Key-words: eusociality, insect agriculture, delayed dispersal, cooperative fungiculture, alloparental care, helping, haplodiploidy

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Introduction

Eusociality stands for the highest level of social organization, which is characterized by cooperative brood care, overlap of parental and offspring generations, and the presence of non-reproductive castes (Batra 1966). Among insects it is found primarily in Hymenoptera and Isoptera (Wilson 1971). In Coleoptera, the most species-rich order of insects, sociality is surprisingly rare and eusociality has only been described for one species of Platypodid ambrosia beetle (Kent & Simpson 1992). Ambrosia beetles live in the heartwood of trees and cultivate fungi for food. Due to their cryptic life inside wood tunnels they are difficult to study, but a recently optimized laboratory breeding technique allows to observe their behaviour (Saunders & Knoke 1967, Biedermann & al. 2009).

Delayed dispersal of adult daughters, and thus overlapping generations, are typical for the ambrosia beetles *Xyleborinus saxesenii* and *Xyleborus affinis*, although daughters are fully capable of founding their own nests (Peer & Taborsky 2007, Biedermann & al. 2011). Both species are members of the Scolytinae subtribe Xyleborini that is predisposed for kin-selected sociality because of high relatedness of families within one nest, due to sib-mating and haplodiploidy. Indeed, delayed dispersing daughters in several Xyleborini have been found to engage in a multitude of cooperative behaviours (e.g. maintenance and protection of the gallery, care of fungi and brood) during their philopatric period (Bischoff 2004, Biedermann 2007). Apparently, this imposes costs on their future reproduction. Mature *X. affinis*-daughters, for instance, produce more eggs when induced to disperse early compared to voluntarily dispersing females that have undergone a philopatric period (Biedermann & al. 2011). Apart from investing in siblings, however, daughters could potentially also invest in own reproduction before dispersing from the natal nest.

Here we describe the typical phenology of *Xyleborinus saxesenii* in laboratory galleries, which illustrates that parental and offspring generations overlap. Furthermore we dissected the ovaries of all daughters present in field galleries to determine if they reproduced in the natal nest.

Material and Methods

Collection and artificial rearing

We collected adult females of *Xyleborinus saxesenii* from field galleries by dissecting stumps of beech (*Fagus sylvatica*) with active galleries in the Spilwald forest near Bern, Switzerland. Individuals used for artificial rearing were immediately transferred to the laboratory, surface-sterilized (by submerging them first in ethanol (95%) and then in distilled water for a few seconds), and afterwards individually put in glass tubes filled with an artificial rearing medium based on sawdust and agar (modified medium; for details see Biedermann & al. 2009).

Typically, adult females put onto the medium immediately start to excavate a tunnel system (gallery), the walls of which they inseminate with spores of their mutualistic ambrosia fungus *Ambrosiella sulfurea* Batra and several other auxiliary fungi (Francke-Grosmann 1975). After establishment of the fungus layers, which is a delicate process that is successful only in about 20% of cases, successful foundresses start to lay eggs. Offspring development and brood care can be observed when tunnels and brood chambers are constructed next to the glass of the tubes.

Gallery phenology

For our observations we selected six laboratory galleries that provided almost full insight. We observed these galleries every second day for a period of four months and recorded the numbers of eggs, larvae, pupae, immature females, mature females, and males. Females have a brownish coloration as long as they are immature and turn black when fully sclerotized / mature. Sexes are easily discernable by morphology and body size (see figures in Fischer 1954). The dates of the first visible egg, the first mature female offspring, the first dispersal of female offspring and the death of the foundress were determined. Dispersal was defined as emergence from the gallery, i.e., when individuals were found on the surface of the medium under the cap of the tube (Biedermann 2007). A single dead female appeared in one third of all galleries within the first 100 days after gallery foundation. Most likely this was the foundress, because (i) they were the oldest members of the gallery, (ii) it was always only one individual and (iii) dead adult beetles are only very rarely found inside galleries in general (pers. obs.). Therefore, the moment of 'death of the foundress' was defined as the day when a single dead female was found on the surface (dead individuals, sawdust and faeces are thrown out of the nest by adult group members). To increase the sample size for a reliable estimate of this date we used our whole sample of laboratory galleries, which comprised 66 observations in total.

Reproduction of daughters

For 16 dissected field galleries we recorded the number of eggs, larvae, pupae, immature and mature females and males. Dispersal had been monitored with dispersal traps (Peer & Taborsky 2007) in 13 of these galleries from the time the gallery was founded until it was dissected ($\tilde{x}$ = 72 days, range = 19 –309 d). Dispersal had started in nine of these galleries in the meantime and all dispersing females were preserved. After gallery collection, females were stored in 95% ethanol. Dissection was accomplished from the dorsal surface with high precision tweezers under a binocular (6.4 × – 40 × magnification). We discriminated between immature ovaries (no oocytes visible) and egg-carrying ovaries (the four ovarioles contain one or more oocytes; see figures in Fischer 1954).

In order to determine the number of breeders in the galleries, we dissected all females collected from galleries containing eggs. For galleries without eggs, a minimum of 15 females each were dissected. Females that were accidentally damaged when the gallery was opened could not be dissected (proportion dissected females: $\tilde{x}$ = 93.5%, Range = 19 –100%, N = 16 galleries). Thus, we consider the number of egg-layers found in a gallery as a minimum estimate. However, we found that the galleries that could not be fully dissected all had more than one egg-layer; i.e. the number of galleries with only a single breeder was correctly determined. We also dissected 45 dispersing females from four different galleries to check their reproductive status.

Results

Gallery phenology

After the successful establishment of a fungal layer on the gallery walls, females started to lay eggs between days 11 and 26 ($\bar{x}$ ± se: 18 ± 2.2; N = 6 galleries) after gallery foundation. First mature daughters appeared between 16 and 34 days ($\bar{x}$ ± se: 26.2 ± 2.8; N = 6) after the first egg-laying. These daughters delayed their dispersal from the natal nest for 17 to 38 days ($\bar{x}$ ± se: 22.8 ± 3.2; N = 6). Foundresses were found dead on the gallery surface between 34 and 97 days ($\bar{x}$ ± se: 72.2 ± 1.8; N = 66) after gallery foundation.

Two to three peaks of egg-laying, followed by two clear peaks of larval numbers (Fig.1), were found by averaging the offspring numbers of the six selected galleries every day, over the whole observation period (day 18 as the mean date of the first visible egg was set as reference for all six galleries). Egg numbers were smaller than larval numbers because (i) developmental periods are different (eggs : 5 days; 1st to 3rd larval instars: 8 – 17 days) and (ii) eggs are less visible especially at the beginning of gallery development. The first offspring peak was obviously produced by the foundress, but the others were probably the result of egg-laying daughters, as the foundress likely had died before. Note that the second phase of egg-laying started at about the same time when the first mature daughters started to disperse (Fig.1). Offspring production strongly decreased about 90 to 100 days after gallery foundation, when the medium dried out and fungal growth ceased.

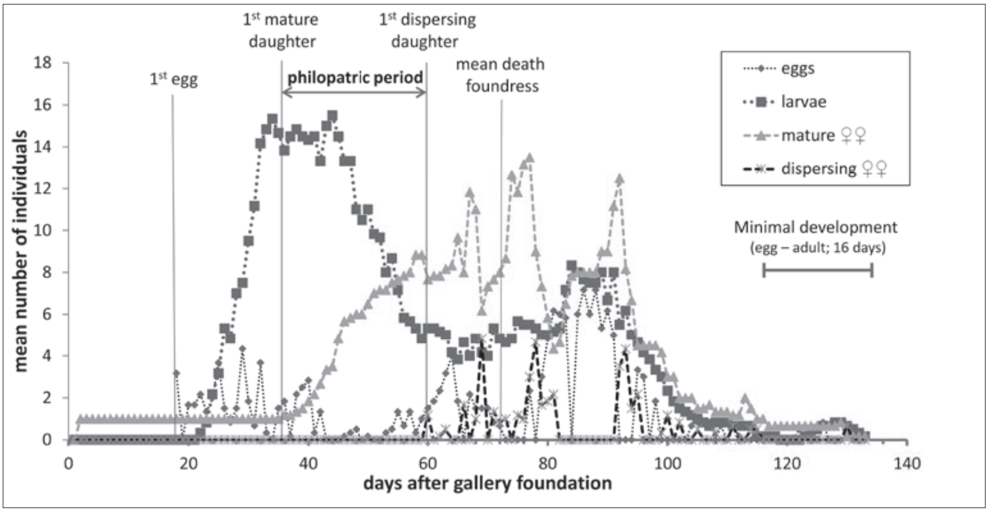

Fig.1. Average phenology of six laboratory galleries of Xyleborinus saxesenii. We synchronised the phenologies of the six galleries by matching the appearance of the first eggs (see text).

Reproduction of daughters

A direct benefit of delayed dispersal might be reproduction inside the natal gallery. Four of 16 field galleries contained at least two egg-laying females, (with ovarioles containing oocytes). By contrast, all dispersing females (N = 45 females from 4 galleries) did not lay eggs, i.e. their ovarioles contained no oocytes. More than one egg-layer was only found in galleries where female dispersal had already started (N = 9). Before the onset of dispersal, there was always a single reproductive female (i.e., the foundress; N = 4). In all galleries, numbers of sub-adult offspring (only larvae and pupae) and eggs present at the time of dissection were positively associated with the number of egg layers found. This was not a byeffect of gallery size, as there was no correlation between the total number of mature females in the gallery and the number of egg-layers (Table 1).

Table 1. Correlations (Kendall's Tau) between total number of eggs, sub-adults (larvae and pupae), mature females (fully sclerotized) and the number of egg laying females in field galleries. Significant p-values are printed in bold; α = 0.0083 (Bonferroni corrected α = 0.05/6; N = 14 -16 galleries as noted).

		Sub-adults total	Mature females total	Egg-layers total
Eggs total	T	0.536	0.000	0.681
	p (2-tailed)	0.007	1.000	0.003
	N	16	16	14
Sub-adults total	T		0.199	0.638
	p (2-tailed)		0.295	0.004
	N		16	14
Mature ♀♀ total	T			0.131
	p (2-tailed)			0.556
	N			14

Discussion

The first mature female offspring in *X. saxesenii* galleries stayed and helped in the care of fungi and brood for at least 17 days, which is longer than reported for *Xyleborus affinis* (7 days; ROEPER & al. 1980). Even after dispersal had started, females accumulated in the gallery, which supports the hypothesis that females usually delay their dispersal. This was already suggested by an earlier study, where we showed that delayed dispersal does not serve the accumulation of reserves, but, on the contrary, reduces the fertility of *X. affinis* females (BIEDERMANN & al. 2011). As these females are mature and potentially fully capable of breeding independently, delayed dispersal must entail other fitness gains if it was not maladaptive. Here we showed that, besides indirect fitness benefits to females through cooperative behaviours raising the production of close kin (BIEDERMANN 2007), some females also benefit directly by producing own offspring. The numbers of females and egg layers did not correlate with each other, however, and on average about three quarters of females were not laying eggs. Interestingly, both in the laboratory and field daughters apparently did not reproduce before the onset of dispersal in the respective galleries. Nevertheless, aggressive interactions, which would suggest competition over reproduction, have never been observed.

Future studies need to explore the factors affecting dispersal, helping and breeding decisions of female Xyleborini. Sociality in ambrosia beetles probably evolved in close association with fungus agriculture in wood. Indeed, advanced sociality within Coleoptera is only known from the Scolytinae, Platypodinae, and Passalidae, which are all wood-living insects associated with microbial symbionts. Interestingly, it has been hypothesized that sociality of ants and termites also evolved within the same habitat (HAMILTON 1978).

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