The importance of blocking the gallery entrance in the ambrosia beetle *Xyleborinus saxesenii* Ratzeburg (Coleoptera; Scolytinae)

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Zusammenfassung: Die Bedeutung der Blockierung des Eingangstunnels beim Kleinen Holzbohrer *Xyleborinus saxesenii* Ratzeburg (Coleoptera; Scolytinae)

Die Evolution von Kooperation und Altruismus im Tierreich lässt sich in vielen Systemen durch Hamiltons Theorie der Verwandtenselektion (1964) erklären. Sie besagt, dass Helfer indirekt die eigene Fitness steigern, indem sie in die Nachkommen von Verwandten investieren. Hilfe wird jedoch nicht immer freiwillig geleistet und neue Studien zeigen, dass Tiere in manchen sozialen Gruppen dazu gezwungen werden. Gerade reproduktiv aktive Individuen könnten ein grosses Interesse daran haben, dass andere Gruppenmitglieder auf eigene Fortpflanzung verzichten und stattdessen ihren Nachwuchs pflegen.

Beim Kleinen Holzbohrer *X. saxesenii* verbleiben junge Weibchen im gemeinschaftlichen Nest, obwohl sie bereits fähig wären auszufliegen. Da sie von ihren Brüdern befruchtet wurden, könnten sie eigene Kolonien gründen. Stattdessen verbleiben sie aber für einige Wochen im Geburtsnest, wo sie sich an der Fürsorge der fremden Brut beteiligen. An Laborkolonien untersuchten wir, ob dieses Verhalten von anderen Weibchen erzwungen wird, indem sie den Eingangstunnel blockieren. Eine negative Korrelation der Frequenz des Blockierverhaltens mit der Anzahl ausfliegender Weibchen lieferte dafür Hinweise. Wir haben künstliche Eingänge zu den Kolonien geschaffen, um eine alternative Ausflugsmöglichkeit zum blockierten Eingang zu bieten. Die neuen Eingänge wurden von den Koloniemitgliedern allerdings nicht angenommen und stattdessen oftmals mit Sägespänen und Kot verstopft. Wir deuten dieses Verhalten als eine gemeinschaftliche Reaktion auf eine potenzielle Gefährdung des Nests. Eine experimentelle Bestätigung, dass das Blockierverhalten den Ausflug von helfenden Weibchen verhindert und so die Produktivität des Nestes erhöht, konnte mit diesem Versuch nicht erbracht werden.

Key-words: coercion, eusociality, insect fungiculture, enforced cooperation, delayed dispersal, helping, cooperative breeding

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Introduction

Cooperation is ubiquitous in nature, although natural selection favours organisms that selfishly maximize their own reproduction (Darwin 1859). This apparent contradiction has been explained by the inclusive fitness theory of William D. Hamilton (1964) and the insight that altruistic behaviour among related individuals serves the selfish propagation of own genes through others. But even among relatives, cheating by withholding altruistic help can be selected within non-clonal groups (West & al. 2002). Therefore, in addition to kin selection, coercion, punishment and policing have been proposed as important means to maintain cooperation in social groups (Bourke 2011). Coercive behaviours are widespread in many social taxa (Tebbich & al. 1996) and are expected to be particularly important in highly social species with totipotent group members, which provide a high potential for reproductive cheating.

Beetles are the most species-rich order of animals, constituting almost 25% of all known life-forms (Hunt & al. 2007). Higher sociality is surprisingly rare in beetles, however, and known only from bess beetles (Passalidae) and some wood-boring weevils (Platypodinae and Scolytinae). The latter are termed ambrosia beetles, as they cultivate ambrosia fungi for food, a trait that apparently can only evolve in cooperative societies with division of labour (Kirkendall & al. 1997; Farrell & al. 2001). The fruit-tree pinhole borer *Xyleborinus saxesenii* that is native to Europe has been recently shown to be facultatively eusocial. Daughters of a founder female do not leave the natal nest immediately after maturation, but delay their dispersal for some weeks to help the mother and sisters with brood care and fungiculture. Although daughters have already mated with a brother and thus are fully fertile, only about 20 % co-breed in the natal nest (Biedermann & al. 2012). Cooperative tasks, like the expansion of the nest, the removal of frass and sawdust, the control of pathogens, the tending of the fungus gardens and the protection of the nest, are shared among philopatric adults and helping larvae, which is unique among holometabolous insects (Biedermann & Taborsky 2011).

In *X. saxesenii,* gallery protection is provided exclusively by adult females, which simply sit in the narrow entrance-tunnel. This blocking behaviour is only shown by egg-laying females (Biedermann & Taborsky 2011). It is of particularly interest, because it may serve both nest protection and regulation of the microclimate (for details see Kirkendall & al. 1997), but also hinder the free dispersal of adults. Thereby it might coerce philopatry and helping (Biedermann & Taborsky 2011). Dispersal of adults depends on the number of needy offspring in the gallery (Peer & Taborsky 2007) and increases after experimental removal of the blocking female (Biedermann & Taborsky 2011). This may either suggest that dispersal is triggered by the absence of an egg-laying female, or the blocker indeed enforces the philopatry of adults. Here we aimed to disentangle these two possibilities by testing if the experimental creation of a second entrance tunnel in laboratory nests – which would allow adult females to freely disperse – leads to a higher dispersal rate also when a blocking female is present.

Materials and Methods

Artificial rearing & treatments: Adult females of *Xyleborinus saxesenii* used for artificial rearing in this study came from a population that had been kept for about ten generations in the laboratory. Foundresses of this population were originally collected in the Spilwald forest near Bern, Switzerland. For artificial rearing we put females individually into plastic tubes filled with an artificial rearing medium based on sawdust and agar (modified medium; for details see Biedermann & al. 2009), which we stored in a climate chamber at 25 °C and a 11:13 hours light:dark cycle. Beetles readily excavate a tunnel system into this medium, and if the tunnels are constructed close to the transparent tube walls, the behaviours of colony members can be recorded. 31 tubes provided sufficient visibility for our experiment and observations. We created artificial entrances to the galleries by drilling a hole through the back end of the tube when a tunnel system had been established, using a driller and a heated metal wire. Other methods used in pilot trials did not prove to be successful (Nuotclà 2013). Nests were sorted according to the number of visible individuals and then allocated pairwise to a treatment (N = 15 plus one tube that had no matching control) and a control group (N = 15). We prolonged test tubes with another tube and closed the bottom with a plastic cap to create space for dispersal. Dispersing females were meant to be collected from this chamber. Control tubes we treated in exactly the same way to control for the manipulation, with the exception that the artificial entrance tunnel was blocked with a drop of hot glue to prevent females from dispersing through it.

Behavioural observations and statistical analyses: We started behavioural scans when a colony exceeded a minimum number of five adult inhabitants, between 33 and 45 days after nest foundation. Using a microscope, we scanned all visible tunnel systems and recorded the number of individuals (larvae, not fully sclerotized females, and adult females) and the behaviours they exhibited at the moment when we saw them for the first time. Behaviours were then classified as either gallery maintenance or hygienic tasks (see Table 1 for details about the recorded behaviours). For the analysis we used these two grouped behaviour classes only. Each colony was scanned seven times during two days before and seven times during two days after the manipulation (tubes where manipulated on the second day, right after the seventh scan). During every scan we also checked for blocking females in both the natural and artificial entrances, and we recorded if individuals had dispersed through one of these tunnels. Blocking was analysed as the relative blocking frequency, i.e. the proportion of scans per day when blocking was present. In the final analyses we used only those six control and treatment nests, where we had recorded the behaviours of at least 60 individuals in total during the four days of the experiment. With generalised linear mixed models (GLMMs; lmer in R) we determined the effects of treatment, colony age, number of individuals and changes in air pressure (independent variables) on the frequency of the different behaviours (dependent variable), controlling for gallery identity and scan (random variables). Effects on dispersal were analysed by exact Wilcoxon signed rank tests (EWSRT; paired data and zero values occurred in the data). Data on daily air pressure were obtained from the archive of MeteoSwiss. All statistical analyses were performed with R (version 2.14.1 32 bit with additional packages: lme4, MASS, coin and languageR; Core Team 2010) which was implemented in the multi-language software development environment eclipse (version IDE 3.7 with additional package: StatET).

Tab. 1: Ethogram of the behaviours shown by larvae (L), not-fully sclerotized females (J) and adult females (A) (after Biedermann & Taborsky 2011).

Behaviour	Shown by	Definition	Occurrence	Class
Inactivity	L, J, A	Not moving	Often	
Locomotion	L, J, A	Walking	Often	
Blocking	A	Plugging the entrance tunnel with the body	Often	
Feeding	L, J, A	Feeding on fungus (J, A) or fungus infested wood (L)	Very often	Maintenance
Digging	J, A	Excavating new tunnels	Rare	Maintenance
Grooming	L, J, A	Allogrooming of others	Often	Hygiene
Shuffling	J, A	Moving frass and sawdust	Very rare	Hygiene
Cannibalism	L, J, A	Feeding on conspecifics	Very rare	Hygiene
Balling	L	Forming balls of frass and sawdust	Very rare	Hygiene

Results

In response to the experimental treatment, the beetles closed the artificial entrances with sawdust and frass, and they did not disperse through them. The treatment did also not affect the number of females dispersing through the natural entrance (EWSRT: $P > 0.05$), and therefore we excluded this factor from all subsequent analyses. A statistical model with dispersal as response variable showed that the number of dispersing females was negatively correlated with the time a gallery was blocked per day (GLMM: Coeff. ± SE = -2.523 ± 0.812, $Z = -3.110$, $P = 0.002$; Fig. 1). In this model, dispersal showed to be significantly higher in older colonies (Coeff. ± SE = 0.227 ± 0.090, $Z = 2.513$, $P = 0.012$) and independent of colony size and air pressure ($P > 0.05$; excluded from final model).

In a second model with blocking time as the response variable, blocking was positively affected by a rise in air pressure over the past 24 hours (Coeff. ± SE = 2.706 ± 0.772, $Z = 3.506$, $P < 0.001$), whereas absolute air pressure had no significant effect ($P > 0.05$; excluded from final model). Blocking time correlated positively with the number of adult female colony members (Coeff. ± SE = 0.555 ± 0.163, $Z = 3.401$, $P < 0.001$; Fig. 1) and with the frequency of larval (Coeff. ± SE = 4.897 ± 1.566, $Z = 3.128$, $P = 0.002$) and adult maintenance behaviours (Coeff. ± SE = 7.1156 ± 1.8618, $Z = 3.822$, $P < 0.001$), and negatively with the number of larvae in the colony (Coeff. ± SE = -0.3895 ± 0.120, $Z = -3.245$, $P = 0.001$).

Discussion

Adult females did not use the artificial ducts for dispersal, but instead closed these openings with sawdust and frass. This might have been caused by an unnatural shape of the duct walls or missing layers of fungi. Therefore, the experiment does not allow us to draw conclusions about the functions of blocking. The closing of the artificial openings by the colony members shows for the first time that ambrosia beetles secure their home by repair of substrate, which is comparable to the closing of artificial gall-openings by social aphids (Uematsu & al. 2010). Furthermore, correlative analyses of the behavioural data provided some insight into the function of blocking: In accordance with the hypothesis that blocking retains helpers in the nest (Biedermann & Taborsky 2011) (i) we found a negative correlation between blocking activity and female dispersal and (ii) we observed a few times that adult females were pushed back into the

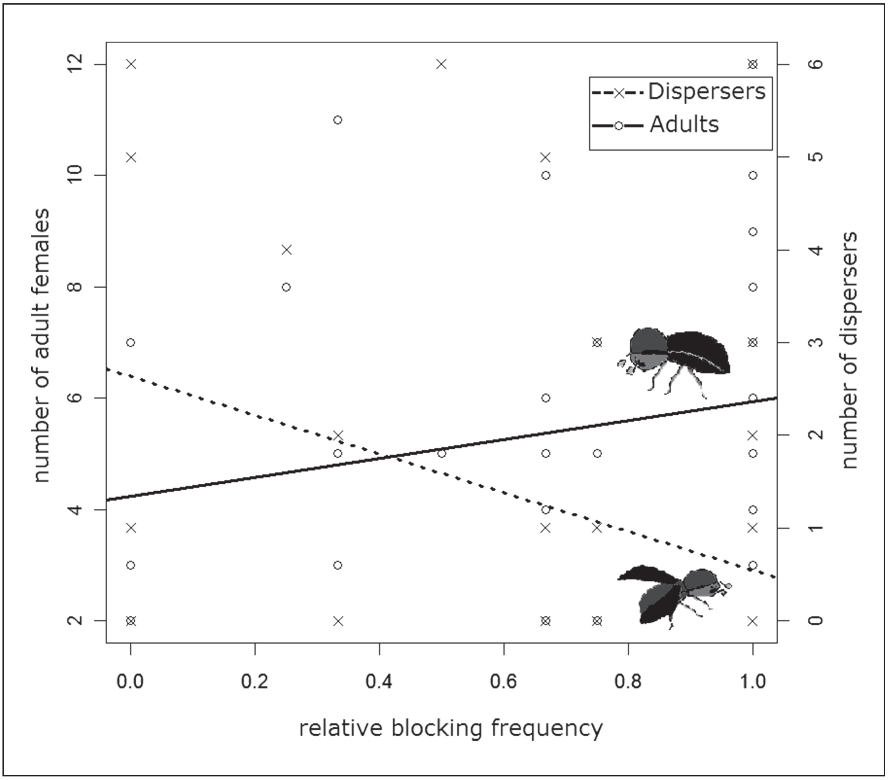

Fig. 1: Correlations of the relative blocking frequency with the number of adult females in the gallery (GLMM: $p < 0.001$), and the number of dispersers ($p < 0.001$).

brood chamber by the blocking females. The latter may have caused the positive correlations between female gallery maintenance and the time a gallery was blocked. Female dispersal increased with colony age (see also Biedermann & Taborsky 2011; Biedermann. 2012), which might reflect decreasing inclusive fitness gains for females in older, deteriorating nests. Absolute air pressure did not affect dispersal in our study, but this needs to be interpreted with caution because of a limited sample size and experimental period. In contrast, we found a significant positive influence of rising air pressure over the last 24 hours on the time females spent blocking the entrance tunnels. Results of large-scale studies on *X. saxesenii* (Biedermann 2012) and other small arthropods (Wellington 1946; Zettel 1984) strongly suggest that dispersal is enhanced by raising air pressure, probably because it indicates improving weather conditions. The observation that blocking increased when air pressure was rising (which probably indicates good dispersal conditions) is compatible with the coercion hypothesis. When there is a greater incentive to disperse because of favourable environmental conditions, blocking may be necessary to prevent that too many females would leave the gallery.

Philopatry of daughters is fundamental to the social system of many ambrosia beetles (Biedermann 2012) and other arthropods (Costa 2006). Our results suggest that female blocking behaviour does not only serve gallery protection, but may also hinder female dispersal and thus coerce individuals to stay and help in the natal nest instead of leaving to reproduce independently elsewhere. Empirical examples of such coercive behaviours in social taxa are scarce, but theoretical models suggest that they might be more common than envisaged (Lehmann & Keller 2006). Firm evidence for a potential causal link between dispersal and blocking activity can only be gained by further experiments.

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