



Reemergence and sister brood establishment in the bark beetle *Polygraphus proximus* (Coleoptera: Curculionidae: Scolytinae) under laboratory conditions

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Abstract

Reemergence and sister broods (second broods) are commonly observed in bark beetles (Coleoptera: Curculionidae: Scolytinae), particularly in species with male-initiated polygyny and female-initiated monogyny. *Polygraphus proximus* Blandford, a male-initiated monogynous bark beetle, is a quarantine species in the EU, which has been introduced from east Asia into both Siberia and central European Russia, causing mortality of trees across large areas of *Abies sibirica* forests. To clear whether *P. proximus* females reemerge to lay additional broods (sister broods), we observed the reproduction of reemerged females under laboratory conditions. We prepared 25 females reproducing in galleries collected by peeling the infested logs and 25 females reemerged from such logs. These females were put into the tubes vertically attached onto the surfaces of logs. Consequently, approximately 40% of both the reproducing females in galleries and the reemerged females bored into the logs and established sister broods without repeated mating. Moreover, 21% of these reproducing females established sister broods again using the same procedures, suggesting that the females can establish sister broods more than once. Our results indicated that the fecundity of *P. proximus* is higher than previously assumed, suggesting that its population levels can rapidly increase in weakened or dead trees, ultimately leading to the ability to attack healthy trees during the epidemic phase.

Keywords *Abies* · Male-initiated monogyny · Nonaggressive bark beetle · Scolytinae · Second brood

Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are important components of forest ecosystems. Most species live in dead or dying trees, serving as early decomposers in forest ecosystems. However, several species can infest and kill healthy trees, thereby serving as major agents of disturbance in forest ecosystems (Raffa et al. 2015).

Reemergence and sister broods (second broods) are commonly observed in bark beetles, particularly in species with male-initiated polygyny and female-initiated monogyny (Kirkendall 1983; Anderbrant 1989). Female bark beetles

reemerge after laying one brood and subsequently attack other hosts to lay sister broods. The probable advantages of reemergence include seeking new materials for regenerative feeding, laying other broods in suitable habitats for the development of offspring, and reducing intraspecific competition (Anderbrant 1989; Davidková and Doležal 2017). Reemergence and subsequent offspring (i.e., sister broods) generation lead to a continuous supply of beetles that are readily able to attack and colonize new trees and may significantly reduce the effectiveness of two commonly applied protective practices: the removal of infested timber and the installation of trap trees, as females may reemerge from the timbers and trap trees.

Polygraphus proximus Blandford (Coleoptera: Curculionidae: Scolytinae) is a nonaggressive phloem-feeding bark beetle. This species feeds on the following *Abies* species from the Far East: *Abies firma* Sieb. et Zucc., *A. holophylla* Maxim., *A. homolepis* Sieb. & Zucc., *A. mariesii* Masters, *A. nephrolepis* (Trautv. ex Maxim.) Maxim., *A. sachalinensis* (Fr. Schmidt) Masters, *A. sibirica* Ledeb., and *A. veitchii* Lindl. (Koizumi 1977; Kerchev 2014a; Takagi 2022, 2023).

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Since the late 2000s, *P. proximus* has infested trees in Russia and West Siberia of the European continent, causing the mortality of trees across large areas of *A. sibirica* forests (Baranchikov et al. 2010; Kerchev 2014a). Mass attacks and mortality events of *Abies* trees caused by *P. proximus* have also been observed in its native range in Japan (Tokuda et al. 2008; Chiba et al. 2020; Takagi et al. 2018, 2021).

Although little is known regarding the life cycle of *P. proximus*, it has a bivoltine cycle in its native distribution (EPPO 2014; EFSA et al. 2020). The emergence period and subsequent flight of the first generation of adults occur at the end of spring or the beginning of summer (May–July), and the second generation emerges from infested trees at the end of summer (August–September) (EPPO 2014; EFSA et al. 2020). Beetles may overwinter under the bark. Although the exact composition of the *P. proximus* aggregation pheromone is yet to be determined, two male-specific compounds, (Z)-2-(3,3-dimethylcyclohexylidene)-ethanol and 3-methyl-2-buten-1-ol, have been proven to attract both male and female *P. proximus* adults in the field (Viklund et al. 2022). Thus, males of *P. proximus* create entrance holes and bore through the bark of the host, and the females then enter the entrance hole to mate. These entrance holes are distributed uniformly to avoid intraspecific competition (Takei et al. 2021), and the females produced horizontal mother galleries that are typically one- or two-armed beneath the bark where they laid their eggs (Kōbayashi et al. 2022).

The mating system of *P. proximus* is male-initiate monogamy (Kerchev 2014b; Kōbayashi and Takagi 2020). Kōbayashi and Takagi (2020) showed that 70.4% of galleries contained no males and 26.6% contained no females, suggesting that *P. proximus* can reemerge. Despite the growing sense of urgency for a more comprehensive understanding of *P. proximus*, it remains unclear whether *P. proximus* reemerges to lay sister broods after laying an initial brood. The lack of information on reemergence and sister broods in *P. proximus* makes it difficult to estimate the reproductive potential and provide effective protective practices. Increased understanding of the reemergence and sister broods may lead to more efficient control methods being devised and implemented, such as predicting population dynamics and interfering with beetle reproduction. Hence, we conducted laboratory experiments to investigate the reemergence of females and males, and the laying of sister broods in *P. proximus*.

Materials and methods

Preparations of logs infested by *P. proximus* and the reemerged females

To obtain logs infested by *P. proximus* adults for experiments, we exposed fresh-cut logs in the University of Tokyo Hokkaido Forest (UTHF) (43° 15' N, 142° 30' E), at an elevation of 300 m. a.s.l., in Furano City, Hokkaido, Japan. We cut three sound *A. sachalinensis* trees on April 27, 2022, and cut them into 30 logs measuring 70 cm in length. To prevent drying, paraffin was applied to both ends of the logs. Twenty logs were randomly selected and placed vertically in the UTHF on the day. On July 19, after the offspring larvae of the first-generation adults started to make larval galleries, 17 logs were retrieved from the study site and transported to the laboratory at the Tokyo Metropolitan University, Tokyo, Japan. The air temperature in the laboratory was maintained at 25°C.

We peeled the five logs (mean diameter \pm SD = 16.7 \pm 1.58 cm) to confirm infestation by *P. proximus*. We determined number of the parental adults in each gallery, whether the adults were dead or alive, their sex, and the developmental stages of the offspring generation. These were conducted on July 25–27 and 29, as well as on August 2.

To determine the reemergence of *P. proximus*, we used the photoeclector method. Eight infested logs retrieved from the study site were placed in five rearing boxes in the laboratory. An additional log was placed in a separate rearing box on July 28, and three more logs were placed in two separate rearing boxes on August 2. Eventually, a total of 12 logs (mean diameter \pm SD = 13.5 \pm 1.33 cm) were placed in eight rearing boxes. The rearing boxes (27.5 cm wide, 27.5 cm high, and 66 cm long, RV BOX 800, IRIS OHYAMA Inc., Sendai, Japan) were made of nontransparent dark green plastic. A hole (ca. 10 cm in diameter) was opened on one side of each box, and a clear bottle (9 cm in diameter, 21 cm depth, Pack Clean 1000 mL, AS ONE Corp. Osaka, Japan) with KimTowel® (Nippon Paper Crexia Co. Ltd, Tokyo, Japan) inside was connected to collect emerged adult beetles obtained using the photoeclector method (Online Resource 1). We counted the number of pigmented males and females in the bottle from July 27 to August 6.

Infection experiments using reproducing females in galleries and reemerged females

Uninfested logs were obtained for the infection experiments by cutting down 10 sound *A. sachalinensis* trees in

the UTHF on July 20 and 21, 2022, into 68 logs measuring 70 cm in length. Paraffin was applied to both cut ends of the logs to prevent drying. The logs were transported immediately to the laboratory.

We used 25 female adults found on July 26, 27, and August 2 in the three peeled logs laid in field (reproducing females in galleries). We also used 25 female adults collected in the bottles by the photoelector method on July 27 and 29, and August 3 (reemerged females). The sexes of the insects were identified based on secondary sexual characteristics (Kerchev 2014b) using a stereomicroscope. Then, we put each female into a transparent 1.5 mL tube (9.2 mm in diameter, 38.5 mm depth, AS ONE Corp., Osaka, Japan) and attached the open tube to the five logs using a glue gun within 24 h of being obtained (Online Resource 2). Ten tubes for five reproducing females in galleries and five reemerged females were attached to each log, at least 15 cm apart.

The bark from the logs was peeled between August 31 and September 7, 2022, and the presence or absence of frass, entry holes, mother gallery construction, egg niche, and larval gallery construction were recorded. If the installed females were found alive, they were placed in another set of logs using the same methods employed during the initial observation immediately after log peeling. Subsequent processes were repeated as described before. We installed 19 females on two logs. We peeled the logs in February 2023

and recorded the presence or absence of frass, entry holes, mother galleries, egg niches, and larval gallery construction.

Results

We found 495 galleries from the five logs laid in field. Offspring adults had uncolored, nonpigmented elytra in a brown shade (Fig. 1), whereas the parental adults had pigmented, tanned black elytra. Of the 495 galleries, 338 galleries lacked parental adults but contained offspring. Of the 157 galleries harboring parental adults, 8.9% galleries harbored single pigmented male, 77.7% harbored single pigmented female, 2.5% harbored more than one female, and 1.3% harbored single pair (Table 1). The parental males and females were collected by the photoelector method (Table 2).

Approximately 40% of both the reproducing females in galleries and the reemerged females bored into the logs (i.e., made frass) and reproduced (Table 3). Namely, of the 50 adult females we installed, 23 attempted to bore into the logs, and 20 successfully bore into the logs. All the remaining 30 died in the tubes without boring into the logs (Table 3). All of the 20 females that successfully bore into the logs had constructed mother galleries, and 19 oviposited along the mother galleries (Table 3). All 19 gallery systems contained larval galleries (Fig. 1). There were no differences

Fig. 1 Sister brood gallery system constructed by a reemerged female. Arrows indicate offspring adults



Table 1 Number of galleries with single pigmented (adult) male, single pigmented female, and a pigmented pair in each log

Log ID	Number of galleries					
	No adults	Single male	Single female	More than one female	Single pair	Single adult with unknown sex
No. 1-1	59	0	29	3	0	1
No. 58-1-1	60	1	40	0	1	3
No. B-1-2	64	3	19	0	0	0
No. 58-1-2	79	4	19	0	0	7
No. 58-1-3	76	6	15	1	1	4
Total	338	14	122	4	2	15

Table 2 Number of male and female adults of *Polygraphus proximus* collected by the photoelector method

Date	No. of logs	No. of males	No. of females
July 25	8	4	14
July 26	8	4	16
July 27	8	2	18
July 29	9	5	22
Aug 1	9	1	22
Aug 2	9	2	16
Aug 3	12	5	22
Aug 5	12	4	18

in these numbers between the reproducing females and the reemerged females (Table 3). Of the 19 females attached twice, 7 successfully constructed mother galleries, and four oviposited along the galleries.

Discussion

This study revealed that the females of tree-killing bark beetle *P. proximus*, which employs male-initiated monogyny reemerged after laying one brood. The results from our study are consistent with the findings by Kōbayashi and Takagi (2020), which suggested reemergence in *P. proximus*. The findings from both the photoelector method and the galleries without parental adults in the present study indicate

that both males and females reemerged. The reemergence observed in the present study suggests that the galleries containing only a single male were the ones from which females reemerged. Additionally, the galleries that were inhabited exclusively by females were the ones from which males reemerged or ones bored by females that had already reemerged and successfully recolonized. There were more galleries with a single female than those with a single male, and a higher number of females were collected via the photoelector method compared to males. These suggest that males of *P. proximus* reemerged earlier than females.

Reemergence and sister broods (second broods) are commonly observed phenomena within the subfamily Scolytinae (Kirkendall 1983; Mendel et al. 1985; Anderbrant 1989). For example, *P. poligraphus* L., a secondary pest of Norway spruce *Picea abies* (L.) Karst reemerged and established a sister brood (Weiser et al. 2002). However, the requirements for mating while establishing sister broods remained uncertain in the genus *Polygraphus*. Additionally, *P. proximus* employs male-initiated monogynous mating system. To our knowledge, our findings are the first report of the brood initiation by the reemerged female without re-mating in bark beetle species with a male-initiated mating system. This study proves that *P. proximus* lays other broods in suitable habitats without repeated mating.

Approximately 40% of both the reproducing females and the reemerged females established sister broods, and there were no differences in their performance. The reproducing females might have been just before their reemergence, and

Table 3 Number of females that attempted to bore, successfully bore, established mother galleries, oviposited in logs, and successfully reproduced

Log ID	Collection source of females ^a	Number of females					
		Total	Attempted to bore into the log ^b	Successfully bored into the logs	Successfully established mother gallery	Successfully oviposited	Larval gallery construction
Log 1-3	G	5	3	1	1	1	1
	R	5	0	0	0	0	0
Log 2-3	G	5	3	3	3	3	3
	R	5	5	5	5	5	5
Log 4-5	G	5	2	2	2	1	1
	R	5	3	3	3	3	3
Log 6-4	G	5	5	4	4	4	4
	R	5	2	2	2	2	2
Log 10-5	G	5	0	0	0	0	0
	R	5	0	0	0	0	0
Total	G	25	13	10	10	9	9
	R	25	10	10	10	10	10

^aG indicates the reproducing females in galleries collected by peeling logs and R indicates the reemerged females collected by the photoelector method

^bThe existence of frass produced by the females was observed

this may have caused the absence of differences between them. Moreover, 21% (four out of 19) of the females reproducing the sister broods established the sister broods again. These results suggested that *P. proximus* females can establish sister broods more than once. Thus, our results indicate that the fecundity of *P. proximus* is higher than previously assumed by Yamaguchi (1963), because the previous study did not take the fecundities of reemerged females into the account. The logs cut in July were used for the first and second infestation tests. This duration difference between cutting and testing may affect log quality and result in differences in the number of eggs oviposited; therefore, we could not compare the number of eggs oviposited in the first and second sister broods.

Kerchev and Pousheva (2016) reported that *P. proximus* females initially penetrate host trees. In contrast, Viklund et al. (2022) reported male-specific components that are biologically active parts of *P. proximus* aggregation pheromones. Kerchev (2014b) and Kōbayashi and Takagi (2020) demonstrated that *P. proximus* employs a male-initiate monogyny mating system. The present study indicated that *P. proximus* could establish sister broods in suitable habitats without the need for repeated mating, which is consistent with the findings of previous studies on the sexes that initially bore into hosts. At some point in its life cycle, *P. proximus* mates under the bark. Males initially bore into their hosts, attracting females for mating and other males to participate in mass attacks. However, when females who have already mated recolonize a new host, they can attract other females and males to participate in mass attacks by emitting pheromones. Further studies are needed to investigate whether *P. proximus* females emit pheromones that attract other females and males.

Freshly cut logs were used in this study. Unlike living trees, logs are largely undefended substrates and incapable of inducing defense. However, during endemic population phases, nonaggressive bark beetles utilize weakened or dead trees as suitable habitats. Suppose that these nonaggressive bark beetles reemerge and establish sister broods within weakened or dead trees. In that case, the population levels can rapidly increase, ultimately lending them the ability to attack healthy trees during the epidemic. Our findings provide compelling evidence of the high fecundity of *P. proximus* during its endemic phase, challenging prior assumptions and shedding new light on its reproductive potential.

The phenomenon of beetles reemerging and reproducing multiple times, resulting in successive broods, can ensure that a continuous population of beetles can readily infest and colonize new trees. Additionally, the reemergence and development of sister broods may challenge the effectiveness of two commonly employed protective measures: removing infested timber and using trap trees. Reemergence and sister brood establishment are affected by attack densities,

temperature, and climate conditions (Anderbrant 1986, 1989; Davídková and Doležal 2017). The present study did not identify any factors associated with reemergence or sister broods. Further investigation of the factors that influence reemergence and sister brood establishment will provide valuable insights into the population dynamics of *P. proximus*.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13355-023-00860-7>.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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