AGRIVITA VOLUME 38 No. 1 FEBRUARY - 2016 ISSN : 0126 - 0537

THE BEHAVIORAL ROLE OF MALES OF *Platypus quercivorus* Murayama IN THEIR SUBSOCIAL COLONIES

Hagus Tarno [1*)], Hongye Qi [2)], Michimasa Yamasaki [3)], Masahide Kobayashi [4)] and Kazuyoshi Futai [2)]

[1)] Department of Plant Protection, Faculty of Agriculture, University of Brawijaya, Malang, Indonesia
[2)] Laboratory of Terrestrial Microbial Ecology, Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Japan
[3)] Laboratory of Forest Biology, Division of Forest and Biomaterials Science, Graduate School of Agriculture, Kyoto University, Japan
[4)] Graduate School of Life and Environmental Science, Kyoto Prefectural University, Japan
*) Corresponding author Email: h_gustarno@ub.ac.id

Received: November 8, 2015/ Accepted: January 13, 2016

ABSTRACT

The behavioral role of male ambrosia beetles, *Platypus quercivorus*, in subsocial colonies both field and laboratory has been investigated. The entrance tunnels, where the male beetles are staying, are short, with a mean 4 cm long and mostly incline upwards from outside to inside at an angle of around 20º. To examine the role of males, another male or female (as the invader) was placed into a tunnel. When inhabitant males stayed in the tunnel they quickly expelled the invaders, regardless of their sex. However, when an inhabitant male was removed, an introduced male or female could freely enter the tunnel and was accepted by the inhabitant female. Upon replacing an inhabitant male with an invader male or female and then putting another invader male or female into the tunnel, no rejection occurred, suggesting that invader males and females play no role in guarding the tunnel. Based on the results, an inhabitant male seems to have three responsibilities; protect the gallery from invaders, protect the progeny (larva) from falling and keep the gallery clean from frass. The 20º angle of the entrance tunnel tends to aid both in gallery protection and in frass clearing.

Keywords: entrance tunnel; gallery protection; inhabitant male; *Platypus quercivorus*; sociality

INTRODUCTION

Ambrosia beetles in the subfamilies Scolytinae and Platypodinae are one of the few groups of insects that have evolved farming of mutualistic fungi, a practice that has evolved at least seven times in Scolytinae weevils and once in the Platypodinae (Farrell *et al.*, 2001). There are also other similarities between the two groups, i.e. behavior such as parental care and colonial breeding are both widespread in the two related groups (Kirkendall *et al.*, 1997). Worldwide, Platypodinae contains over 1,400 species (Beaver and Shih, 2003), including important pests such as the ambrosia beetle *Platypus quercivorus* (Murayama) causing Japanese oak wilt on some Fagaceae species (Sone *et al.*, 1998; Kubono and Ito 2002; Kobayashi and Ueda, 2003, 2005; Kinuura and Kobayashi, 2006; Yamasaki and Futai, 2008).

Regarding the mating system, *P. quercivorus* beetles are monogamous and the male initiates tunnel boring into trunks of host trees to form a gallery system (Kobayashi *et al.*, 2001). *Platypus quercivorus* also has a complicated mating behavior similar to those of other Platypodids species (Kobayashi and Ueda, 2002). The term 'subsocial insect' refers to those insects that provide parental care for their offspring. Such subsocial behavior has been regarded as one of the early stages of eusociality in insects. Males and females both play an important part in social insect. Kent and Simpson (1992) studied the social behavior of the ambrosia beetle, *Austroplatypus incompertus* (Scheld) which lives in galleries in the heartwood of living Eucalyptus trees. However, the social behavior of *P. quercivorus*, which belongs to the same family as *A. incompertus*, has not been thoroughly studied.

The research objective was to elucidate the role of the male in the gallery system of *P.*

Cite this as: Tarno, H., H. Qi, M. Yamasaki, M. Kobayashi and K. Futai. 2016. The behavioral role of males of *Platypus quercivorus* Murayama in their subsocial colonies. AGRIVITA. 38(1): 47-54. doi: 10.17503/agrivita.v38i1.778
Accredited: **SK No.81/DIKTI/Kep/2011**
Permalink/DOI: **http://dx.doi.org/10.17503/agrivita.v38i1.778**

quercivorus. Research was aimed to study the role of male beetles of *P. quercivorus* from behavioral points of view including (1) gallery protection against invaders, (2) protection of larvae from falling down and (3) clearing of frass from galleries. Some members of the public tried to prevent emergence of *P. quercivorus* from infested trees by plugging all entrance holes with toothpicks, and most of those toothpicks slightly inclined downward, suggesting the tunnel near entrance hole also inclined downward from inside to outside. So the slope of the tunnel was also measured in relation to the above-mentioned male beetle behavior.

MATERIALS AND METHODS

Beetles

Beetle-infested oak logs, *Quercus crispula* (Blume), were collected from the Ashiu Forest Research Station, Kyoto University, located in the northeastern part of Kyoto Prefecture. The logs were dissected to collect male and female adults. These males and females were used as invaders (alien beetles) in following experiments.

The Angle and the Length of Entrance Tunnels

Eight, *Quercus serrata* (Thunb) logs (46.0-57.0 cm long, 26.0-34.0 cm in diameter) infested with *P. quercivorus* were used to measure the angle and the length of 33 entrance tunnels (the distance from the entrance to the point of the first branch). First, a toothpick was inserted into the entrance tunnel, and the angle formed between the toothpick and a vertical wool thread was measured using a protractor (Figure 1). The logs were placed vertically while measuring of the angle of entrance tunnel. Then the logs were dissected and the lengths of the entrance tunnels were measured.

Removing and Replacing Beetles

Micropipette tips (7.0 cm long, 0.8 cm diameter opening at the terminal tip) and wooden tooth picks (6.5 cm long, 0.2 mm in diameter) were used to remove and replace inhabitant beetles from tunnels. Healthy active males and females were used as invaders (alien beetles) in field experiments that were done at Yoshida–yama hill, Kyoto city, 125 m above sea level (asl).

The inhabitant males were removed as follows: male or female (alien beetle) was placed into a micropipette tip, the apical point of which was then inserted into the tunnel entrance. The alien beetle in the micropipette tip then moved from the tip into the tunnel entrance. After a few minutes, the introduced beetle walked back to the micropipette tip, and the inhabitant male pushed it out and consequently both beetles were captured in the micropipette tip. Replacing an inhabitant male with an invader male or female was done using the same technique.

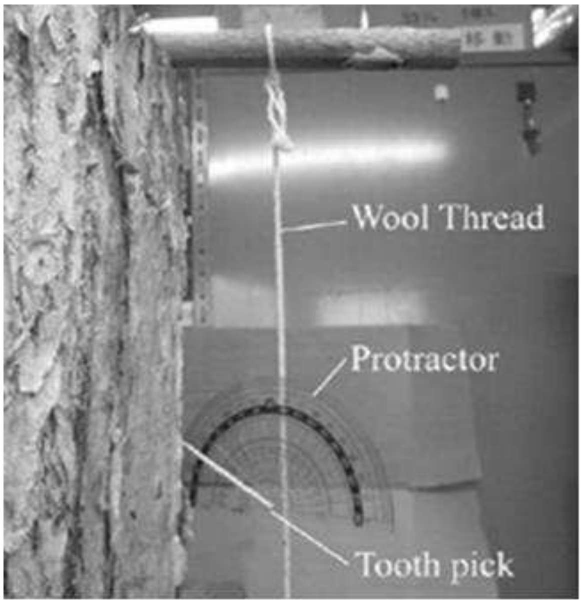

Figure 1. Measurement of *Platypus quercivorus* entrance tunnel angle

Field Experiment to Observe Gallery Protection

At Yoshidayama hill, three treatments were done: 1) forcing an invader male, or a female (as alien beetle) to enter a tunnel with an inhabited male and female, 2) after removing an inhabitant male from the tunnel, an invader male or female were forced to enter the tunnel with an invader male, and 3) after replacing an inhabitant male in a tunnel with an invader male or a female, another invader male or female were forced to enter the tunnel. Ten minutes after introducing the second invader, a 10.0 cm long x 1.5 cm diameter plastic test tube sealed with a plastic cap was inserted into the entrance hole of tunnel using a 1.0 cm long x 0.4 cm diameter metal connecting tube attached to the plastic cap (Figure 2). The plastic tube was kept in the entrance tunnel for 3 days to examine if the invader was accepted or not. All three treatments were replicated 10 times.

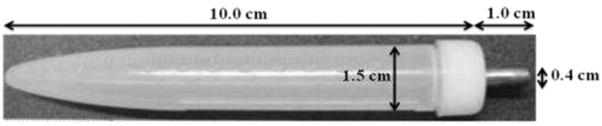

Figure 2. Plastic tube, used for capturing of fallen down beetle, capped with a cap and attached with metal tube

Log Experiment to Observe Gallery Protection

To confirm the results obtained at Yoshida -yama hill, the same experiments were conducted in a greenhouse by using five, oak *(Q. crispula)* logs infested with *P. quercivorus*. Observations were conducted using a video camera to record the behavior of male and female beetles for 5-10 minutes after introduction of an invader beetle.

Video Monitoring for Beetle Behavior in Wood

To observe the behavior of an invader in the tunnel after removing of inhabitant male, the following method (here on refer to as the 'sandwich method') was adopted (Figure 3). Twelve small logs, ca. 15-25 cm in diameter and 10 cm in length, were immersed in tap water for 5 days before using to increase their water content and maintain humidity for the growth of symbiotic fungi (Kobayashi *et al*., 2004). Two transparent plates, ca. 25 x 25 cm with a hole on each corner, were attached tightly by using four bolts to the small log. Introduction of a male then a female into the logs was conducted as in Tarno *et al.* (2011). Behavior of inhabitant females and invader males were observed by using a video camera for 10 minutes after removing of inhabitant male. Removing the inhabitant male and replacing by an invader male were done after the inhabitant female had mated with the original inhabitant male and had laid eggs in the gallery.

Effect of Entrance Tunnel Slope on Gallery Protection

The effect of the tunnel slope (angles of the entrance tunnel) on gallery protection by inhabitant male was examined as follows. For this purpose, an infected log of *Q. serrata*, ca. 25 cm diameter and ca. 60 cm long, was obtained from Yoshida Mountain in August 2010. The log was sealed on both ends by paraffin to prevent desiccation. Five tunnels that produced a lot of powdery frass were selected for tests. To confirm the presence of an inhabitant male in each tunnel, an invader beetle was introduced to the tunnel. When the inhabitant male came out, immediately the tunnel was used for this experiment.

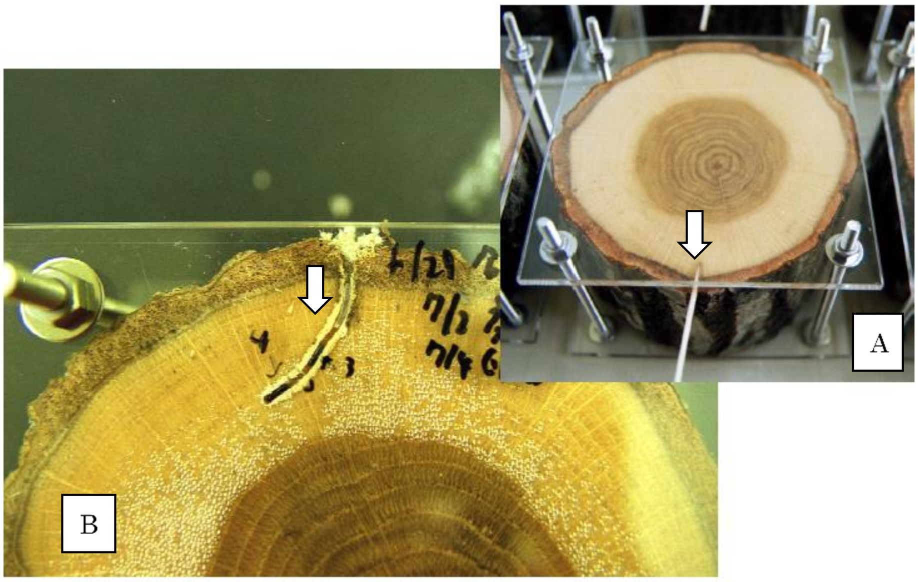

Figure 3. A sandwich method and a toothpick showing the artificial tunnel (A) and gallery made by adults on a sandwich method (B)

For each test, the log was positioned to keep the entrance tunnel at either of three angles (20°, 0° and -20°) with a protractor. To keep the entrance tunnel at -20°, the log was positioned upside down. By using a video camera with a macro lens, the time between introduction of an invader beetle and its expulsion by a resident male was measured for 10 minutes at most.

Protection of Larvae from Falling

To reveal the role of an inhabitant male in protecting his progeny the numbers of larvae that had fallen from tunnel were compared between the galleries with and without an inhabitant male. Experiments were carried out using trees infested with *P. quercivorus* at Yoshida-yama hill. To remove an inhabitant beetle, the aforementioned method was adapted. Briefly, a plastic test tube, as mentioned above, was inserted into the entrance hole of the tunnel on each tree. In 2009, ten trees were used for each treatment (with and without a male) and two to seven successful tunnels per tree were used to examine the numbers of larvae fallen from each tunnel. In 2010, five trees were selected and two to four successful tunnels per tree were examined. For both years, a total of forty and sixty tunnels were examined for the tunnels with and without males, respectively.

Effect of Entrance Tunnel Slope on Frass Clearing

Based on the mean angle of the entrance tunnel slopes measured, efficiency of frass clearing was compared among three different angles, 20°, 0° and -20°. Five infested logs of *Q. serrata* having produced powdery frass were used to compare frass clearing among entrance tunnels with different slope angles. The frass amount ejected from each entrance hole was compared as an indicator to determine the efficiency of frass clearing. A plastic test tube (as described above) was attached to one entrance hole for each log, and then put on a desk at room conditions (23°C). All logs were placed on a desk maintained at one of the three angles, 20°, 0° and -20°, and the angles were changed which started from 0°, 20° and finally -20° coincide for all logs every third day, and plastic tube was replaced with new one to harvest frass ejected during the period. Frass was desiccated in the drier (41°C) for two hours and then weighed on the digital balance (A&D Co. Ltd.).

RESULTS

The Angle and the Length of the Main Tunnel

The mean angle of 33 entrance tunnels observed were ca. 20º (19.72° ± 9.96), and the mean length of the entrance tunnels was 4 cm (4.01 ± 0.95) (Figure 4).

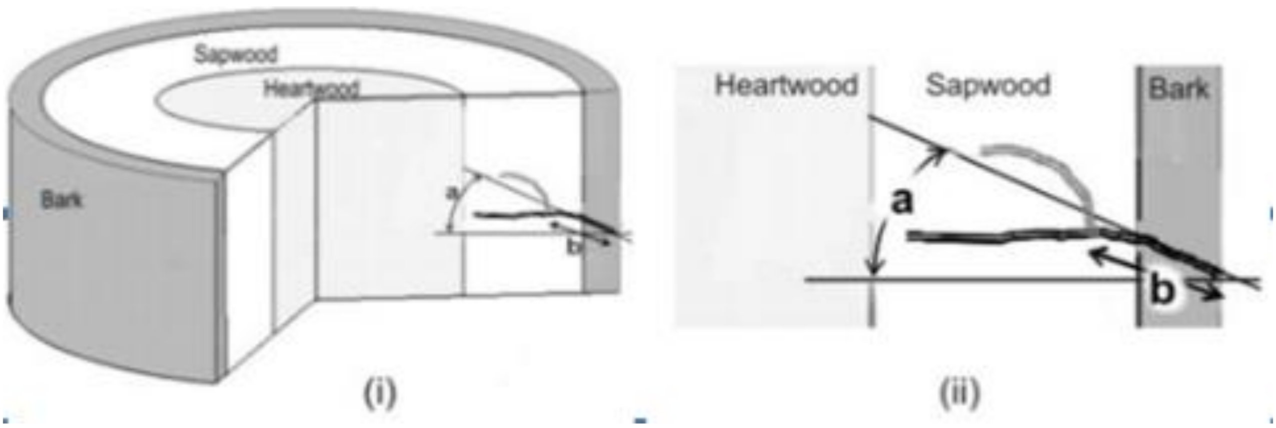

Figure 4. Characteristic of *P. quercivorus* entrance tunnel in 3D (i) and 2D (ii), a. angle (mean = 19.72° ± 9.96, n = 33), b. length of entrance tunnel (mean = 4.01 cm ± 0.95, n = 33)

Gallery Protection

In the first treatment, when an invader beetle was forced to enter a tunnel, the inhabitant male quickly rejected the invader from the tunnel (Figure 5a). In the second treatment, when the inhabitant male was removed and then an invader was introduced into the tunnel, no rejection of the invader occurred even though the inhabitant female remained in the tunnel (Figure 5b). In the third treatment, an inhabitant male in the tunnel was replaced with an invader male or female, then another invader beetle was introduced into the tunnel, but no rejection occurred, suggesting that an invader male or female introduced into a tunnel accepted a subsequently applied invader (Figure 5c). Responses of the inhabitant beetles in the galleries to the invader beetles observed both in the field and greenhouse are summarized in Table 1.

Video camera monitoring of beetle behavior in host logs revealed that inhabitant females did not reject an invader male when introduced into her gallery. An invader male, a substitute male for inhabitant one, also did not disturb the inhabitant female activity performed in front of him. Thus, the invader male seemed to be accepted by the inhabitant female.

The time needed for inhabitant males to evict the invader ranged one to seven minutes for the entrance tunnels with slope ca. 20°. In the case of 0° and ca. -20°, they need more than ten minutes. It means the entrance tunnel with slope ca. 20° can be helpful for inhabitant male to expel the invader from the tunnel.

Protection of Larvae from Falling

Of 40 tunnels from which inhabitant males had been artificially removed, 43 larvae were confirmed to fall from 10 tunnels. It indicated that without the inhabitant male, larvae fell from 25% of tunnels. In the case of 60 control tunnels, that contain an inhabitant male, larval falling down was confirmed only from three tunnels and the number of larvae that fell from the tunnels was just ten. For control, fallen larvae were found for 5% of tunnels. Occurrences of the fallen larvae in the tunnels were described in Table 2.

Frass Clearing

The amount of powdery frass ejected from the entrance tunnel was the largest (0.3866 ± 0.0095 g; n=5) when the tunnel slope was at an angle of ca. 20º. The amount of frass was intermediate (0.3706 ± 0.0031 g; n=5) when the tunnel slope was positioned at ca. 0º, and the smallest (about 0.3667 ± 0.0082 g, n=5) when the tunnel slope was positioned at ca. -20º. By using Tukey B, there was a significant difference ($p < 0.05$) in frass amount ejected among entrance tunnels with different angles.

Table 1. Acceptance responses of *P. quercivorus* beetles inhabiting in tunnels to invader beetles (field and green house experiments)

Added beetle	Beetles inhabiting in a tunnel (∑ acceptance / ∑ replications)					
	♂ + ♀ (inhabitant)		♀ (inhabitant)*)		♂ / ♀ (invader) + ♀ (inhabitant)*)	
	Field	Greenhouse	Field	Greenhouse	Field	Greenhouse
♂	0/10	0/10	10/10	9/10	10/10	10/10
♀	0/10	0/10	10/10	10/10	10/10	10/10

Remarks: All treatments were replicated 10 times; *) = beetle responses were observed for 10 minutes and then observed again after 3 days

Table 2. Occurrences of fallen larvae in the tunnels those were treated with and without male

Tunnel condition	Treatments (∑ Tunnels)		∑ Tunnels
	With ♂ (control)	Without ♂	
Fallen down larvae	3	10	13
No fallen down larvae	57	30	87
Total	60	40	100

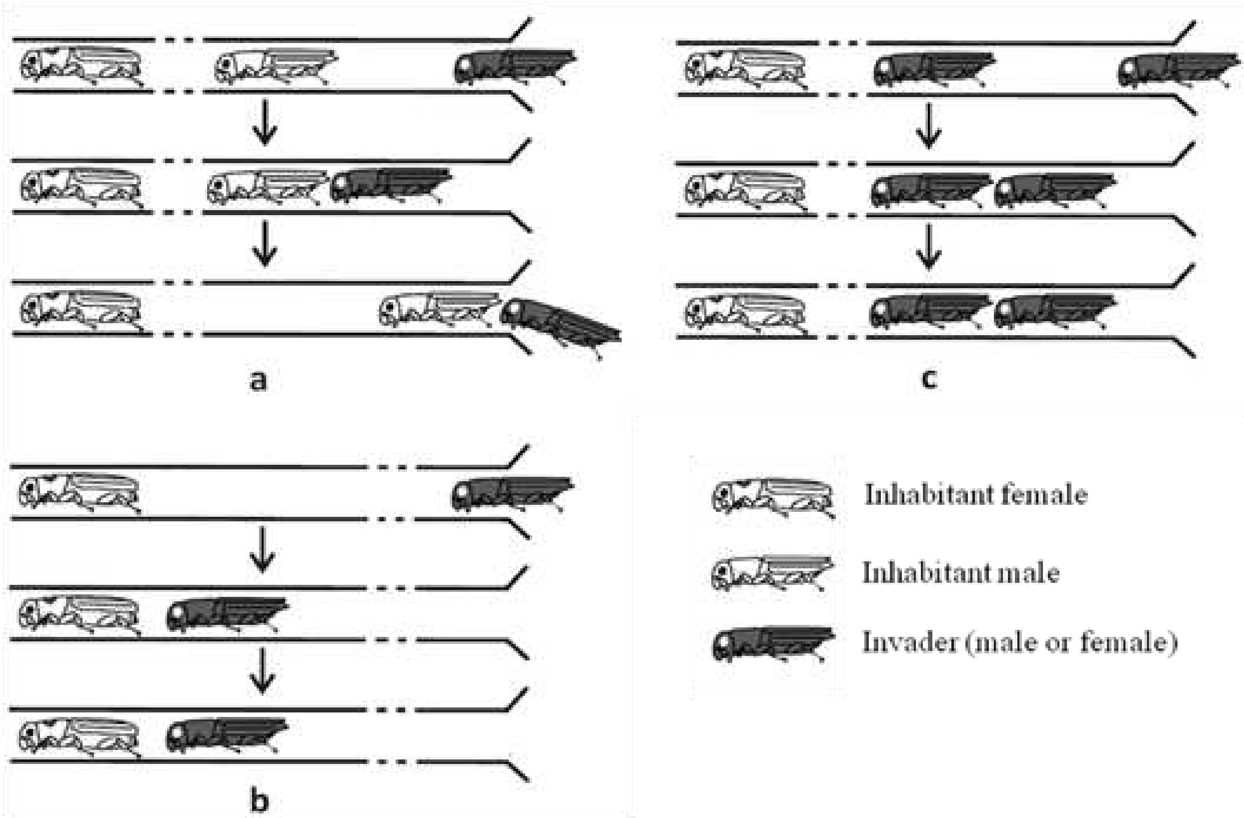

Figure 5. Response of inhabitant male to invader male or female (a), inhabitant female versus invader male or female (b) and an invader male to another invader male or female (c)

DISCUSSION

The experiments on the gallery protection behavior of *P. quercivorus* ambrosia beetles against invaders gave clear results. The inhabitant males always rejected conspecific invaders irrespective of sex. In contrast, the invaders male were accepted when a female remained in a tunnel without an inhabitant male, and also when an invader male occupied a tunnel. So the role of male and female are clearly different. The male has the role of guarding his tunnel system also the same phenomenon was found among other ambrosia beetle such as *Ips pini* (Say) (Reid and Roitberg 1994; Kirkendall *et al.*, 1997).

Males of *P. quercivorus* have a strategy to protect their galleries by blocking the tunnel hole. Kirkendall *et al.* (1997) suggest that an obvious possible function of blocking the entrance hole by *I. pini* is excluding predators, parasites, inquilines, or competitors. Ryan (1997) also explained that males offer parental care by protecting offspring from danger. Reid and Roitberg (1994) demonstrated the defense of offspring by males of *I. pini*, where removal of the males for only three days led to a major increase of predators in the tunnels.

Tunnel blocking allows males to control the number of females gaining access to the gallery system. In a monogynous system, however, it is not evident if the second female is excluded by the male in the tunnel or only chooses not to enter the tunnel which is already occupied by a female (Kirkendall *et al.*, 1997). In the case of *P. quercivorus*, our results suggest that a male maintains a monogynous system by expelling an invader female, but inhabitant females play no such role. However, more studies need to be done to confirm this.

Kirkendall *et al.* (1997) state that tunnel blocking by males also regulates humidity and thus keeps high moisture content in the wood which facilitates growth of ambrosia fungi. By remaining near the entrance hole the male can also protect the offspring from falling out of the tunnel. Kirkendall *et al.* (1997) showed that the males are responsible for keeping larvae and eggs in the gallery. Males of two platypodids (*Doliopygus* spp.) have been observed rescuing eggs in danger of being ejected along with wood fragments (Kirkendall *et al.*, 1997). In this study many more larvae fell from tunnels when the inhabitant male of *P. quercivorus* had been removed. The same has been observed by Kirkendall *et al.* (1997) when males of *Platypus cylindricus* were removed.

The angle of the entrance tunnel was ca. 20° and this obviously helps inhabitant males to expel invaders from their galleries. Also the slope of the entrance tunnel must facilitate frass clearing, though it may increase the risk of larva falling. Males usually remain near the entrance hole, but when they find frass in front of them, they move back and forth in the main tunnel (Tarno, unpublished data, 2010). Thus, the male keeps the gallery free of waste and frass. The slope of the entrance hole near the entrance can also help males when they are discarding frass because males would use less energy to push the frass out of the tunnel. The responsibility of male of *P. quercivorus* was related to keeping the gallery clean by removing frass in the gallery, as well as in the case of male *I. pini* (Lissemore, 1997). Jackson and Hart (2009) explained that most social animals have solved the problems of waste by depositing it directly in a dedicated place away from colony or by removing it from the colony as it is produced.

An entrance tunnel of 4 cm is long enough for the male to stay near the entrance hole, and move back and forth to remove frass. In Platypodids, males may often stay near the entrance hole throughout their life. It is apparently common for both parents to break off tarsal segments and claws, thereby rendering them incapable of walking outside their tunnel systems. Males usually remain with females in these tunnel systems, controlling and expelling refuse (Kirkendall *et al.*, 1997).

The case of *A. incompertus*, breeding in living trees of *Eucalyptus* spp. in southeastern Australia, is unusual among ambrosia beetles since males do not stay and help their mates (Kent and Simpson, 1992). For *A. incompertus*, the role of the male was assumed by females to protect their gallery against predators and to remove frass (Kent and Simpson, 1992; Kirkendall *et al.*, 1997). This differs from *P. quercivorus*, where females never assume the male duties.

CONCLUSION

An inhabitant male seems to have three responsibilities; protect the gallery from invaders, protect the progeny (larva) from falling and also keep the gallery clean from frass. In addition, the 20º angle of the entrance tunnel tends to aid both in gallery protection and in frass clearing.

REFERENCES

Beaver, R.A. and H.T. Shih. 2003. Checklist of Platypodidae (Coleoptera: Curculionoidea) from Taiwan. Plant Protection Bulletin. 44: 75-90.

Farrell, B.D., A.S. Sequeira, B.C. O'Meara, B.B. Normark, J.H. Chung and B.H. Jordal. 2001. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). Evolution. 55(10): 2011-2027. doi: 10.1111/j.0014-3820.2001.tb01318.x

Jackson, D.E. and A.G. Hart. 2009. Does sanitation facilitate sociality?. Animal Behaviour. 77(1): e1-e5. doi: 10.1016/j.anbehav.2008.09.013

Kent, D.S. and J.A. Simpson. 1992. Eusociality in the beetle *Austroplatypus incompertus* (Coleoptera: Curculiomdae). Naturwissenschaften. 79(2): 86-87. doi: 10.1007/BF01131810

Kinuura, H. and M. Kobayashi. 2006. Death of *Quercus crispula* by inoculation with adult *Platypus quercivorus* (Coleoptera: Platypodidae). Applied Entomology and Zoology. 41(1): 123-128. doi: 10.1303/aez.2006.123

Kirkendall, L.R., D.S. Kent and K.F. Raffa. 1997. Interactions among males, females and offspring in bark and ambrosia beetles: The significance of living in tunnels for the evolution of social behavior. In: Choe, J.C. and B.J. Crespi (Eds.), The evolution of social behavior in insect and arachnids. Cambridge: Cambridge University Press. p. 181-214.

Kobayashi, M. and A. Ueda. 2002. Preliminary study of mate choice in *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). Applied Entomology and Zoology. 37(3): 451-457. doi: 10.1303/aez.2002.451

Kobayashi, M. and A. Ueda. 2003. Observation of mass attack and artificial reproduction in *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) (in Japanese with English summary). Japanese Journal of Applied Entomology and Zoology. 47(2): 53-60. doi: 10.1303/jjaez.2003.53

Kobayashi, M. and A. Ueda. 2005. Wilt disease of fagaceae trees caused by *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) and the associated fungus: Aim is to clarify the damage factor (in Japanese with English summary). Journal of the Japanese Forest Society. 87(5): 435-450. doi: 10.4005/jjfs.87.435

Kobayashi, M., A. Nozaki and A. Ueda. 2004. Influence of water content of host trees on attacking behavior of *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) and on fungi in the galleries bored by the beetles (in Japanese with English summary). Japanese Journal of Applied Entomology and Zoology. 48(2): 141-149. dweoi: 10.1303/jjaez.2004.141

Kobayashi, M., A. Ueda and Y. Takahata. 2001. Inducing infection of oak logs by a pathogenic fungus carried by *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). Journal of Forest Research. 6(3): 153-156. doi: 10.1007/BF02767086

Kubono, T. and S. Ito. 2002. *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle *(Platypus quercivorus)*. Mycoscience. 43(3): 255-260. doi: 10.1007/s102670200037

Lissemore, F.M. 1997. Frass clearing by male pine engraver beetles (*Ips pini*; Scolytidae): paternal care or paternity assurance?. Behavioral Ecology. 8(3): 318-325.

Reid, M.L. and B.D. Roitberg. 1994. Benefits of prolonged male residence with mates and brood in pine engravers (Coleoptera: Scolytidae). Oikos. 70(1): 140-148. doi: 10.2307/3545709

Ryan, M.J. 1997. Sexual selection and mate choice. In: Krebs, J.R. and N.B. Davies (Eds.), Behavioural ecology: An evolutionary approach (4th edition). London: Blackwell Science. p. 179-202.

Sone, K., T. Mori and M. Ide. 1998. Life history of the oak borer, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). Applied Entomology and Zoology. 33(1): 67-75. doi: 10.1303/aez.33.67

Tarno, H., H. Qi, R. Endoh, M. Kobayashi, H. Goto and K. Futai. 2011. Types of frass produced by the ambrosia beetle *Platypus quercivorus* during gallery construction, and host suitability of five tree species for the beetle. Journal of Forest Research. 16(1): 68-75. doi: 10.1007/s1 0310-010-0211-z

Yamasaki, M. and K. Futai. 2008. Host selection by *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) before and after flying to trees. Applied Entomology and Zoology. 43(2): 249-257. doi: 10.13 03iaez.2008.249