

# Larval helpers and age polyethism in ambrosia beetles

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Division of labor among the workers of insect societies is a conspicuous feature of their biology. Social tasks are commonly shared among age groups but not between larvae and adults with completely different morphologies, as in bees, wasps, ants, and beetles (i.e., Holometabola). A unique yet hardly studied holometabolous group of insects is the ambrosia beetles. Along with one tribe of ants and one subfamily of termites, wood-dwelling ambrosia beetles are the only insect lineage culturing fungi, a trait predicted to favor cooperation and division of labor. Their sociality has not been fully demonstrated, because behavioral observations have been missing. Here we present behavioral data and experiments from within nests of an ambrosia beetle, *Xyleborinus saxesenii*. Larval and adult offspring of a single foundress cooperate in brood care, gallery maintenance, and fungus gardening, showing a clear division of labor between larval and adult colony members. Larvae enlarge the gallery and participate in brood care and gallery hygiene. The cooperative effort of adult females in the colony and the timing of their dispersal depend on the number of sibling recipients (larvae and pupae), on the presence of the mother, and on the number of adult workers. This suggests that altruistic help is triggered by demands of brood dependent on care. Thus, ambrosia beetles are not only highly social but also show a special form of division of labor that is unique among holometabolous insects.

altruism | cooperative fungiculture | insect agriculture | larval workers | mutualism

Division of labor enhances work efficiency and is fundamental to the biological evolution of social complexity (1). This conspicuous feature of social insects largely explains their ecological success (2). Task specialization in insects usually occurs between different age groups. Individuals either pass through consecutive molts during development (Hemimetabola; e.g., termites, aphids), with immatures resembling small adults and division of labor occurring typically between such larval nymphs that may show morphological specializations [e.g., first-instar soldier aphids (3)]; or individuals metamorphose by dramatically reorganizing their morphology during the pupal stage (Holometabola; e.g., bees, wasps, ants, beetles), which predestines larvae and adults to specialize in different tasks because of their morphological and physiological differentiation. Indeed, larvae of the eusocial Hymenoptera, for example, may produce nest-building silk [weaver ants (4)] and may supply adults with extra enzymes and nutrients [trophallaxis in several wasps and ants (2, 5, 6)]. However, all known cooperative actions of holometabolous larvae are apparently completely under adult control (2), and despite the potential for the evolution of highly specialized immature helper morphs, larvae of these taxa are largely immobile and helpless and in need of being moved, fed, and cared for by adults (7). In ambrosia beetles, however, in which cooperative breeding (8) and eusociality (9) also have been assumed, larvae can move and forage independently in the nest, which provides great potential for division of labor between larvae and adults.

Division of labor sets the stage for the origin of fungiculture in fungus-growing ants and termites (10). Ambrosia beetles originate from solitary or colonial ancestors, and their fungus agriculture may have coevolved with sociality (8, 10, 11). However,

the role of division of labor is unknown. Ambrosia beetles live inside trees, which is a habitat extraordinarily favoring social evolution (12), apparently having fostered at least seven independent origins of fungiculture in beetles (13). Hence, they represent a unique model system to study the evolution of sociality in relation to fungiculture. Interestingly, ambrosia beetles vary in their mating system (inbreeding vs. outbreeding species) and ploidy level (haplodiploid vs. diploid species), which are factors that have been assumed to contribute to social evolution, although their respective roles in social evolution are controversial (1). The ambrosia beetle subtribe Xyleborini is characterized by regular inbreeding, haplodiploidy, and fungiculture (8, 14). High relatedness and haplodiploidy in combination with an extremely female-biased sex ratio are factors predisposing them to advanced sociality (1). Additionally, cooperation in fungiculture is likely because a single individual can hardly maintain a fungus garden on its own (10). Indeed, it was shown that adult female offspring delay dispersal from their natal gallery, which results in an overlap of eggs, larvae, pupae, and at least two generations of adult offspring within a colony (8). A helper effect of philopatric females has been indicated by the fact that the number of staying females that do not reproduce correlates positively with gallery productivity in *Xyleborinus saxesenii* Ratzeburg (8). Behavioral observations of ambrosia beetles within their galleries have been missing so far, however, because it is virtually impossible to observe them in nature inside the wood. The only report on eusociality in ambrosia beetles is not based on behavioral data, but reproductive roles have been inferred by destructive sampling of active nests of *Austroplatypus incomptus* (9). To facilitate observations of beetle behaviors inside galleries, we developed artificial observation tubes to contain entire colonies of reproducing beetles (15, 16). Here we use this breeding technique of *X. saxesenii* to ask (i) whether offspring produced in a gallery engage in alloparental brood care and fungus maintenance, (ii) whether different types of individuals specialize in divergent tasks, and (iii) whether decisions to help and to disperse relate to the number of potential beneficiaries and the number of potential workers present in the colony. Furthermore, we evaluate experimentally (iv) whether female dispersal depends on the presence of an egg-laying foundress, because her removal should affect the need for alloparental care. We compare our results with the patterns of sociality known from other major insect taxa.

## Results

**Age- and Sex-Specific Behavior.** Gallery maintenance and brood care were allocated differently between different age classes within a nest (Fig. 1 and Table S1). The gallery was extended mainly by larvae (*digging*), which also reduces the spread of mold (*SI Text* and Fig. S1), whereas fungus care (*cropping*) and brood protection (*blocking*) were exclusively conducted by adults. *Blocking* was only

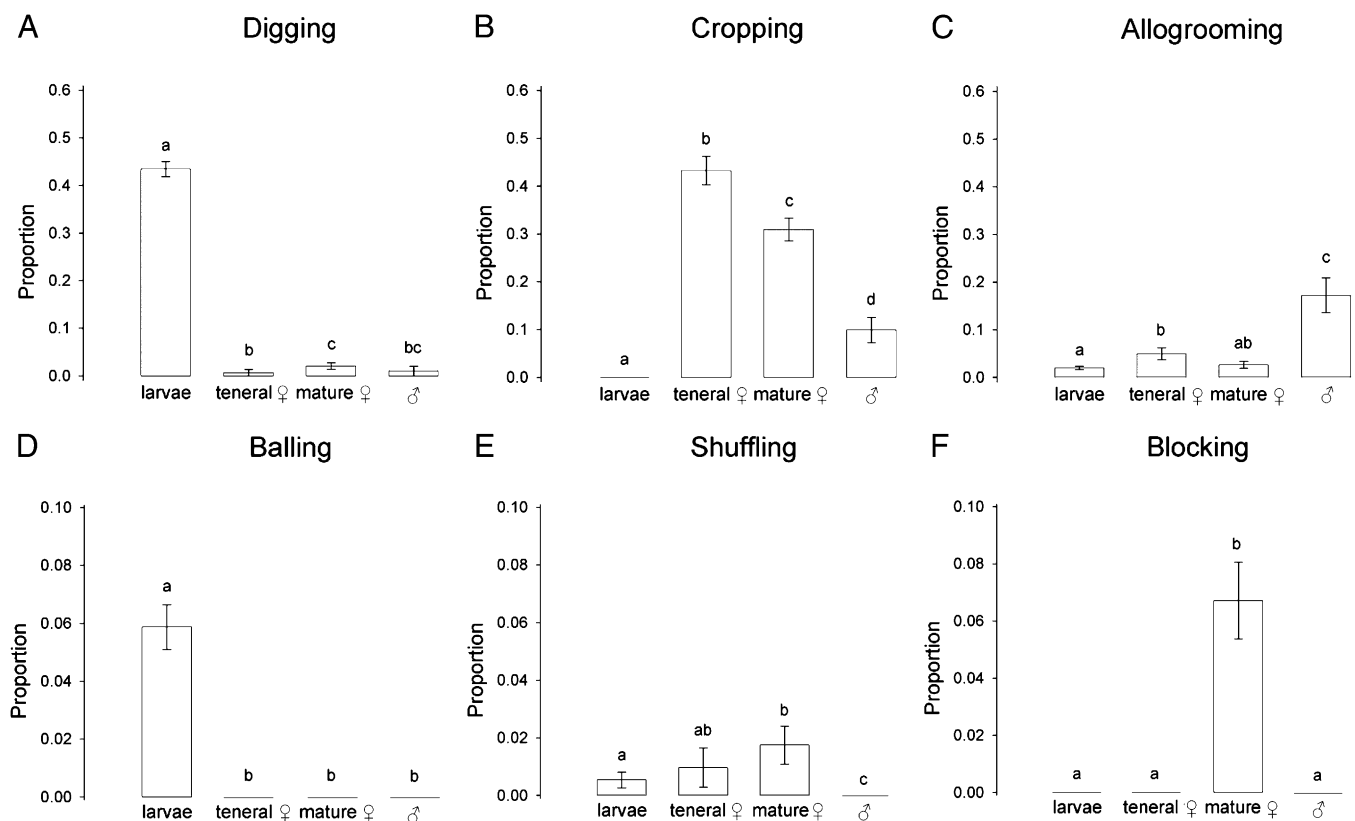
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**Fig. 1.** Division of labor and age polyethism between age and sex classes in *X. saxesenii*. Bars show the mean ( $\pm$ SE) proportions of time larvae, teneral females, mature females, and males performed different cooperative tasks. Statistically significant differences between the classes are denoted by different letters ( $P < 0.05$ ; GEE, details in Table S1). Note scale differences between A–C and D–F.

performed by foundresses and mature females, and in 33 of 35 cases only one individual blocked at a time. In the 25 cases (in 19 of 93 galleries) when we did not observe a blocking female, the following behavioral scan revealed that larvae had crawled out of the gallery. At least 71 larvae ( $\bar{x} = 3.7$  larvae per gallery) died in this manner, which suggests that an important function of *blocking* is to prevent larvae from getting accidentally lost.

Larvae and adults took different shares in hygienic behaviors. Only larvae compressed dispersed waste into compact balls (*balling*; Movie S1). Frass-balls, pieces of wood, or parts of dead siblings were moved within the gallery and pushed out of the entrance (*shuffling*; Movie S2) mainly by mature females but to some extent also by larvae and teneral females (Fig. 1 and Table S1). The ultimate waste disposer was always the mature female that was *blocking* the entrance. *Cannibalism* was directed toward adult beetles (1 case), pupae (3 cases), and larvae (37 cases) that were already dead (in most cases) or that did not respond to being groomed. In such cases the groomer (larva or adult) would use its mandibles to open the body of the groomed sibling within seconds. *Allogrooming* was shown by all stages and both sexes, and it seemed to be crucial for individual survival: in an experiment with 10 pupae that were singly kept either with one or six larvae, the pupae survived in five of five cases with six larvae, but they survived in only one of five cases with one larva (Fisher exact test:  $P = 0.048$ ,  $n = 10$ ; details in SI Text and Fig. S2). In the other four cases with one larva, the pupae were overgrown and killed by fungi. In addition, the body surface of single foundresses that had not yet successfully established a brood was overgrown by a fungal layer (mainly *Paecilomyces variotii* and *Fusarium merismoides*) within a few weeks, which caused the death of at least 7 of 29 solitary females. Whenever individuals of different stages encountered each other, they removed the visible

fungal layer on each other's bodies by *allogrooming*. In males, *allogrooming* was frequently followed by a mating attempt; it may thus serve also to obtain information about female mating status.

**Adult Female Behaviors Depending on Gallery Composition.** The proportion of time adult females exhibited *cropping* and *allogrooming* and the occurrence of *blocking* were higher during gallery stages when brood dependent on care was present in the colony (*preadult* and *larval-adult* gallery stages) than during other times (*postlarval* gallery stage; Table S2). *Shuffling* was shown equally often before, during, and after larval presence within a gallery, whereas adult female *digging* tended to increase per capita after pupation of the last larva. *Cannibalism* was not shown before the hatching of adult daughters, and it occurred more often when dependent offspring were still present. Dispersal of adult females seemed to be contingent on brood care demands: it significantly increased in the 10 d after a major proportion of larvae had completed their development relative to the 10 d before (Wilcoxon:  $z = -3$ ,  $P < 0.001$ ,  $n = 23$ ; Fig. S3). Because brood numbers likely correlate with fungus productivity, dispersal could be triggered by alternative factors, however, like the quality of the wood/medium or fungus. Nevertheless, in summary there are hints that some behavioral tasks of adult females functionally relate to the demands of care-dependent brood.

In a second analysis we tested for the relationship between adult female behaviors and the numbers of adult females and brood (pupae and larvae) present in the galleries during the *larval-adult* gallery stage only (Table S2). Analyzed per capita, adult female *digging*, *shuffling*, *cannibalism*, and *blocking* were all independent of the numbers of adult females and younger nestmates. However, per capita *allogrooming* and fungus *cropping* activities significantly increased with increasing numbers of pu-

pae and larvae, whereas with increasing adult female numbers *allogrooming* significantly decreased. Adult female dispersal correlated negatively with the number of dependent offspring [generalized estimation equation (GEE):  $P = 0.003$ ; Fig. 2A and Table S2] and positively with adult female numbers (GEE:  $P < 0.001$ ; Table S2).

In 34 of 43 galleries with mature offspring, females delayed their dispersal from the natal nest after maturation. This philopatric period (i.e., the latency from the first appearance of a mature female within a gallery until the first dispersal event) correlated positively with the average number of dependent brood (eggs, larvae, pupae) per adult female present during this period (Spearman rank correlation:  $R_s = 0.379$ ,  $P = 0.027$ ,  $n = 34$ ).

**Effect of Foundress Removal.** Eggs are produced primarily by the foundress, and in 4 of 16 galleries dissected in the field eggs were produced also by at least one daughter (on average reproduced 23.9% of all females in these 4 galleries: range, 2–4 egg-layers on 4–22 females in total; details in ref. 17). If fewer eggs are produced, the need for alloparental care declines. Therefore, we predicted that the number of dispersing daughters will increase if the foundress is experimentally removed from the gallery. Our experimental interference raised the dispersal activity of daughters (relative to the same galleries before the manipulation; Fig. 2B) in the treatment (Wilcoxon:  $z = -2.637$ ,  $P = 0.008$ ,  $n = 10$ ) and in the control groups (Wilcoxon:  $z = -1.82$ ,  $P = 0.034$ ,  $n = 10$ ). Removal of the foundress, however, raised the dispersal of daughters much more strongly than the control situation (i.e., removal of the medium without the foundress;  $U$  test:  $z = -3.708$ ,  $P < 0.001$ ,  $n = 10 + 10$ ).

## Discussion

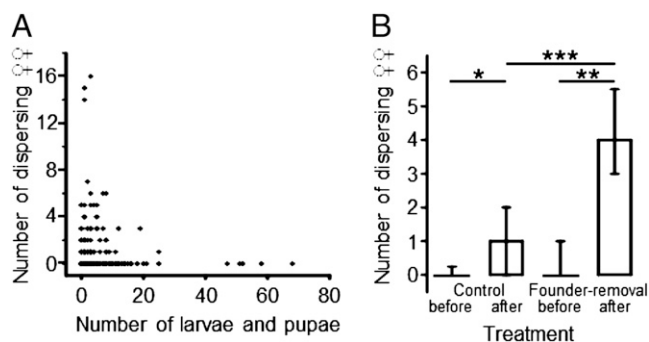
Here we report on division of labor and age polyethism in Coleoptera, which apparently relate to the fungiculture in ambrosia beetles. It confirms the predicted high degree of sociality of ambrosia beetles (9, 11). In *X. saxesenii*, all group members contribute to the divergent tasks of gallery maintenance and brood care, and there is correlative evidence that female offspring delay their dispersal depending on the number of potential beneficiaries present in their natal gallery. Tasks are typically shared differentially between larval and adult colony members, which resembles the division of labor reported from termites and other highly social insect taxa (Table 1). Among holometabolous insects, however, *X. saxesenii* is the only species to date known to exhibit an active behavioral task specialization between larvae and adults. Furthermore, adult daughters in this species spe-

cialize in different tasks than the colony foundress, and they seem to adjust their work effort flexibly to the varying size of the brood produced by the latter. Dispersal propensity of adult females (i) correlates positively with low numbers of dependent young and high numbers of adult workers, and (ii) is increased by experimental removal of the foundress. This suggests that philopatry may be related to indirect fitness benefits, because the group members sharing a gallery are all very highly related (for another xyleborine beetle see ref. 14). There is no morphological differentiation among adult females, and they are all fully capable of breeding and establishing their own gallery. However, dissections of all females in a number of field galleries showed that daughters cobred in their natal gallery in only a quarter of colonies (17). In summary, the social and reproductive patterns of *X. saxesenii* conform to primitive eusociality, defined by overlapping generations, cooperative brood care, and some reproductive division of labor, despite totipotency in reproduction of all individuals.

**Division of Labor Between Larvae and Adults.** Task sharing in *X. saxesenii* is unequal between the sexes and age classes for most of the social behaviors described (Fig. 1 and *SI Text*). Regarding gallery hygiene, for example, larvae form balls from dispersed frass, whereas the transport and removal of these balls is mainly performed by adult females. Gallery extension is almost exclusively accomplished by larvae. A social role of larvae has never been reported in ambrosia beetles, and gallery maintenance and fungiculture have been attributed solely to the foundress (11, 15, 18). Larvae might serve a cooperative function not only in Xyleborini but also in other beetles, as anecdotal observations and speculations suggest from the Scolytinae [e.g., *Dendroctonus* sp.(19); the Xyloterini (20)], Platypodinae [e.g., *Trachyostus ghanaensis* (21); *Doliopygus conradti* (22)], and Passalidae [*Passalus cornatus* (23)]. The ultimate cause for the larval specialization in digging may relate to their repeated molting: as the mandibles gradually wear during excavation (for wood-dwelling termites see ref. 24), adult females showing extensive digging behavior would suffer from substantial long-term costs. In contrast, larval mandibles regenerate at each molt.

Teneral and mature females take over fungus care. *Blocking* of the gallery entrance is done exclusively by mature females and almost only by the foundress (see ref. 14 for similar observations in *Xylosandrus germanus*). Direct brood care by *allogrooming* is performed by all age classes. Males groom females at high rates, which may primarily serve courtship because mating attempts are always initiated by *allogrooming* (cf. 20). Males do not take part in other social behaviors except for low levels of *digging* and *cropping*. Asymmetries in relatedness caused by haplodiploidy should favor females to become helpers (25, 26). Inbreeding can nevertheless reduce relatedness asymmetries and thus favor also males to help (27). Male soldiers and brood-caring males have been documented in haplodiploid and sib-mating thrips and *Cardiocondyla* ants, respectively (27). In Xyleborini, very few males are produced [approximately 5–12% of offspring (28)], and males do not seem to contribute significantly to gallery function, hygiene, and fungus growth. Instead, they seem to specialize in their reproductive role by continually attempting to locate and fertilize their sisters. Nevertheless, both male larvae and adults are fully capable of performing all of the behaviors exhibited by females, as demonstrated in galleries that contain only males [approximately 2% of *X. saxesenii* galleries are founded by unfertilized females that produce solely male broods (16, 28)].

The life-history trajectory of *X. saxesenii* is most similar to that of social aphids and some termite families, where individuals serve as helpers (e.g., workers and soldiers) in their natal colonies before maturation (Table 1). Either sex may help in these taxa, and their flexible developmental period allows individuals to adjust the length of their immature stage to maximize their



**Fig. 2.** (A) Adult female dispersal correlates negatively with the number of cared brood in the gallery (larvae and pupae; GEE:  $P = 0.003$ ; statistical details in Table S2). (B) Effects of removal of the blocking foundress. The numbers of dispersing *X. saxesenii* adult females (medians and quartiles) are shown 4 d before and 4 d after the experimental treatments. Wilcoxon tests: \* $P < 0.05$ , \*\* $P < 0.01$ ; Mann-Whitney  $U$  test: \*\*\* $P < 0.001$ ; sample sizes were 10 control galleries and 10 galleries from which the foundress was removed.



**Table 1. Forms of division of labor in exemplary species of the most prominent social insect taxa**

Social insect species	Behavioral task-specialization			Mode of nesting and nourishment	Reference
	Between immatures* and adult stages	Among adult females	Cooperation by males		
<b>Hemimetabola</b>					
<i>Cryptotermes cavifrons</i> (Kalotermitidae)	+	–	–	Wood dweller, gut symbionts	45
<i>Hodotermopsis japonica</i> (Termopsidae)	+	–	+	Wood dweller, gut symbionts	46
<i>Reticulitermes fukienensis</i> (Rhinotermitidae)	+	–	?	Subterranean, gut symbionts	47
<i>Macrotermes subhyalinus</i> (Termitidae)	+	+	+	Subterranean and mound building, fungus cultivation	48
<i>Pemphigus spyrothecae</i> (Aphidae)	+	–	–	Plant galls, sap sucking	49
<i>Kladothrips intermedius</i> (Thysanoptera)	–	+	+	Plant galls, sap sucking	50
<b>Holometabola</b>					
<i>Lassioglossum zephyrum</i> (Halictinae)	–	+	–	Ground nesting, pollen and nectar	51
<i>Exoneura bicolor</i> (Allodapinae)	–	+	–	Stalk-nesting, pollen and nectar	51
<i>Apis mellifera</i> (Apidae)	–	+	–	Cavity nesting, pollen and nectar	52
<i>Liostenogaster flavolineata</i> (Stenogastrinae)	–	+	–	Free nesting, arthropods and nectar	53
<i>Vespula germanica</i> (Vespinae)	–	+	–	Free nesting, arthropods and nectar	7
<i>Atta texana</i> (Formicidae)	–	+	–	Subterranean, fungus cultivation	2
<i>Oecophylla longinoda</i> (Formicidae)	– <sup>†</sup>	+	–	Tree nesting, arthropods and honeydew	4
<i>Austroplatypus incompertus</i> (Platypotinae)	–	+	+	Wood boring, fungus cultivation	9
<i>Xyleborinus saxesenii</i> (Scolytinae)	+	+	+	Wood boring, fungus cultivation	This study

+, present; –, absent; ?, unknown.

\*Nymphal and larval stages.

<sup>†</sup>Larvae of weaver ants produce nest-building-silk, but the weaving action is controlled by the worker ants (2, 4).

inclusive fitness. They may either remain in an immature helper phase at the nest or develop into adults that can disperse or reproduce in their natal colony (3, 29–32). Likewise, the developmental period of second- and third-instar larvae of *X. saxesenii* can vary between 4 and 17 d (16). In addition, there is a striking similarity of these taxa in ecology and mating patterns. They all inhabit defensible nests, often within wood, which favors local mating and inbreeding—conditions claimed as strongly favoring social evolution (12). In many of these cases helping tasks do not seem to curtail a helper's future reproduction (i.e., because helping is risk free and does not reduce a helper's energy stores), which may weaken the tradeoff between helping and future reproduction (33, 34).

**Role of Individual Selection and Kin Selection.** We defined a behavior as cooperative if social partners potentially benefit from its performance, independently of whether this behavior entails net costs to the actor (35). This definition includes (i) mutualistic behaviors that are regarded as selfish acts generating benefits to other individuals (common goods) as a by-product (36), and (ii) altruistic behaviors that bring about net costs to the actor, which are compensated through indirect fitness benefits via kin-selection; they will thus only evolve in groups of relatives (25). In the course of social evolution and task specialization shaped by kin selection, mutualistic behaviors may lose their original function and change into truly altruistic behaviors (36). High relatedness and spatial separation between groups are very favorable to the evolution of cooperative behaviors (12), as long as local competition between relatives does not oppose this force (37).

In Xyleborini, ideal conditions for the evolution of both mutualism and altruism seem to prevail: (i) they are well pre-adapted to brood care because they originate from a beetle lineage (Scolytinae) with parental care (38); (ii) they breed in isolated galleries that are founded by one reproductive that dominates offspring production [i.e., the foundress (17)]; (iii) they are haplodiploid and mate predominantly among full siblings [e.g., inbreeding coefficients of approximately 0.9 in *Xylosandrus germanus* Reiter (8)], which increases relatedness within natal colonies; and (iv) they disperse solitarily after maturation to reproduce elsewhere. In addition, (v) colony members benefit greatly from cooperation due to their dependence on fungiculture; and (vi) the wood used as a resource for shelter and substratum for fungi is virtually nondepreciable, which renders resource competition negligible. Larval *digging*, for example, which might be regarded as a by-product of selfish larval feeding, reduces competition because it generates a common good (i.e., space and substratum for fungiculture). Similarly, other group members benefit from gallery hygiene resulting as a by-product from the seemingly selfish adult feeding activities *cropping* and *cannibalism*. By contrast, *balling*, *shuffling*, *allogrooming*, *blocking*, and adult *digging* may rather be altruistic. These behaviors apparently benefit other group members at the expense of time and energy costs to the actors, without immediate benefits to the latter. Particularly dangerous are *allogrooming* and *blocking*, because they expose the performers to pathogens, parasites, and predators.

Adult *X. saxesenii* females showed a strong incentive to stay and cooperate in a productive natal nest. Partly this may be

selfish, because up to one quarter of females may get the chance to reproduce (17), and females might build up reserves during their philopatric period for subsequent dispersal and nest foundation. An experimental study in the ambrosia beetle *Xyleborus affinis* suggests, however, that females do not build up reserves during their philopatric period but rather suffer direct fitness costs (39). Alternatively, indirect fitness benefits may be involved in helping to raise siblings (8). Three results suggest that adult female cooperation is triggered by the demands of brood dependent on care: (i) helping effort of adult females rose with a greater number of brood dependent on care, (ii) adult females dispersed at a higher rate with an increasing number of workers in the colony, and (iii) dispersal rate of females increased in response to experimental removal of the foundress, which indicates that delayed dispersal of females is not primarily motivated by the potential to breed in the natal gallery (see also ref. 8).

In conclusion, the high degree of sociality in ambrosia beetles seems to result from a combination of four major factors: (i) parental care as a preadaptation for the evolution of sociality in the ancestors of modern ambrosia beetles (38); (ii) very high relatedness within families due to haplodiploidy and inbreeding; (iii) a proliferating, monopolizable resource providing ample food for many individuals, which needs to be tended and protected; and (iv) high costs of dispersal (for another scolytine beetle see ref. 40) due to the difficulties of finding a suitable host tree, of nest foundation, and a successful start of fungiculture (11, 16), which render predispersal cooperation particularly worthwhile. *X. saxesenii* larvae are predisposed to assume certain tasks like balling of frass and gallery enlargement (digging) because of their body morphology and the frequent renewal of mandibles by molting. Thus, behavioral tasks are shared between larval and adult stages. This has not been shown for beetles, and the described division of labor between immature and adult stages seems to be unique among holometabolous social insects at large.

## Materials and Methods

**Study System.** *X. saxesenii* galleries are founded by individual females that transmit spores of the species-specific ambrosia fungus *Ambrosiella sulfurea* Batra in their gut from the natal to the new gallery (41, 42). This fungus forms a yellow layer of fruiting cells on the surface of gallery walls (Fig. S4A). After landing on a tree trunk, the foundress excavates a straight tunnel into the xylem with a small egg niche at its end. As soon as fungus beds emerge, she feeds on them and starts egg-laying (16). During offspring development the egg niche is enlarged to a single flat brood chamber of up to a few square centimeters in size and  $\approx 1$ -mm height. There, most of the fungus garden grows, and individuals of all age classes live in close contact with each other (Fig. S4C). In this study we bred *X. saxesenii* in glass tubes filled with artificial medium that mainly contained sawdust (for details on this method see SI Text and ref. 16).

**Behavioral Recordings and Analyses.** In total, 93 of roughly 500 galleries were founded successfully in the laboratory (i.e., eggs were laid), and in 43 of these galleries individuals reached adulthood. These galleries were used for behavioral analyses. We distinguished 11 behaviors (Table S3), which comprised seven cooperative behaviors that apparently raise the fitness of colony members. Every second to third day we performed scan observations of all individuals visible within a gallery (details in SI Text).

**Age- and sex-specific behavior.** We used GEEs (details below) with an exchangeable correlation structure of the response variable within a cluster (= gallery identity) to identify effects of the four classes of individuals (larvae, teneral females, mature females, males) on the proportion of time spent with a certain behavior, using binomial error distributions (Fig. 1 and Table S1).

**Adult female behaviors depending on gallery composition.** To check for potential effects of gallery age on behavior, we discriminated between the following successive stages of gallery development: (i) *preadult* gallery stage: founder female and dependent offspring (larvae and pupae) with or without eggs present ( $n = 2$  galleries); (ii) *larval-adult* gallery stage: all age classes (mature/teneral beetles, larvae, and pupae) with or without eggs present ( $n = 31$  galleries); and (iii) *postlarval* gallery stage: only adults without eggs, larvae, or pupae present ( $n = 16$  galleries). Variation in sample size of galleries was caused by the fact that not all age classes were visible in every gallery. We used a first series of GEEs to identify the effect of the particular stage of gallery development on the proportion of time adult females (= teneral and mature females) spent with a certain behavior. In a second GEE series performed only with data of the *larval-adult* gallery stage, we analyzed whether and how task performance of adult females related to the number of adult females present and to the number of pupae and larvae present in the gallery (Table S2).

**Female dispersal.** For each gallery we measured the retention period between the first appearance of a mature daughter (fully sclerotized, ready to disperse) within the gallery and the first female dispersal event (i.e., the female had left through the gallery entrance and sat on top of the medium, where it tried to escape through the cap). Using a Spearman rank correlation analysis we tested whether the dispersal delay interval related to the mean number of offspring attended, divided by the mean number of adult females present at that time.

**Influence of foundress on offspring dispersal.** In the entrance tunnel foundresses either block (sit still and fully close the tunnel) or move back and forth when shuffling material to the dumps. To determine the influence of the foundress' presence on the behavior and dispersal propensity of mature females, we experimentally removed the first centimeter of the entrance tunnel when a female was present in there ( $n = 11$  galleries), at a stage when eggs, larvae, and adult daughters were present together with the foundress. We determined the reproductive status of the removed female by dissection to check whether we had successfully removed the foundress. We excluded one gallery from the treatment group, where we found an immature female blocking instead of the foundress. In the control group ( $n = 10$  galleries) we removed the first centimeter of medium when no female was present in this part of the entrance tunnel. Experimental galleries were randomly assigned to treatment and control groups. Dispersal of the daughters was measured in both groups for 4 d before and 4 d after the intervention by collecting the females on top of the medium that had left the gallery through the entrance and tried to disperse through the cap of the tube (Fig. 2B).

**Statistics.** We used a series of GEEs [Imer in R (43)], which are an extension of generalized linear models with an exchangeable correlation structure of the response variable within a cluster (= gallery identity), to analyze effects of dependent variables on correlated binary response variables (proportional data were transformed to binary data) and to identify factors affecting the relative behavioral frequencies per class (larvae, adult females, and males) (44). First, we tested whether the larvae, teneral females, mature females, and males show different tendencies to express the cooperative behaviors (Fig. 1 and Table S1). Second, we compared these frequencies between foundresses and their mature daughters (SI Text and Table S4). In a third series of GEEs we determined the influence of a particular developmental stage of the gallery (*preadult*, *larval-adult*, and *postlarval* gallery stages) on the relative behavioral frequency per class (Table S2). Finally, we modeled whether larvae and adult female numbers affected the relative frequencies of cooperative behaviors in adult females (Table S2). For the removal experiment we compared behavioral frequencies and dispersal activity between the groups using Mann-Whitney *U* tests, and within groups using Wilcoxon matched-pairs signed-ranks tests (Fig. 2B). All statistical analyses were performed with SPSS version 15.0 and R version 2.8.1 (43).

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1. Bourke AFG (2011) *Principles of Social Evolution* (Oxford Univ Press, Oxford).
2. Hölldobler B, Wilson EO (1990) *The Ants* (Belknap Press of Harvard Univ Press, Cambridge, MA).
3. Stern DL (1997) Foster WA. *Social Behaviour in Insects and Arachnids*, eds Choe JC, Crespi BJ (Cambridge Univ Press, Cambridge, U.K.), pp 150–165.
4. Wilson EO, Hölldobler B (1980) Sex differences in cooperative silk-spinning by weaver ant larvae. *Proc Natl Acad Sci USA* 77:2343–2347.

5. Ishay J, Ikan R (1968) Food exchange between adults and larvae in *Vespa orientalis* F. *Anim Behav* 16:298–303.
6. Hunt JH, Baker I, Baker HG (1982) Similarity of amino-acids in nectar and larval saliva - the nutritional basis for trophallaxis in social wasps. *Evolution* 36:1318–1322.
7. Wilson EO (1971) *The Insect Societies* (Belknap Press of Harvard Univ Press, Cambridge, MA).

8. Peer K, Taborsky M (2007) Delayed dispersal as a potential route to cooperative breeding in ambrosia beetles. *Behav Ecol Sociobiol* 61:729–739.
9. Kent DS, Simpson JA (1992) Eusociality in the beetle *Austroplatypus incompertus* (Coleoptera: Platypodidae). *Naturwissenschaften* 79:86–87.
10. Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR (2005) The evolution of agriculture in insects. *Annu Rev Ecol Syst* 36:563–595.
11. Kirkendall LR, Kent DS, Raffa KF (1997) *The Evolution of Social Behavior in Insects and Arachnids*, eds Choe JC, Crespi BJ (Cambridge Univ Press, Cambridge, U.K.), pp 181–215.
12. Hamilton WD (1978) *Diversity of Insect Faunas*, eds Mound LA, Waloff N (Blackwell, Oxford), pp 154–175.
13. Farrell BD, et al. (2001) The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodidae). *Evolution* 55:2011–2027.
14. Peer K, Taborsky M (2005) Outbreeding depression, but no inbreeding depression in haplodiploid Ambrosia beetles with regular sibling mating. *Evolution* 59:317–323.
15. Saunders JL, Knoke JK (1967) Diets for rearing the ambrosia beetle *Xyleborus ferrugineus* (Fabricius) in vitro. *Science* 15:463.
16. Biedermann PHW, Klepzig KD, Taborsky M (2009) Fungus cultivation by ambrosia beetles: Behavior and laboratory breeding success in three xyleborine species. *Environ Entomol* 38:1096–1105.
17. Biedermann PHW, Peer K, Taborsky M (2011) Female dispersal and reproduction in the ambrosia beetle *Xyleborinus saxeseni* Ratzeburg (Coleoptera: Scolytinae). *Mitt Dtsch Ges Allg Angew Entomol*.
18. Francke-Grosman H (1967) *Symbiosis*, ed Henry SM (Academic Press, New York), pp 141–205.
19. Deneubourg JL, Grégoire JC, LeFort E (1990) Kinetics of larval gregarious behaviour in the bark beetle *Dendroctonus micans* (Coleoptera: Scolytidae). *J Insect Behav* 3:169–182.
20. Wichmann HE (1967) Die Wirkungsbreite des Ausstoßreflexes bei Borkenkäfern. *Anzeiger für Schädlingskunde. J Pest Sci* 40:184–187.
21. Roberts H (1968) Notes on biology of ambrosia beetles of genus *Trachyostus* Schedl (Coleoptera - Platypodidae) in West Africa. *Bull Entomol Res* 58:325–352.
22. Browne FG (1963) Notes on the habits and distribution of some Ghanaian bark beetles and ambrosia beetles (Coleoptera: Scolytidae and Platypodidae). *Bull Entomol Res* 54:229–266.
23. Miller WC (1932) The pupa-case building activities of *Passalus cornutus* Fab. (Lamellicornia). *Ann Entomol Soc Am* 25:709–712.
24. Roisin Y (2000) *Termites: Evolution, Sociality, Symbioses, Ecology*, eds Abe T, Bignell DE, Higashi M (Kluwer Academic, Dordrecht, The Netherlands), pp 95–119.
25. Hamilton WD (1964) The genetical evolution of social behaviour. I. *J Theor Biol* 7:1–16.
26. Alexander RD (1974) The evolution of social behavior. *Annu Rev Ecol Syst* 5:325–383.
27. Hamilton WD (1972) Altruism and related phenomena, mainly in social insects. *Annu Rev Ecol Syst* 3:193–232.
28. Biedermann PHW (2010) Observations on sex ratio and behavior of males in *Xyleborinus saxeseni* Ratzeburg (Scolytinae, Coleoptera). *Zookeys* 56:253–267.
29. Thorne BL (1997) Evolution of eusociality in termites. *Annu Rev Ecol Syst* 28:27–54.
30. Crespi BJ (1997) Mound LA. *The Evolution of Social Behaviour in Insects and Arachnids*, eds Choe JC, Crespi BJ (Cambridge Univ Press, Cambridge, U.K.), pp 166–180.
31. Chapman TW, Crespi BJ, Perry SP (2008) *Ecology of Social Evolution*, eds Korb J, Heinze J (Springer, Berlin), pp 57–83.
32. Korb J (2008) *Ecology of Social Evolution*, eds Korb J, Heinze J (Springer, Berlin), pp 151–174.
33. Queller DC, Strassmann JE (1998) Kin selection and social insects. *Bioscience* 48:165–175.
34. Korb J (2008) *Ecology of Social Evolution*, eds Korb J, Heinze J (Springer, Berlin), pp 245–259.
35. Brosnan S, de Waal F (2002) A proximate perspective on reciprocal altruism. *Hum Nat* 13:129–152.
36. Lin N, Michener CD (1972) Evolution of sociality in insects. *Q Rev Biol* 47:131–159.
37. Hamilton WD (1975) *Biosocial Anthropology*, ed Fox R (John Wiley & Sons, New York), pp 133–155.
38. Jordal BH, Sequeira AS, Cognato AI (2011) The age and phylogeny of wood boring weevils and the origin of subsociality. *Mol Phylogenet Evol* 59:708–724.
39. Biedermann PHW, Klepzig KD, Taborsky M (2011) Costs of delayed dispersal and alloparental care in the fungus-cultivating ambrosia beetle *Xyleborus affinis* Eichhoff (Scolytinae: Curculionidae). *Behav Ecol Sociobiol* 65:1753–1761.
40. Garraway E, Freeman BE (1981) Population-dynamics of the juniper bark beetle *Phloeosinus neotropicus* in Jamaica. *Oikos* 37:363–368.
41. Batra LR (1966) Ambrosia fungi: Extent of specificity to ambrosia beetles. *Science* 153:193–195.
42. Francke-Grosman H (1975) Zur epizoischen und endozoischen Übertragung der symbiotischen Pilze des Ambrosiakäfers *Xyleborus saxeseni* (Coleoptera: Scolytidae). *Entomologica Germanica* 1:279–292.
43. R Development Core Team (2008) R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, Vienna, Austria).
44. Zeger SL, Liang KY, Albert PS (1988) Models for longitudinal data: A generalized estimating equation approach. *Biometrics* 44:1049–1060.
45. Crosland MWJ, Traniello JFA, Scheffrahn RH (2004) Social organization in the dry-wood termite, *Cryptotermes cavifrons*: Is there polyethism among instars? *Ethol Ecol Evol* 16:117–132.
46. Machida M, Miura T, Kitade O, Matsumoto T (2001) Sexual polyethism of founding reproductives in incipient colonies of the Japanese damp-wood termite *Hodotermopsis japonica* (Isoptera: Termopsidae). *Sociobiology* 38:501–512.
47. Crosland MWJ, Ren SX, Traniello JFA (1998) Division of labour among workers in the termite, *Reticulitermes fukiensis* (Isoptera: Rhinotermitidae). *Ethology* 104:57–67.
48. Badertscher S, Gerber C, Leuthold RH (1983) Polyethism in food-supply and processing in termite colonies of *Macrotermes subhyalinus* (Isoptera). *Behav Ecol Sociobiol* 12:115–119.
49. Benton TG, Foster WA (1992) Altruistic housekeeping in a social aphid. *Proc R Soc Lond B Biol Sci* 247:199–202.
50. Crespi BJ (1992) Behavioral ecology of Australian gall thrips (Insecta, Thysanoptera). *J Nat Hist* 26:769–809.
51. Choe JC, Crespi BJ (1997) *The Evolution of Social Behaviour in Insects and Arachnids* (Cambridge Univ Press, Cambridge, U.K.).
52. Lindauer M (1953) Division of labour in the honey bee colony. *Bee World* 34:63–73.
53. Korb J, Heinze J (2008) *Ecology of Social Evolution* (Springer, Berlin).



# Supporting Information

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## SI Text

**Activity of Individuals Dependent on Light and Gravity.** All behavioral observations of *Xyleborinus saxesenii* in this study were done under a microscope with a 6-W artificial light source. For storage, however, tubes were wrapped in paper to keep the gallery dark as if in wood. Therefore, we tested whether the changing of light conditions and of the axis of gravity would affect beetle activity.

Five galleries during the *larval-adult* gallery stage were used to obtain five activity measures after three different treatments: the numbers of active and inactive individuals (larvae and adults combined) in each gallery were counted (i) right after uncovering the tubes, (ii) after 30 min of exposure to a 6-W light source, and (iii) after 60 min of light exposure, right after changing the axis of gravity by 90°.

Separate pairwise comparisons (Wilcoxon matched-pairs signed-ranks test) of the five observations within each gallery were combined in a metaanalysis according to Sokal and Rohlf (1):  $\chi^2(2 \times N) = -2 \times \sum \ln(\pi)$ .

This revealed no significant influence of the light [ $\chi^2(10) < 18.31$ ;  $P > 0.05$ ] and gravity treatments ( $P > 0.05$ ) on the activity of both larvae and adults.

Behaviors of the bark beetle *Ips pini* also do not differ between individuals kept in the dark or exposed to light, nor between individuals reared in chambers in a vertical position or in a horizontal position (2).

**Tendency of *X. saxesenii* Larvae to Aggregate.** For this experiment we removed all individuals and tunnels from seven galleries during the *larval-adult* gallery stage, leaving only some centimeters of fungus-infested medium within the tubes. In these seven tubes we created three artificial chambers of  $\approx 20$  mm<sup>2</sup> (and  $\approx 1$ -mm height) within the medium, extending next to the glass from the top of the medium alongside the tube wall. Six to twelve second/third-instar larvae were placed on top of the medium in each of the tubes and allowed to move freely. After 24 h we recorded the location of all larvae.

In six replicates, all larvae had moved inside one and the same of the three chambers. In only one replicate had larvae split up and were distributed over two chambers. This suggests that *X. saxesenii* larvae show a strong tendency to aggregate.

**Effect of Larval Numbers on the Survival of Pupae and on Fungus Growth.** For this experiment we removed all individuals from 13 galleries during the *larval-adult* gallery stage. In each of these galleries we created two flat chambers with a height of  $\approx 1$  mm and an area between 22.86 mm<sup>2</sup> and 91.89 mm<sup>2</sup> next to one of the existing tunnels and next to the tube glass (to allow observations). Thereafter, one chamber was filled with one larva and the other one with six larvae (chambers were randomly assigned). In 5 of the 13 galleries we also placed one pupa each in both chambers, which contained one or six larvae, respectively. For the next 14 d we tracked the survival of the pupae (in 5 galleries) and the appearance of mold on the walls of the chambers (in all 13 galleries). Several fungi coexist in the medium at this gallery stage, and mold fungi are expected to overtake if they are not controlled by larvae. Therefore, we took pictures of the chambers on day 14 after the treatment and analyzed the chamber area covered and uncovered by mold according to larval numbers, by using the program ImageJ (version 1.44p). We predicted that (i) fungal layers growing on the body surface of pupae should be removed more frequently by six *allogrooming* larvae than by one *allogrooming* larva, which may

affect survival of pupae, and that (ii) six *digging* larvae should be more successful than one *digging* larva to hinder the spread of mold on the chamber walls.

Survival of pupae was significantly affected by the number of coinhabiting larvae (Fisher exact test:  $P = 0.048$ ,  $n = 10$ ). All five pupae survived in the chambers with six larvae, whereas only one of the five pupae survived in the chamber with one larva (Fig. S2). Pupae died because they were overgrown by fungi.

The percentage of chamber wall area covered with mold after 14 d was significantly lower when the chamber was inhabited by six larvae (median 24.1%) than by one larva [median 90.5%; generalized estimation equation (GEE): coefficient  $\pm$  SE =  $-3.072 \pm 0.163$ ,  $z = 18.86$ ,  $P < 0.001$ ,  $n = 26$ ; Fig. S1]. Mold fungi covered 93% of the chamber area in two chambers that were left empty for 14 d.

In summary, this experiment revealed the importance of (i) larval *allogrooming* for the survival of pupae and of (ii) larval *digging* to hinder the spread of mold fungi within the gallery.

**Behaviors of Foundresses Vs. Mature Daughters.** Usually the foundress cannot be distinguished from her mature daughters once the latter are fully sclerotized. In six galleries, however, the foundress could be distinguished from her mature daughters because they remained lighter than their mothers for an extended period. In these galleries we made 10 focal observations of foundresses and 14 of mature daughters (10 min each). GEE models showed that foundresses and their mature daughters differed significantly in the frequency of cooperative behaviors, except for *allogrooming* (Table S4) and *cannibalism*, which were never observed by foundresses. The individual *blocking* the gallery entrance was always the foundress during the focal animal observations. In the removal experiment, however, 1 of 11 dissections of the *blocking* female revealed immature ovaries, indicating that daughters may also *block* the gallery entrance occasionally. Per capita, foundresses showed more fungus *cropping* and *shuffling* behavior but less gallery extension behavior (*digging*) than their mature daughters. In summary, foundresses spent more time with cooperative behaviors than their mature daughters.

**Details on Function of Different Cooperative Behaviors. Gallery extension—digging.** An important common good created by larval *digging* is the enlargement of the gallery. Nevertheless, it is probably a mutually beneficial behavior with selfish benefits for the larvae because it is also part of their feeding on fungal hyphae penetrating the wood. The consumed wood passes the gut without any sign of digestion (3, 4), however, because the enzymes required to digest wood are likely missing (5, 6). Because hyphae of several fungi cooccur together with the ambrosia fungus in the medium/wood (especially when the gallery gets older), larval *digging* apparently also hindered the spread of mold (see above and Fig. S1). Mold fungi did not completely overgrow chambers in the presence of larvae. They probably serve the larvae as additional food source, despite the fact that these fungi are usually toxic to arthropods. Previous studies have shown that toxic secondary metabolites produced by mold fungi as a response to feeding by arthropods can be overcome if the arthropods feed gregariously on these fungi (7–9). *X. saxesenii* larvae showed a strong tendency to aggregate within the gallery (see above).

The *digging* of adults is clearly different from feeding and solely serves gallery extension.

Gallery enlargement by *digging* is a mutual benefit to colony members, because it increases the surface where the ambrosia

fungi can grow, in this way lowering within-family competition for food and space (10). Gallery surface area positively affects fungus and thus colony productivity (11–13). The contribution of adults to gallery extension is negligible compared with larvae.

**Fungus care—cropping.** Adult females are constantly walking and screening the fungal layers with their large, disk-shaped antennae (Fig. S4D). They stop frequently to move their hairy, comb-like mouthparts through the fungus (Fig. S4E), probably brushing off sprouting ambrosia structures (Fig. S4B). This *cropping* behavior of the adults apparently serves both nutrient intake and fungus care. It induces the characteristic ambrosial growth of the fungus, that is, the formation of copious ambrosia cells (fruiting bodies) and sporodochia [clusters of fungus spores (14–18)] (Fig. S4 A and B). The presence of beetles is thus crucial for the growth of consumable ambrosia fungus structures (15, 19). Additionally, *cropping* is likely to prevent invasions of the ambrosia garden by foreign fungi and microbes (14, 20, 21). Oral secretions of *X. saxesenii* contain various bacteria (e.g., refs. 22 and 23) that possibly support fungus growth either by providing nutrients or by controlling the spread of pathogens, as shown for bacterial mutualists of fungus-culturing ants (24–26).

**Gallery hygiene—balling, shuffling, and cannibalism.** Gallery hygiene is important for a flourishing fungus garden as well as a healthy colony. The larval *balling* behavior has not been described before. Bending their bodies ventrally, larvae form cordlike frass into balls that can be shifted within the gallery or dumped out of the entrance more easily (Movie S1). Frass balls are shifted by *shuffling* (Movie S2) them toward certain areas, where they are used either to close parts of tunnels, possibly to regulate humidity (14); to isolate diseased areas [so-called “death chambers” (27, 28)]; or to be recycled by the fungus. We observed that the wood chippings in the larval frass are often integrated into the fungal beds (see also ref. 27), which suggests that the mastication of the wood may facilitate resource utilization by the ambrosia fungi. This would explain why nitrogen from beetle excretions had been detected in growing fungi (29). Perhaps most importantly, frass and debris are disposed, because space is required for fungi to grow and for beetles to move (3, 12). Occasionally we observed sibling *cannibalism*, which may serve both nutrient recycling and the removal of dead and diseased specimens. The latter is probably essential for hindering the spread of diseases and parasites [including mold (30, 31)], which is particularly threatening for highly inbred communities (32, 33).

**Gallery protection—blocking.** *Blocking* of the entrance by a gallery member is ubiquitous in ambrosia beetles, although it exposes the *blocking* individual to predation, for instance by birds (34, 35) or predatory beetles (36). Parasitoids also have been reported to lay eggs on *blocking* ambrosia beetles (37).

*Blocking* provides a variety of essential services to the gallery members (see ref. 38 for review), like regulating the microclimate, preventing larvae from falling out of the gallery, and excluding parasites, parasitoids, predators, and foreign fungi from the gallery, which are common threats in bark and ambrosia beetles. Additionally, it may hinder other ambrosia beetles from entering a proliferating gallery and foreign males from mating with females in the gallery, which can detrimentally affect their future reproductive success due to an outbreeding depression (39). Our study cannot distinguish between these potential, not mutually exclusive functions, but the data suggest that *blocking* by adult females is essential for the safety of larvae. Larvae are very mobile, and in addition to our own results, the only previous removal experiment of a *blocking* female in scolytine beetles we know of also resulted in the loss of larvae [and eggs; species: *Coccotrypes dactyliperda* Fabricius (40)]. In line with this, *blocking* is most common during the presence of larvae and tends to decrease in frequency after the first female offspring has matured (Table S2). In addition, we found that *blocking* might also

retain workers in the nest, because females dispersed at higher rates after the removal of the *blocking* foundress (Fig. 2B).

**Brood care and body hygiene—allogrooming.** *Allogrooming* was frequently observed between individuals of all stages, and its importance was shown by (i) pupae being overgrown by fungi and dying more frequently in the presence of one *allogrooming* larva compared with six *allogrooming* larvae (see above), and (ii) the repeated observation of single foundresses (before a brood was successfully produced) dying due to a lack of getting groomed. Four solitary foundresses died because they got adhered to the gallery wall by a fungal layer on their elytra, or fungi grew underneath the elytra and caused them to swell so that moving was prevented. Additionally, it has been reported from ambrosia beetles that eggs do not hatch (41) and larvae die (21) in the absence of the grooming foundress. The gregarious life of all age classes that constantly *groom* each other might also explain the puzzling low frequency of parasitoids and mites found in nests of ambrosia beetles compared with those of their close relatives, the nongregarious, phloem-feeding bark beetles (35, 42, 43). Recent studies of other taxa have shown that grooming can greatly decrease the success of parasitoids [e.g., by grooming their eggs (44)], parasites [e.g., phoretic mites (45, 46) and entomopathogenic fungi (47, 48)]. Costs of grooming include the time and energy spent and the risk of parasite transmission to the groomer (30, 49, 50).

#### Detailed Materials and Methods. Study system and laboratory breeding.

Of 350 galleries of *X. saxesenii* studied in the field, the majority produced approximately 10–25 dispersing individuals, 3.6% produced more than 100 individuals, and one gallery exceeded 300 individuals (51). The beetles show a strong sexual dimorphism: the rare males [the average sex ratio is approximately 1:8 to 1:20 male/female (52)] do not fully sclerotize, stay small, and are unable to fly. Most of them die in their natal gallery after the females have dispersed (52).

We bred *X. saxesenii* in glass tubes filled with artificial medium that mainly contained sawdust (“test medium” described in ref. 53). We used one founder female per tube that had either been collected in the field or originated from a brood raised in the laboratory. Females excavated galleries and brood chambers largely along the transparent tube wall, which enabled observations of activity and development (Fig. S4A). When a gallery had been successfully established, the tube was wrapped in paper to keep it dark as if in wood, but light could enter through the entrance at the top of the tube. The tube was kept in a constant light/dark cycle (11 h light/13 h dark) at 28 °C/22 °C and 70% humidity, and the paper wrap was only removed during observations under a microscope (×6.4 to ×16 magnification) with an artificial light source (maximum 6 W). Behavior of scolytine beetles is affected neither by light (kept in the dark vs. exposed to light) nor when the gallery is turned by 90°, changing the axis of gravity (see above and ref. 2).

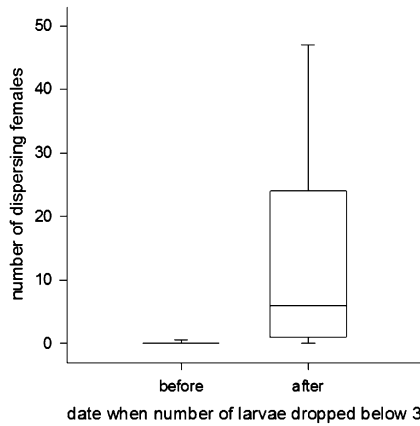
**Behavioral recordings and analyses.** Every second to third day we performed scan observations of all individuals visible within a gallery: we noted the gallery identity, the number of visible individuals, and the respective behaviors they showed at that moment, the number of dispersing individuals found on the surface of the medium (where they tried to escape through the cap), and we counted the number of eggs and pupae. In each scan all gallery parts were browsed one time for visible individuals as described. We did not discriminate between the three larval instars. Pupae and adult beetles were sexed on the basis of morphology and size. We discerned teneral adult females that had recently hatched and showed weak sclerotization and brownish elytra, and mature adult females that were fully sclerotized with dark brown to black elytra.

From each scan observation we noted the proportion of individuals per class (larvae, teneral females, mature females, males) performing a respective behavior. In total we conducted 500 scan observations of 43 galleries per age class ( $\bar{x}$  = 11.9 scans per gallery, range 2–40).

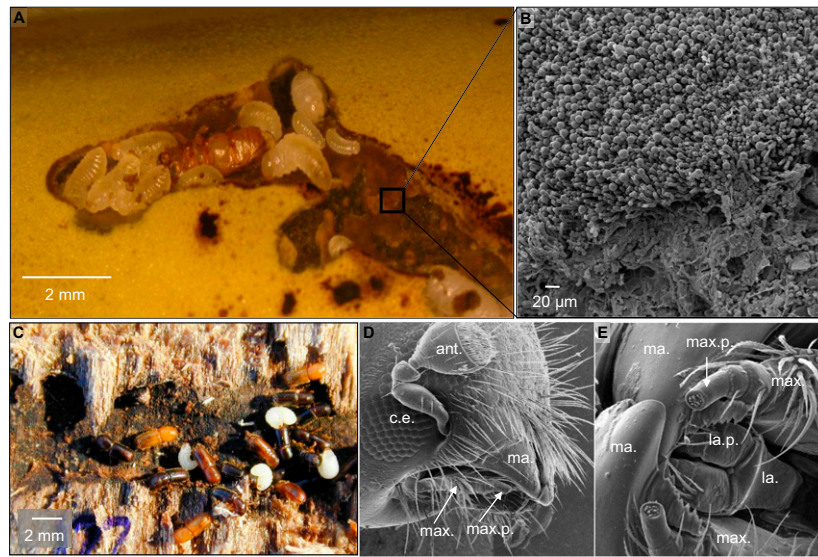


1. Sokal RR, Rohlf FJ (1995) *Biometry: The Principles and Practice of Statistics in Biological Research* (W. H. Freeman, New York).
2. Schmitz RF (1972) Behavior of *Ips pini* during mating, oviposition, and larval development (Coleoptera, Scolytidae). *Can Entomol* 104:1723–1728.
3. Francke-Grosmann H (1956) Hautdrüsen als Träger der Pilzsymbiose bei Ambrosiakäfern. *Z Morphol Oekol Tiere* 45:275–308.
4. Batra LR (1963) Ecology of ambrosia fungi and their dissemination by beetles. *Trans Kans Acad Sci* 66:213–236.
5. Martin MM, Kukor JJ, Martin JS, O'Toole TE, Johnson MW (1981) Digestive enzymes of fungus-feeding beetles. *Physiol Zool* 54:137–145.
6. Martin MM (1983) Cellulose digestion in insects. *Comp Biochem Physiol A* 75:313–324.
7. Rohlfs M, Churchill ACL (2011) Fungal secondary metabolites as modulators of interactions with insects and other arthropods. *Fungal Genet Biol* 48:23–34.
8. Rohlfs M, Obmann B, Petersen R (2005) Competition with filamentous fungi and its implication for a gregarious lifestyle in insects living on ephemeral resources. *Ecol Entomol* 30:556–563.
9. Rohlfs M, Hoffmeister TS (2004) Spatial aggregation across ephemeral resource patches in insect communities: An adaptive response to natural enemies? *Oecologia* 140:654–661.
10. Bright DE (1973) *The Bark and Ambrosia Beetles of California, Coleoptera: Scolytidae and Platypodidae* (Univ California Press, Berkeley, CA).
11. Kajimura H, Hijii N (1994) Reproduction and resource utilization of the ambrosia beetle, *Xylosandrus mutilatus*, in-field and experiment populations. *Entomol Exp Appl* 71:121–132.
12. Kajimura H, Hijii N (1992) Dynamics of the fungal symbionts in the gallery system and the mycangia of the ambrosia beetle, *Xylosandrus mutilatus* (Blandford) (Coleoptera, Scolytidae). *Ecol Res* 7:107–117.
13. Tarno H, et al. (2010) Types of frass produced by the ambrosia beetle *Platypus quercivorus* during gallery construction, and host suitability of five tree species for the beetle. *J For Res* 16:68–75.
14. Schneider-Orelli O (1913) Untersuchungen über den pilzzüchtenden Obstbaum-borkenkäfer *Xyleborus (Anisandrus) dispar* und seinen Nährpilz. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene II* 38:25–110.
15. Batra LR, Michie MD (1963) Pleomorphism in some ambrosia and related fungi. *Trans Kans Acad Sci* 66:470–481.
16. Batra LR (1966) Ambrosia fungi: Extent of specificity to ambrosia beetles. *Science* 153: 193–195.
17. French JRJ, Roeper RA (1972) Interactions of ambrosia beetle, *Xyleborus dispar* (Coleoptera, Scolytidae), with its symbiotic fungus *Ambrosiella hartigii* (Fungi imperfecti). *Can Entomol* 104:1635–1641.
18. Norris DM (1979) *Nutrition, Mutualism, and Commensalism*, ed Batra LR (Allanheld, Osmun & Company, Montclair, NJ), pp 53–63.
19. Francke-Grosmann H (1967) *Symbiosis*, ed Henry SM (Academic Press, New York), pp 141–205.
20. Lengerken H (1939) *Die Brutfürsorge- und Brutpflegeinstinkte der Käfer* (Akademische Verlagsgesellschaft, Leipzig).
21. Norris DM (1993) *Xyleborus ambrosia* beetles—a symbiotic ideal extreme biofacies with evolved polyphagous privileges at monophagous prices. *Symbiosis* 14:229–236.
22. Haanstad JO, Norris DM (1985) Microbial symbiotes of the ambrosia beetle *Xyletorinus politus*. *Microb Ecol* 11:267–276.
23. Grubbs KJ, et al. (2011) Genome sequence of *Streptomyces griseus* strain XylebKG-1, an ambrosia beetle-associated actinomycete. *J Bacteriol* 193:2890–2891.
24. Currie CR, Stuart AE (2001) Weeding and grooming of pathogens in agriculture by ants. *Proc Biol Sci* 268:1033–1039.
25. Mueller UG, Gerardo N (2002) Fungus-farming insects: Multiple origins and diverse evolutionary histories. *Proc Natl Acad Sci USA* 99:15247–15249.
26. Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR (2005) The evolution of agriculture in insects. *Annu Rev Ecol Syst* 36:563–595.
27. Hubbard HG (1897) in *Some Miscellaneous Results of the Work of the Division of Entomology* (US Department of Agriculture Bureau of Entomology Bulletin No. 7), ed Howard LO (US Department of Agriculture, Washington, DC), pp 9–13.
28. Peer K, Taborsky M (2007) Delayed dispersal as a potential route to cooperative breeding in ambrosia beetles. *Behav Ecol Sociobiol* 61:729–739.
29. Kok LT, Norris DM (1972) Symbiotic interrelationships between microbes and ambrosia beetles 6. Aminoacid composition of ectosymbiotic fungi of *Xyleborus ferrugineus* (Coleoptera, Scolytidae). *Ann Entomol Soc Am* 65:598–602.
30. Cremer S, Armitage SAO, Schmid-Hempel P (2007) Social immunity. *Curr Biol* 17: R693–R702.
31. Wilson-Rich N, Spivak M, Fefferman NH, Starks PT (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu Rev Entomol* 54: 405–423.
32. Hamilton WD (1972) Altruism and related phenomena, mainly in social insects. *Annu Rev Ecol Syst* 3:193–232.
33. Hamilton WD, Axelrod R, Tanese R (1990) Sexual reproduction as an adaptation to resist parasites (a review). *Proc Natl Acad Sci USA* 87:3566–3573.
34. Moore GE (1972) Southern pine beetle mortality in North Carolina caused by parasites and predators. *Environ Entomol* 1:58–65.
35. Kenis M, Wermelinger B, Gregoire JC (2004) *Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis*, ed Lieutier F (Kluwer Academic Publishers, Dordrecht, The Netherlands), pp 237–290.
36. Wichmann HE (1967) Die Wirkungsbreite des Ausstoßreflexes bei Borkenkäfern. *Anzeiger für Schädlingskunde. J Pest Sci* 40:184–187.
37. Beaver RA (1986) The taxonomy, mycangia and biology of *Hypothenemus curtippennis* (Schedl), the first known cryphaline ambrosia beetle (Coleoptera: Scolytidae). *Ent Scand* 17:131–135.
38. Kirkendall LR, Kent DS, Raffa KF (1997) *The Evolution of Social Behavior in Insects and Arachnids*, eds Choe JC, Crespi BJ (Cambridge Univ Press, Cambridge, U.K), pp 181–215.
39. Peer K, Taborsky M (2005) Outbreeding depression, but no inbreeding depression in haplodiploid Ambrosia beetles with regular sibling mating. *Evolution* 59:317–323.
40. Herfs A (1950) Studien an dem Steinnußborkenkäfer, *Coccotrypes tanguanus* Eggers. *Höfchen Briefe* 3 and 1:1–57.
41. Roeper R, Treeful LM, O'Brien KM, Foote RA, Bunce MA (1980) Life history of the ambrosia beetle *Xyleborus affinis* (Coleoptera: Scolytidae) from *in vitro* culture. *Great Lakes Entomol* 13:141–144.
42. Schedl W (1964) Biologie des gehöckerten Eichenholzbohrers, *Xyleborus monographus* (Scolytidae, Coleoptera). *Z Angew Entomol* 53:411–428.
43. Eichhorn O, Graf P (1974) Über einige Nutzholzborkenkäfer und ihre Feinde. *Anzeiger für Schädlingkunde-Journal of Pest Science* 47:129–135.
44. Vincent CM, Bertram SM (2010) Crickets groom to avoid lethal parasitoids. *Anim Behav* 79:51–56.
45. Buchler R, Drescher W, Tornier I (1992) Grooming behavior of *Apis cerana*, *Apis mellifera* and *Apis dorsata* and its effect on the parasitic mites *Varroa jacobsoni* and *Tropilaelaps clareae*. *Exp Appl Acarol* 16:313–319.
46. Delfinado-Baker M, Rath W, Boecking O (1992) Phoretic bee mites and honeybee grooming behavior. *Int J Acarol* 18:315–322.
47. Rosengaus RB, Maxmen AB, Coates LE, Traniello JFA (1998) Disease resistance: A benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termitidae). *Behav Ecol Sociobiol* 44:125–134.
48. Fernández-Marín H, Zimmerman JK, Rehner SA, Wcislo WT (2006) Active use of the metapleural glands by ants in controlling fungal infection. *Proc Biol Sci* 273: 1689–1695.
49. Rosengaus RB, Traniello JFA (1997) Pathobiology and disease transmission in dampwood termites *Zootermopsis angusticollis* (Isoptera: Termitidae) infected with the fungus *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes). *Sociobiology* 30:185–195.
50. Schmid-Hempel P (1998) *Parasites in Social Insects* (Princeton Univ Press, Princeton).
51. Hosking GB (1972) *Xyleborus saxesenii*, its life-history and flight behaviour in New Zealand. *N Z J For Sci* 3:37–53.
52. Biedermann PHW (2010) Observations on sex ratio and behavior of males in *Xyleborinus saxesenii* Ratzburg (Scolytinae, Coleoptera). *Zookeys* 56:253–267.
53. Biedermann PHW, Klepzig KD, Taborsky M (2009) Fungus cultivation by ambrosia beetles: Behavior and laboratory breeding success in three xyleborine species. *Environ Entomol* 38:1096–1105.





**Fig. S3.** Relationship between larval numbers present in a gallery and the dispersal activity of adult females. In 23 galleries with a single offspring generation larval numbers dropped below three at 21–77 d (mean 46 d) after gallery foundation. Numbers of dispersing females were counted 10 d before and 10 d after this date. Significantly more females dispersed after than before this date, that is, when all but the last two larvae had pupated (Wilcoxon matched-pairs signed-ranks tests:  $z = -3$ ,  $P < 0.001$ ,  $n = 23$  galleries). This suggests that female dispersal might be triggered by demands of larvae dependent on female care. Productivity of the fungus, which is probably decreasing at the same time (because no new eggs are laid), is a confounding factor of this analysis, however, that we could not control for. Box-whisker plots with medians, 10th, 25th, 75th, and 90th percentiles are shown.



**Fig. 54.** Morphology of *X. saxesenii* galleries and their inhabitants (beetles and fungi). (A) Morphology of a brood chamber in artificial medium, with different larval instars and a teneral female. The orange layer on the gallery walls is formed by fruiting structures of the ambrosia fungus *Ambrosiella sulfurea* Batra. (B) Morphology of the fungal layer depicted by scanning electron microscopy with  $\times 300$  magnification. The round "balls" are fruiting structures of *A. sulfurea*. (C) Morphology of a brood chamber in the field, with several third-instar larvae, teneral (light brown), and mature (black) females. A thin, orange fungus layer is lining the gallery walls. (D and E) Head and mouthparts of a *X. saxesenii* female depicted by scanning electron microscopy with  $\times 200$  and  $\times 500$  magnification, respectively. ant., antenna; c.e., compound eye; la., labium; la.p., labial palp; ma., mandible; max., maxilla; max.p., maxillary palp.



**Table S1. Separate GEE models to examine differences ( $P < 0.05$ ) between the proportion of time the different age and sex classes spent with the observed cooperative behaviors (see Fig. 1 in main text)**

Behavior	Parameter	Coeff. $\pm$ SE	$z$	$P$
Digging	Mean (teneral ♀♀)	$-6.33 \pm 1.02$	-6.2	<b>&lt;0.001</b>
	Contrast teneral ♀♀ vs. larvae	$6.13 \pm 1.01$	-6.04	<b>&lt;0.001</b>
	Contrast teneral ♀♀ vs. adult ♀♀	$2.28 \pm 1.04$	2.2	<b>0.028</b>
	Contrast teneral ♀♀ vs. ♂♂	$1.39 \pm 1.43$	0.97	0.33
	Contrast larvae vs. adult ♀♀	$-3.85 \pm 0.26$	-14.79	<b>&lt;0.001</b>
	Contrast larvae vs. ♂♂	$-4.74 \pm 1.01$	-4.67	<b>&lt;0.001</b>
Cropping	Contrast adult ♀♀ vs. ♂♂	$-0.89 \pm 1.04$	-0.85	0.39
	Mean (♂♂)	$-2.24 \pm 0.3$	-7.52	<b>&lt;0.001</b>
	Contrast ♂♂ vs. adult ♀♀	$1.09 \pm 0.29$	-3.78	<b>&lt;0.001</b>
Balling	Contrast ♂♂ vs. teneral ♀♀	$1.82 \pm 0.29$	-6.17	<b>&lt;0.001</b>
	Contrast adult ♀♀ vs. teneral ♀♀	$0.73 \pm 0.14$	5.37	<b>&lt;0.001</b>
	Mean (larvae)	$-3 \pm 0.13$	-22.31	<b>&lt;0.001</b>
Shuffling	Mean (larvae)	$-6.29 \pm 0.46$	-13.57	<b>&lt;0.001</b>
	Contrast larvae vs. teneral ♀♀	$0.98 \pm 0.68$	1.43	0.15
	Contrast larvae vs. adult ♀♀	$1.11 \pm 0.54$	2.05	<b>0.041</b>
Cannibalism	Contrast teneral ♀♀ vs. adult ♀♀	$-0.13 \pm 0.65$	-0.20	0.84
	Mean (larvae)	$-6.67 \pm 0.49$	-13.57	<b>&lt;0.001</b>
	Contrast larvae vs. teneral ♀♀	$3.43 \pm 0.46$	7.46	<b>&lt;0.001</b>
	Contrast larvae vs. adult ♀♀	$1.38 \pm 0.56$	2.46	<b>0.014</b>
	Contrast larvae vs. ♂♂	$1.74 \pm 0.86$	2.02	<b>0.043</b>
	Contrast teneral ♀♀ vs. adult ♀♀	$-2.05 \pm 0.42$	-4.91	<b>&lt;0.001</b>
Allogrooming	Contrast teneral ♀♀ vs. ♂♂	$-1.69 \pm 0.79$	-2.14	<b>0.033</b>
	Contrast adult ♀♀ vs. ♂♂	$0.36 \pm 0.85$	0.426	0.67
	Mean (larvae)	$-3.91 \pm 0.16$	-24.5	<b>&lt;0.001</b>
	Contrast larvae vs. teneral ♀♀	$0.80 \pm 0.25$	3.18	<b>0.001</b>
	Contrast larvae vs. adult ♀♀	$0.27 \pm 0.25$	1.07	0.29
	Contrast larvae vs. ♂♂	$1.92 \pm 0.28$	6.82	<b>&lt;0.001</b>
Blocking	Contrast teneral ♀♀ vs. adult ♀♀	$-0.53 \pm 0.30$	-1.76	0.079
	Contrast teneral ♀♀ vs. ♂♂	$1.12 \pm 0.33$	3.4	<b>&lt;0.001</b>
	Contrast adult ♀♀ vs. ♂♂	$1.65 \pm 0.32$	5.09	<b>&lt;0.001</b>
Blocking	Mean (adult ♀♀)	$-4.28 \pm 0.56$	-7.68	<b>&lt;0.001</b>

We used GEEs with an exchangeable correlation structure of the response variable within a cluster (= gallery identity) to identify effects of the four age and sex classes on the total amount of time each behavior was observed. Model coefficients are reported as coeff.  $\pm$  SE (SE of the estimate), with the group in parentheses in the first row of the respective model as the reference category (coefficient set to zero). The class with the smallest mean is given first, and a significant  $P$  value (bold) denotes that it differed significantly from zero. The influences of independent variables on the behavioral frequencies are displayed as contrasts between classes. A positive contrast denotes that the "Mean" of the second class is higher than the "Mean" of the first class; a negative contrast denotes the reverse. Each behavior was modeled by a single GEE.

**Table S2. GEE models for examining differences ( $P < 0.05$ ) between the proportion of time adult females spent with each cooperative behavior, according to stage (see footnote)**

Behavior	Parameter	Coeff. $\pm$ SE	z	P	
Digging* <sup>†</sup>	Mean (larval-adult stage)	-6.83 $\pm$ 0.89	-7.68	<0.001	
	Contrast larval-adult stage vs. preadult stage	2.28 $\pm$ 1.86	1.23	0.219	
	Contrast larval-adult stage vs. postlarval stage	2.2 $\pm$ 1.15	1.91	0.056	
Cropping*	Mean (larval-adult stage)	-0.7 $\pm$ 0.14	-5.2	<0.001	
	Contrast larval-adult stage vs. preadult stage	-0.6 $\pm$ 0.49	-1.22	0.22	
	Contrast larval-adult stage vs. postlarval stage	-0.72 $\pm$ 0.26	-2.83	0.005	
	<u>Separate GEE only for larval-adult stage</u>				
	Mean (cropping)	-0.76 $\pm$ 0.2	-3.81	<0.001	
Shuffling* <sup>†</sup>	Number of larvae and pupae	0.03 $\pm$ 0.01	3.94	<0.001	
	Number of adult females	-0.02 $\pm$ 0.02	-1.16	0.246	
	Mean (larval-adult stage)	-4.97 $\pm$ 0.4	-12.46	< 0.001	
	Contrast larval-adult stage vs. preadult stage	1.42 $\pm$ 1.2	1.19	0.24	
	Contrast larval-adult stage vs. postlarval stage	0.09 $\pm$ 0.82	0.11	0.91	
Cannibalism* <sup>†</sup>	Mean (larval-adult stage)	-4.43 $\pm$ 0.51	-8.65	<0.001	
	No cannibalism				
	Contrast larval-adult stage vs. postlarval stage	-3.21 $\pm$ 0.72	-4.46	<0.001	
Allogrooming*	Mean (larval-adult stage)	-3.62 $\pm$ 0.25	-14.2	<0.001	
	Contrast larval-adult stage vs. preadult stage	-0.02 $\pm$ 1.13	-0.019	0.99	
	Contrast larval-adult stage vs. postlarval stage	-2.06 $\pm$ 1.13	-1.82	0.068	
	<u>Separate GEE only for larval-adult stage</u>				
	Mean (allogrooming)	-3.41 $\pm$ 0.4	-8.58	<0.001	
Dispersal*	Number of larvae and pupae	0.04 $\pm$ 0.02	2.56	0.01	
	Number of adult females	-0.1 $\pm$ 0.05	-2.12	0.034	
	Mean (larval-adult stage)	-1.41 $\pm$ 0.31	-4.52	<0.001	
	No dispersal				
	Contrast larval-adult stage vs. postlarval stage	0.97 $\pm$ 0.29	3.33	<0.001	
Blocking <sup>†</sup> (yes/no)	<u>Separate GEE only for larval-adult stage</u>				
	Mean (dispersal)	-2.34 $\pm$ 0.45	-5.23	<0.001	
	Number of larvae and pupae	-0.08 $\pm$ 0.03	-2.99	0.003	
	Number of adult females	0.15 $\pm$ 0.03	5.1	<0.001	
	Mean (larval-adult stage)	-3.47 $\pm$ 0.62	-5.63	<0.001	
Blocking <sup>†</sup> (yes/no)	Contrast larval-adult stage vs. preadult stage	1.8 $\pm$ 0.96	1.88	0.06	
	Contrast larval-adult stage vs. postlarval stage	-1.36 $\pm$ 1.29	-1.06	0.29	
	Contrast preadult stage vs. postlarval stage	-3.16 $\pm$ 1.5	-2.1	0.035	

A separate model was calculated for each behavior exhibited during the larval-adult stage to analyze the influence of the number of dependent offspring (larvae and pupae) and the number of helpers (adult females) on the individual workload of adult females. For all analyses we used GEEs with an exchangeable correlation structure of the response variable within a cluster (= gallery identity) to identify effects of the classes on the total amount of time during which each behavior was observed. Model coefficients are reported as coeff.  $\pm$  SE (SE of the estimate), with the group in parentheses in the first row of the model as the reference category (coefficient set to zero). A significant  $P$  value of the "Mean" (bold) denotes that the class mean differed significantly from zero. The influences of independent variables on the behavioral frequencies are displayed as contrasts between classes. A positive contrast denotes that the "Mean" of the second class is higher than the "Mean" of the first class; a negative contrast denotes the reverse. Each behavior was modeled by a single GEE. Consecutive order of gallery stages: (i) preadult stage (foundress with eggs, larvae, and pupae); (ii) larval-adult stage (foundress with eggs, larvae, pupae, and adult offspring); (iii) postlarval stage (foundress with adult offspring).

\*Contrasts preadult stage vs. postlarval stage were not significant ( $P > 0.05$ ).

<sup>†</sup>Separate GEEs for larval-adult stage were not significant ( $P > 0.05$ ).

**Table S3. Ethogram of the observed behaviors of larvae (L), females (F), and males (M)**

Behavior	Shown by	Definition	Cooperative function
Digging	L, F, M	Enlarging the brood chamber by digging into fungus-infested substrate which may also serve nutritional functions (L); or excavating new tunnels without feeding (F, M)	Gallery extension
Cropping	F, M	Grazing on the fungal layer covering gallery walls with the maxillae and/or mandibles	Fungus care
Balling	L	Forming balls of frass and sawdust by repeated ventral body contractions	Hygiene
Shuffling	L, F	Moving frass and sawdust with the body (L) or the legs and elytra (F)	Hygiene
Cannibalism	L, F, M	Feeding on a larva, pupa, or adult beetle that is usually dead	Hygiene
Allogrooming	L, F, M	Grooming an egg, larva, pupa, or adult beetle with the mouthparts (i.e., maxillae, labium)	Brood care, hygiene
Blocking	F	Staying inactive in the entrance tunnel and plugging it with the body (abdomen directed to the outside)	Protection
Self grooming	F	Grooming oneself with the legs	—
Inactive	L, F, M	Being inactive without moving	—
Locomotion	L, F, M	Creeping (L) or walking on the tibia with back-folded tarsi (F, M)	—
Mating (attempt)	M	Mounting a female or copulating with her	—

The rightmost column lists the (mutually) beneficial function of the behavior for the whole group.

**Table S4. Separate GEE models for examining differences ( $P < 0.05$ ) between the proportion of time mature daughters and foundresses spent with cooperative behaviors**

Behavior	Parameter	Coeff. $\pm$ SE	z	P
Digging	Mean (mature daughter)	-14.45 $\pm$ 9.97	-1.45	0.15
	Contrast mature daughter vs. foundress	-8.5 $\pm$ 3.52	-2.42	<b>0.016</b>
Cropping	Mean (mature daughter)	-0.24 $\pm$ 0.41	-0.58	0.564
	Contrast mature daughter vs. foundress	0.4 $\pm$ 0.05	7.94	<b>&lt;0.001</b>
Shuffling	Mean (mature daughter)	-4.19 $\pm$ 0.34	-12.3	<b>&lt;0.001</b>
	Contrast mature daughter vs. foundress	1.86 $\pm$ 0.09	19.83	<b>&lt;0.001</b>
Cannibalism	Only in mature daughters			
Allogrooming	Mean (mature daughter)	-6.47 $\pm$ 1.58	-4.09	<b>&lt;0.001</b>
	Contrast mature daughter vs. foundress	0.09 $\pm$ 0.08	1.09	0.274
Blocking	Only in foundresses			

We used GEEs with an exchangeable correlation structure of the response variable within a cluster (= gallery identity) to identify effects of the two groups on the total amount of time each behavior was observed. Model coefficients are reported as coeff.  $\pm$  SE (SE of the estimate), with "mature daughters" as the reference category (coefficient set to zero). A significant  $P$  value of the "Mean" (bold) denotes that the class mean differed significantly from zero. The influences of the independent variable (= foundress) on the behavioral frequencies are displayed as contrasts between classes. A positive contrast denotes that the "Mean" of the second class is higher than the "Mean" of the first class; a negative contrast denotes the reverse. Each behavior was modeled by a single GEE.



**Movie S1.** *Balling* behavior by a larval worker within the brood chamber. This sequence shows an example of *balling* by a third-instar larva that is surrounded by other larvae and a pupa. With this behavior larvae collect waste material (sawdust and frass) to form balls that can be shifted in the gallery or dumped out of its entrance.

[Movie S1](#)



