

**Brood size in a subsocial  
bark beetle breeding in live plants**

Master of Science Thesis

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## Abstract

Maternal care is found in many insect species, and maternal care affects both offspring survival and growth significantly. The effect of maternal care was tested on the monogynous bark beetle *Scolytodes gunnerae* which breeds in live *Gunnera* petioles, by experimentally removing females from galleries to see how offspring survival and the number of offspring was affected. No consistent effect of female presence was found, and any effect of maternal presence was small. Female presence is discussed, and could be a case of functional semelparity. The brood size for this species was also found to be extremely small, and this is also discussed herein.

# Introduction

The main aim of my study is to conduct a mother removal experiment to see if maternal care increases offspring survival, and to identify what factors are affecting brood size in a subsocial bark beetle (Coleoptera, Scolytinae) which breeds in live *Gunnera* petioles.

In this thesis optimal clutch size will be regarded as a part of the parental care decision made by the female, in order to maximize lifetime fitness. Life history theory predicts that all organisms should be under selection to allocate resource optimally, in order to maximize lifetime reproductive success (Coleman and Gross 1991). Both parental care theory and optimal clutch size theory are based on this assumption.

## Parental care theory

Trivers defined parental investment as parental behavior that increases the offspring's fitness, at the cost of the parent's future reproduction (Trivers 1972, Zeh and Smith 1985). For parental care to evolve the benefits of providing parental care must be higher than the costs (Trivers 1972, Dawkins and Carlisle 1976, Clutton-Brock 1991, Tallamy and Brown 1999, Mas and Kölliker 2008). Wilson identified four "prime movers" that could explain the evolution of parental care in animals, namely stable structured habitats, physically demanding environments, scarce and specialized food resources and lastly predation (Wilson 1975, Tallamy and Brown 1999).

Patterns of parental care could also be affected by the differences between the sexes. Bateman was one of the first to acknowledge that there is an asymmetry between the sexes when it comes to fitness maximizing, in his paper about intra-sexual selection in *Drosophila melongaster* (Bateman 1948). Bateman found that males and female maximize fitness differently. Male fitness is highly dependent on mating frequency and mating success varies widely between individuals, while female fitness is limited by her physical ability to produce eggs and thus varies little (Bateman 1948). Differential fertility has implications for patterns of intra-sexual selection, firstly since the total number of offspring produced by females

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normally is lower than that for males and secondly because their investment in the offspring is higher, there is a competition among males for mating with females (Trivers 1972). This fundamental difference between the sexes could also have implications for patterns of parental care, since the male could have more to gain by deserting the offspring in terms of fitness by acquiring a new mate (Trivers 1972).

The option to either desert or to care for the offspring is an example where the best strategy depends on the choice made by the other part, and this conflict has to be solved as an Evolutionary Stable Strategy (ESS) (Dawkins and Carisle 1976, Maynard Smith 1977). Since both sexes are selected to maximize their lifetime reproductive success (Trivers 1972), if only uniparental care increases offspring survival, it would pay to leave if the chance of re-mating is high and if the other part remains with the offspring to provide parental care (Maynard Smith 1977). This means that the latter partner is somewhat “stuck” with the offspring, because to leave would reduce the offspring survival and thereby fitness (Maynard Smith 1977).

Maternal care is more common than paternal care in most groups of animals (Gross 2005), and there are three factors that can help to explain this bias. Firstly it is the future investment that is important to consider when it comes to the option to care for or to desert the offspring, but since the females initially invest more than the male in the gamete, the cost of deserting the offspring could be higher in terms of fitness for the female than for the male (Dawkins and Carisle 1976). Secondly because male fitness is more dependent on mating frequency they could also have more to gain by deserting, and lastly internal fertilization leaves the female with the zygote, which has given the male the chance to desert first (Dawkins and Carisle 1976).

Parental care is considered to be rare in insects, but it has been reported in 13 orders and in at least 45 families (Tallamy and Wood 1986, Tallamy and Brown 1999). There are three types of parental care found in insects: protection of offspring, food provisioning and lastly resource protection (Tallamy and Wood 1986). Maternal care is more common than biparental care or paternal care in insects (Tallamy 1983), probably because the male could not assist their mates effectively or because the chance of re-mating is high (Robertson 1998a). Studies on the burying beetle *Nicrophorus* has shown that parental care increases larvae weight and survival (Eggert et al. 1998, Anduaga and Huerta 2001, Smiseth et al. 2007). Maternal care has also been reported in the bark beetle species *Ips pini* (Reid and

Roitberg 1994, Robertson 1998a), in the bark beetle *Monarthrum* and in the ambrosia beetle genus *Xyleborus* (Kirkendall et al. 1997).

## **Optimal clutch size theory**

Optimal clutch size theory was first proposed by David Lack in 1947 to explain clutch size in birds (Lack 1947), but has later also been applied and experimentally tested on insects (Godfray et al. 1991). The theory is based on the existence of a trade-off between the number of offspring and per capita fitness when offspring are laid in discrete clutches (Godfray et al. 1991). Parents, in order to maximize their fitness should be selected to lay an optimal clutch size, which is the clutch size that gives the highest expected fitness per offspring (Brockelman 1975). In insects it has been tested on parasitoid Hymenoptera (Godfray et al. 1991, Godfray 1987), chestnut beetles (Desouhant et al. 2000) and in two species of seed beetle (Fox et al. 1996). The focus in these studies have been on how many eggs to lay on a host plant or animal, in order to see if there exists a trade off between the number of offspring, and their size or fitness (Godfray et al. 1991, Fox et al. 1996, Desouhant et al. 2000). Some studies have found a negative correlation between clutch size, and weight of the individual offspring (Godfray et al. 1991). This is highly important in insects since adult size have proven to be related to fecundity, and thereby fitness (Godfray et al. 1991, Honěk 1993). The observed optimal clutch size has often proven to be smaller than the estimated clutch size, this discrepancy may result from a trade-off between present and future reproduction (Krebs and Charnov 1974, Godfray et al. 1991).

## **Life history traits of bark beetles and *Gunnera* plants**

Bark beetles are a subfamily of weevils (*Curculionidae*), and are a species rich group worldwide with approximately 6000 species (Kirkendall et al. 1997). There are many different mating systems within bark beetles (Kirkendall 1983). The species used in this study *Scolytodes gunnerae* Wood, is a monogynous bark beetle believed to breed exclusively in the petioles and veins of large live leaves of *Gunnera insignis* (Wood 2007).

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The sexes can be distinguished by the difference in frons, which in the male is more flattened and lacks long setae (Wood 2007). For most monogynous bark beetle species the female is the colonizing sex (Kirkendall 1989), but the genus *Scolyodes* galleries are male-initiated (Brueland 1997). For *Scolytinae* the shape of the frons for species with a dimorphic forehead is related to which is the pioneering sex; the pioneering sex is usually convex, while the courting sex is normally flattened or concave (Kirkendall 1983). The colonizing sex often produces long-range pheromones to attract a mate (Kirkendall et al. 1997).

Breeding in petioles is considered an unusual habitat for bark beetles, since most species construct galleries and breed in the inner bark of dead trees. However it has been reported for a variety of species including several *Scolytodes* that breed in *Cecropia* leaf stalks (Jordal and Kirkendall 1998, Jordal 1998). The brood size of the scolytine beetles breeding in *Cecropia* leaf stalks is very low, with only two to ten offspring on average (Jordal and Kirkendall 1998).

In *S. gunnerae* the mother remains in the gallery after oviposition through all developmental stages from eggs to teneral adults; the male on the other hand is believed to leave the gallery soon after mating, which is unusual behavior for bark beetles (L. R. Kirkendall and K. Nishida, unpublished observations). Paternal care is the norm in bark beetles (Kirkendall 1983), and male presence has been found to increase the reproductive success of females in several studies (Helland 1994, Reid and Roitberg 1994 and Robertson 1998a).

Three explanations has been proposed to explain maternal presence in tunnels with eggs or juveniles, in bark beetles: the mother could be providing some kind of post zygotic maternal care such as food provisioning or protection, secondly she can remain to overwinter in the gallery or lastly she may be using the gallery to feed and regenerate flight muscles (Kirkendall et al. 1997). Flight muscle degeneration and regeneration is reported for many species of bark beetles, and has probably evolved to allocate more resources to reproduction (Chapman 1956, Bhaktan at al. 1970, Langor 1987, Robertson 1998b).

Preliminary data from 2005 showed a very low brood size for *S. gunnerae* with only four to six offspring per brood (L.R. Kirkendall and K. Nishida, unpublished observations), which is an extremely small brood size compared to other bark beetles species and for most other animals.

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Competition is believed to affect offspring survival in bark beetles, especially for larvae due to the discrete resources they are utilizing (Schmitz 1972, Salonen 1973, Beaver 1976, Kirkendall 1989, Denno et al. 1995). Both a study of the harem polygynous bark beetle *Ips acuminatus* (Kirkendall 1989), and a study conducted on the species *Scolytus scolytus*, *S. multistriatus* and *Tomicus piniperda* (Beaver 1976) confirmed this. The latter study also showed that increasing density reduced the mean weight of emerging adults (Beaver 1976).

The plant genus *Gunnera* are perennial herbs found in the southern hemisphere, and are the only known angiosperms that are in a facultative symbiosis with nitrogen-fixing cyanobacteria (Bergman et al. 1992). Gunneras have a thick semi-erect stem, lobed leaves and wind pollinated flowers (Palkovic 1978). *Gunneras* are restricted to humid areas with heavy rainfall in high altitudes and in shaded areas (Palkovic 1978, Bergman et al. 1992). There are two species of *Gunnera* found in Costa Rica, *G. insignis* and *G. talamancana* (Bergman et al. 1992, Palkovic 1978). Palkovic also reported of a hybrid between the two species (Palkovic 1978). Palkovic found some morphological traits to identify both the hybrid and the two species, including the degree of leaf-lobbing, ligule colour, and the prickles size (Palkovic 1978).

### Hypothesis 1 - Female removal experiments

Female removal bag: The hypothesis behind this experiment is that if the female is providing some kind of post zygotic parental care to her offspring, the survival of the brood should be reduced when she is experimentally removed from the gallery. If this assumption is violated the hypothesis must be rejected.

The two other female removal experiments will compare total number of offspring, in manipulated and unmanipulated galleries. If maternal care is provided through protection of offspring against predators and/or parasites, there should be a higher number of offspring in unmanipulated than in manipulated galleries. If this is not the case the hypothesis must be rejected.

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### Hypothesis 2 - Frass removal experiment

Bark beetles construct galleries most often in the inner bark of trees, and in this process they produce frass. Frass consists normally of a combination of boring dust and excrement that are expelled by the male or/and female from the gallery (Byers 1981). The hypothesis presented here is based on my observations during the study that there were no obvious larval tunnels (as there are in most bark beetles), plus the observation that many galleries containing larvae also had large amount of frass. To test the possibility that the female is providing the larvae with frass as a food source, I removed adults from all galleries; the frass was removed from experimental galleries, while the control galleries retained the frass already present. If the hypothesis is correct survival should be significantly higher for the controls than for the experimental galleries. If this assumption is violated the hypothesis must be rejected.

### Gallery dissection and brood size

Given the extraordinarily low number of offspring in the galleries, I wanted too identify what factors are affecting brood size for this bark beetle species. This is interesting due to the extremely small brood sizes recorded for this species in 2005 (L. R. Kirkendall and K. Nishida, unpublished data), and secondly because *Scolytodes* breeding in *Cecropia* leaf stalks also showed very small brood sizes (Jordal 1998). The predictor variables which I studied were plant, petiole, location, population, petiole length, petiole diameter, and gallery size.

### Body size measurements

Sexual size dimorphism is not uncommon in *Scolytodes* (Jordal 1998) or for insects in general, and I will therefore test if mean total body length differs between males and females. Female-biased body size dimorphism is common among animals, and size dimorphism is believed to be adaptive (Fairbairn 1990). Size could be highly important especially for females, because there is often a correlation between size and fecundity in insects (Honěk 1993). For some species of bark beetles there is also found a positive correlation between increasing altitude and increasing body size or pronotum width (Jordal 1998). Since *S. gunnerae* is found over a wide altitudinal gradient range it would be interesting to test if they follow this pattern.

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I also recorded the locations and populations of beetles; these data will be used to see if altitude is posing a limitation on colonizing by the beetles.

## Establishment in petioles

I will try to identify who the pioneering sex is for this species. This will be done by trying to get both males and females to establish galleries in petioles, to see if the establishment rate is different for the sexes. If one of the sexes has a much higher establishment rate, that indicates that this is the pioneering sex.

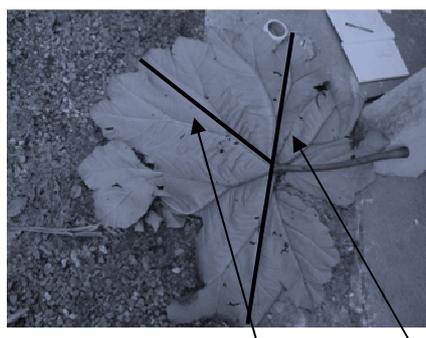
# Materials and methods

## Sampling

For all experimental material and for most of the petiole dissections, sampling was conducted in the Cerro de la Muerte and in Parque Nacional Tapantí, near the parks La Esperanza station. Both these locations consist of high altitude cloud forest, with high annual rainfall and relatively low temperatures. To conduct the sampling a pocket knife or a small hand saw was used to cut down the *Gunnera* petioles. The petioles were cut down as close as possible to the stem, and the petioles including the leaf were then brought back to La Esperanza station, for either experimental usage or dissection. There was also conducted some sampling and dissection of petioles at the main station of Parque Nacional Tapantí nearby Orosí, and at Parque Nacional Braulio Carillio, both located at 1500 m altitude.

## Gallery dissection and brood size

Before opening the galleries, total petiole length was measured, with a measuring tape. Leaf length and diameter were also measured (n = 128). Leaf length is here defined as the length of the vein continuing out from the petiole. Leaf diameter was measured where the leaf was at the widest (Figure 1). The leaf was removed from the petiole, by cutting of the leaf veins with a hand clipper. Galleries in the veins were treated as galleries in the petioles.



**Figure 1.** Leaf length and diameter measurements.

The petioles were divided with a hand clipper, such that one piece consisted of one gallery. First a measure of the petiole diameter was taken nearby the tunnel entrance and the small petiole pieces were then sliced into two half's longitudinally with a pocket knife, such that the entrance tunnel became the splitting point and the gallery was divided in two

parts. The number of adults, their sex (identified with hand lens with 10x enlargement), number of teneral adults, number of eggs, larvae and pupae in the gallery was recorded. Secondly the gallery size was measured with a measuring tape. A method was developed which gave a measure of the relative size of different galleries, this enabled me to compare the size of galleries with different shapes. The first measure taken was the longest direction of the gallery, and was defined as gallery length. The perpendicular direction of the gallery length was measured and defined as gallery width. These two measurements were later multiplied with each other, and the measure is referred to as gallery size.

## **Female removal experiments**

### 1. Female removal: bag

Under this experiment the *Gunnera* petioles were divided into small sections as described for the gallery dissection. I had both manipulated and unmanipulated galleries in the same petioles, and the length of each piece varied because the distance between entrance tunnels varied within and between petioles. After dividing the gallery in two pieces, a probe or a small paint brush was used inside both unmanipulated and manipulated galleries, and lastly the female and if present the male were removed from the latter galleries. After conducting the manipulation the galleries were closed, by putting the petiole piece together with two rubber bands, one in the upper and one in the lower part of the petiole piece. The two petiole ends were then covered with plastic wrap to avoid desiccation, and attached to the petiole with transparent packaging tape. Lastly the petiole pieces were put into sandwich bags (Johnson Ziplock® bags), and then the bags were sealed with the closing mechanism. The bags were stored outside La Esperanza station at 2600 m altitude for 15 days, under a wooden bench to avoid direct sunlight. The experiment was initiated on 24/6 (n = 10), 1/7 (n = 8), 2/7 (n = 10), 4/7 (n = 19), 5/7 (n = 32), 6/7 (n = 6) and 8/7 (n = 4). The 6/7 and the 8/7 were terminated respectively after 14 and 13 days. After termination of the experiment measures of petiole diameter, gallery length and width were taken, offspring survival and the number of offspring at different stages was also recorded.

## 2. Female removal: opening and closing of petioles

The veins and the leaflets were cut off from the petiole, and the cut areas of the veins were covered with plastic wrap and attached to the petiole by transparent packaging tape to avoid desiccation of the petiole. In this experiment the petioles were not divided into pieces, but kept intact and I had both manipulated and unmanipulated galleries in one petiole. In order to access the galleries, two transverse cuts were made down in the petiole on each side of the entrance tunnel with a pocket knife. The knife was then placed below the area between the two cuts and the knife edge was gently lifted, the piece between the two cuts was then removed. The stage of the offspring (egg, larvae, pupae or teneral adult) in the gallery was recorded, and the female and if present the male were removed with a probe from the manipulated galleries. The unmanipulated galleries were equally kept open in the same way, and also had the probe inserted into them, but here the adult beetles were not removed. After this both the manipulated and the unmanipulated galleries were closed, by putting the removed petiole piece in place and attaching it to the rest of the petiole by transparent packaging tape. The entrance tunnels were marked and assigned a gallery number with correction fluid and a pen. The petioles were kept outside of La Esperanza station, in 0.5 liters bottles with the top cut off and filled with water for 15 days. The experiment was initiated on 29/6 ( $n = 26$ ) and 30/6 ( $n = 11$ ). Tape was not working to keep the pieces together, so on the 2/7 it was replaced by small rubber bands. After termination after 15 days, measures of petiole diameter, gallery length and width were taken, and the number of offspring present at the different stages was recorded.

## 3. Female removal: probe

For this experiment the petioles were treated as for the opening and closing of petiole experiment. The female and if present the male were removed from the experimental galleries by using two probes. The first probe was inserted into the *Gunnera* petiole nearby the tunnel to block the beetle to go further into the gallery. Then the second probe was inserted into the abdomen of the beetle, which then was pulled out of the tunnel. Beetles were removed from every second gallery if possible, so there were both manipulated and control galleries in the same petiole. The entrance tunnels were marked and assigned gallery number with correction fluid and a pen. The petioles were kept outside of La Esperanza station for 15 days, in 0.5 liters bottles with the top cut off, and which was filled with water. The experiment was

initiated on 26/6 (n= 26) and on 28/6 (n=28). The experiment was terminated after 15 days, petiole diameter was measured, and the galleries were opened and treated as the described for the gallery dissections. Lastly the number of offspring at different stages was recorded, and also gallery length and width.

## **Frass removal experiment**

Opening of the galleries and sampling was as described above for the gallery dissections, and the experimental galleries were treated as the female removal experiment in bags. For this experiment however only the larval stage was used, and the female and if present the male were removed from both manipulated and unmanipulated galleries. The larvae were divided into four different categories depending on size: small, medium, large feeding and lastly large non feeding larvae (prepupae). The galleries were opened and the larvae were moved around with a probe or paint brush in the gallery, and some larvae were moved from their natal gallery. This was done to keep the number of larvae of the four different categories equal for manipulated and unmanipulated galleries. From the manipulated galleries all the frass was removed with a paint brush and/or a probe, while for the unmanipulated galleries the frass was kept in place. The galleries were then sealed with two rubber bands, and the ends of the petioles wrapped in plastic wrap which was attached to the petiole with packaging tape. Lastly the petioles were put into sandwich bags (Ziploc® bag) and sealed. The bags were stored outside of La Esperanza station, under a wood bench to avoid direct sunlight for 11 days. The experiment was initiated on 6/7 (n = 8), 7/7 (n = 2) and 8/7 (n = 12). After termination of the experiment after 11 days, the number of surviving larvae and which size group they belonged to recorded, and the amount of frass recorded in three categories: no, some or much.

## **Establishment in petioles**

The last experiment conducted was to try to get the beetles to establish new galleries in petioles, this was done to see which sex initiated gallery construction, and to see if the pioneer sex could be using pheromones to attract mates. Establishments were tried in both live and dead petioles, but mainly in the latter. The beetles used for this experiment came from

dissected petioles, and dissection was conducted as described for the petiole dissections. The edge of the knife was inserted into the petiole, in order to make a small hole in the outer surface. The beetles were then placed on the petiole with a small paintbrush or probe, with the pronotum facing the hole. The behavior of the beetle was observed, and I recorded if the beetle went down into the hole. The petioles were checked after one or two days for signs of establishment; the indicator used was frass nearby the entrance tunnel. The experiment was initiated on 22/6 (n = 6) in live petioles, and the latter ones in dead petioles 24/6 (n = 12), 27/6 (n = 6) and lastly 30/6 (n = 7).

### **Body size measurements**

The sampled beetles were kept in vials, with 70 percent ethanol which were refrigerated after arrival in Bergen. The lengths of adults and teneral adults were later measured at 25x in the laboratory at the University of Bergen, using an ocular micrometer in the eyepiece a dissecting microscope with 25 times enlargement. The total number of beetles measured was 230, of these 149 adult females, 18 teneral adult females, 43 adult males and lastly 20 teneral adult males. The beetles were measured with their ventral side down on a Petri dish paper; (90 mm), elytra length and width, and pronotum width and length in mm was recorded to the nearest line of the micrometer. The pronotum length was also measured transversely. The beetles that were not able to stand upright were measured while resting on a small piece of cotton.

A comparison was made to see if of total body length and pronotum width varied between beetles collected at populations found at 1500 m, and populations located above 2500 m altitude. The total number of beetles used for this test (n = 170), of these 48 females and 12 males from 1500 m altitude, and 84 females and 26 males from populations located above 2500 m altitude.

## Distribution of plants and beetles in the Cerro de la Muerte

Two species of *Gunnera* were present in the Cerro de la Muerte, *G. talamancana* and *G. insignis* plus a hybrid between the two species. I identified the different species by morphological traits given by Palkovic (1978), and checked if there were beetles present in the petioles of the different species at several populations and locations. I recorded the positions of populations of the different plant species and of beetle populations by GPS (Garmin 60SCX), this gave me the locations within approximately a 4 m radius.

### Observation of offspring and adults

#### 1. Development of offspring

In order to observe the development of the offspring, some of the different stages were kept in Petri dishes (55 mm diameter), with the lid on and paper in the bottom and mostly with small petiole pieces in them. I kept the Petri dishes indoors at La Esperanza station at 2600 m elevation, and observed their development.

#### 2. Feeding and behavior of adults and offspring

To observe feeding and the behavior of adults in their gallery, the petiole and thereby the gallery were sliced into two pieces with a knife. The biggest half of the gallery was then covered with transparent plastic wrap, which allowed me to access the interior of the galleries. These petioles were kept indoors at La Esperanza station.

This project was based on the species *S. gunnerae*, a recently described species and their biology is therefore not been intensively studied previously. They are also breeding in live plants which are an unusual habitat for bark beetles, and the symbiosis between the plants and the beetles is poorly understood. I therefore had to try many different experiments because I before-hand was not certain which experiments would work and which would fail. In the end however I found methods that was working well, in order to test the hypotheses.

# Statistics

The statistical analyses was performed in the statistical program R (version 2.6.0 (2007-10-03) developed by the R Development Core Team (2007).

## Brood size

To find which variables affected brood size (the total number of offspring) a linear model (lm) was built by the Forward selection method. The forward selection method adds one variable at a time, and at each step each variable is tested for inclusion in the model via the anova function in R. The most significant variable is added to the model at each step. The significant level used is  $P < 0.05$ . The predictor variables used were location, population, plant, petiole, petiole length, petiole width, and gallery size. The R-syntax for this test follows as an appendix.

To test if gallery size varied between the different stages, I used the anova function in R, to test if there is significant difference ( $P < 0.05$ ) between the model with no predictor variables against the model using offspring stage as a predictor variable for gallery size. For this test, data from unmanipulated galleries from the mother removal experiments was used together with data from the dissected petioles. A multiple comparison was done in the multcomp function from the R library, to see which categories were different. I also tested for a correlation between petiole length and diameter, between number of galleries on a petiole against both petiole length and diameter. Lastly I performed a Chi square test to analyze if female presence in the gallery varied between early (egg to pupae) and late offspring stages (pupae + teneral adults and teneral adults).

## **Female removal experiments**

To test the effect of the female removal, I performed three generalized linear mixed effect models (GLMM), in the tests petiole was treated as a random variable. To do this I used the `glmpQL` function from MASS (library) in R (Venables and Ripley 2002). R-syntax for these tests follows as an appendix. For the female removal bag experiment I analyzed if the number of dead and survived offspring was different for manipulated (female removed) and unmanipulated galleries (female present) after 15 days with expected binomial distribution. The opening and closing of petioles and the probe experiment compared the total number of offspring of all stages for manipulated and unmanipulated galleries after 15 days with expected Poisson distribution.

## **Frass removal experiment**

To test if offspring survival was different between manipulated (frass removed) and unmanipulated galleries (frass not removed) after 11 days, I performed a Fisher exact test for count data, with a 95 percent confidence interval.

## **Body size dimorphism**

To analyze if total body length (pronotum length + elytra length) varied between males and females I performed a Welch two sample t- test.

To test if mean total body length and pronotum width of individuals varied between populations located at 1500 m and those above 2500 m, I performed a Welch two sample t- test separately for males, and for females respectively for pronotum width and total body length from the two altitudes.

# Results

## Female removal experiments

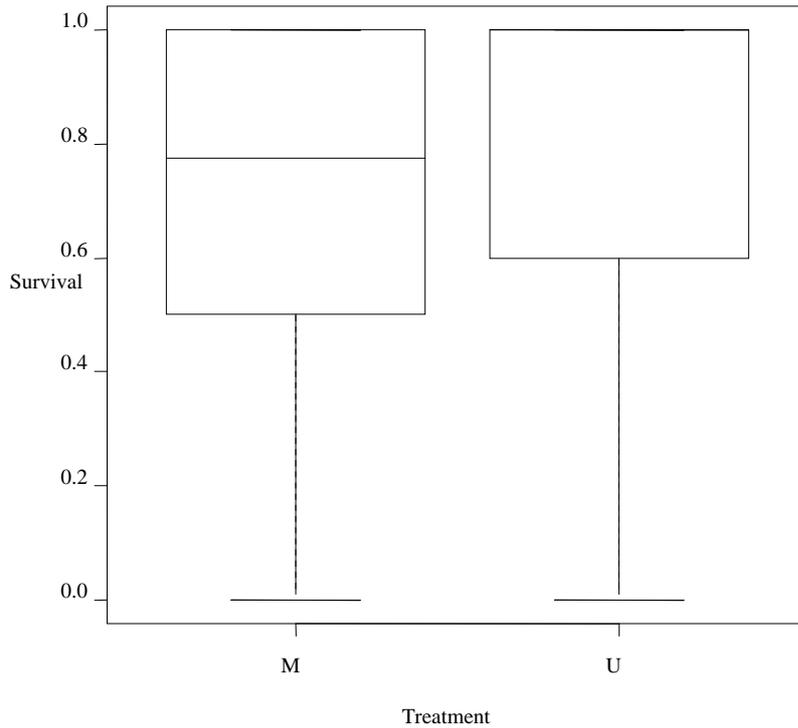
Female removal: bag

There was a small difference in offspring survival between manipulated and unmanipulated galleries when all stages was used, but the difference in survival is not significant (Figure 2 and Table 1). Offspring survival was also lower for manipulated galleries when I analyzed galleries containing only eggs or larvae separately (Table 1). The lowest offspring survival recorded was from galleries containing eggs at the initiation of the experiment (Table 1). However survival was not significantly different between manipulated and unmanipulated galleries for any stage (Table 1). During the experiment eight females had deserted the gallery, and were found in the bags.

**Table 1.** Results female removal: bag. The mean ( $\pm$  SE) offspring survival for manipulated (M), and unmanipulated (U) galleries, depending on start stage. Values given by the generalized linear mixed effect model with expected binomial distribution for difference in offspring survival between the treatments.

Start stage	Treatment	Mean survival $\pm$ SE	P-value (M - U)	Df	T-value
All stages	M	0.68 $\pm$ 0.045	0.2251	67	1.2242
Egg – pupae	U	0.75 $\pm$ 0.052			
Eggs	M	0.64 $\pm$ 0.063	0.5122	31	0.663
	U	0.69 $\pm$ 0.081			
Larvae	M	0.88 $\pm$ 0.05	0.2953	16	1.0819
	U	0.94 $\pm$ 0.041			

## Results



**Figure 2.** Mother removal bag: Offspring survival after 15 days (all stages) depending on treatment, for galleries which were manipulated (female removed) or unmanipulated (female present in gallery). Five galleries had a higher number of offspring after 15 days, than when the experiment was initiated. For these galleries survival is recoded as 1.0 in the plot. Abbreviations: M, female removed: U, female present. The horizontal line in the middle shows the median value. The top of the box is the 75<sup>th</sup> percentile, and the bottom of the box is the 25<sup>th</sup> percentile. The whiskers show maximum and minimum values.

Female removal: opening and closing of petioles

The total number of offspring was significantly different between manipulated and unmanipulated galleries (Table 2); the total number of offspring of all stages is higher in unmanipulated galleries with 26 offspring in 19 galleries against only 8 offspring in 18 galleries for manipulated galleries.

## Results

**Table 2.** Results female removal: opening and closing of petioles. Shows the number of galleries for manipulated (M) and unmanipulated (U), including galleries that had no offspring after 15 days. Values given by the generalized linear mixed effect model with expected Poisson distribution for difference in the total number of offspring between manipulated (M), and unmanipulated (U) galleries after 15 days.

Treatment	Number of galleries	Df	T-value	P-value
M	18	32	-1.4161	0.0250
U	19			

Female removal: probe

The total number of offspring was not significantly different between manipulated and unmanipulated galleries (Table 3).

**Table 3.** Results female removal: probe. The number of manipulated (M) and unmanipulated (U) galleries, including galleries with no offspring after 15 days. Values given by the generalized linear mixed effect model with expected Poisson distribution for difference in the total number of offspring between manipulated (M), and unmanipulated (U) galleries after 15 days.

Treatment	Number of galleries	Df	T-value	P-value
M	23	46	-1.492	0.1425
U	31			

## Gallery dissection and brood size

For the dissected galleries brood size ranged from one to ten, with a mean value of  $3.66 \pm 0.13$  (Table 4). The highest number of galleries had two or three offspring, and very few had six or more (Figure 3). The distribution of offspring between galleries shows a Poisson distribution, as expected for randomly distributed count data (Figure 3). The galleries containing both eggs and larvae had the highest number of offspring, with  $5.46 \pm 0.4$  (Table 4). When the predictor variables was plotted in a forward selection model for linear models (lm), the model that best explained the total number of offspring was petiole and gallery size. The other factors did not significantly improve the model, and I therefore have to remain with this model.

## Results

The number of active galleries per petioles varied from 1 to 20, with two galleries per petiole being the most common followed by five and one gallery (Figure 4). The mean number of galleries per petiole was  $4.6 \pm 0.54$  (Table 5). Of the 586 petioles dissected 44.7 percent were colonized by beetles; having only egg was the most common stage accounting for 18.83 percent of the galleries (Table 6). Plant measures varied also widely between the dissected petioles (Table 5). There is a strong positive correlation between petiole length and diameter ( $t = 2.8031$ ,  $df = 51$ ,  $p = 0.00714$ ), and between petiole diameter and the number of galleries on a petiole ( $t = 3.415$ ,  $df = 44$ ,  $p = 0.001390$ ). The regression line for the relationship between petiole diameter and the number of galleries per petiole, is described by the equation  $y = -1.395 + 2.586(x)$  (figure 5). No correlation was found between petiole length and the number of galleries per petiole ( $t = 0.622$ ,  $df = 52$ ,  $p = 0.5366$ ).

Females were found in galleries with offspring of all stages, from eggs to teneral adults (Table 7), but females is significantly more likely to be found with offspring at early stages (egg to pupae), than with later stages (Pupae + teneral adults and teneral adults) (Chi square:  $X\text{-squared} = 8.7076$ ,  $df = 1$ ,  $p < 0.01$ ). Of the 29 galleries containing a male and a female, 23 was containing eggs, one egg and larvae and the remaining five galleries had larvae (Table 7). No males were recorded for galleries containing pupae or teneral adults (Table 7). 18 out of 64 males (28.1 %) were found solidly and 22 out of 122 (19.2 %) females were found solidly in the galleries. Three galleries had two males and a female.

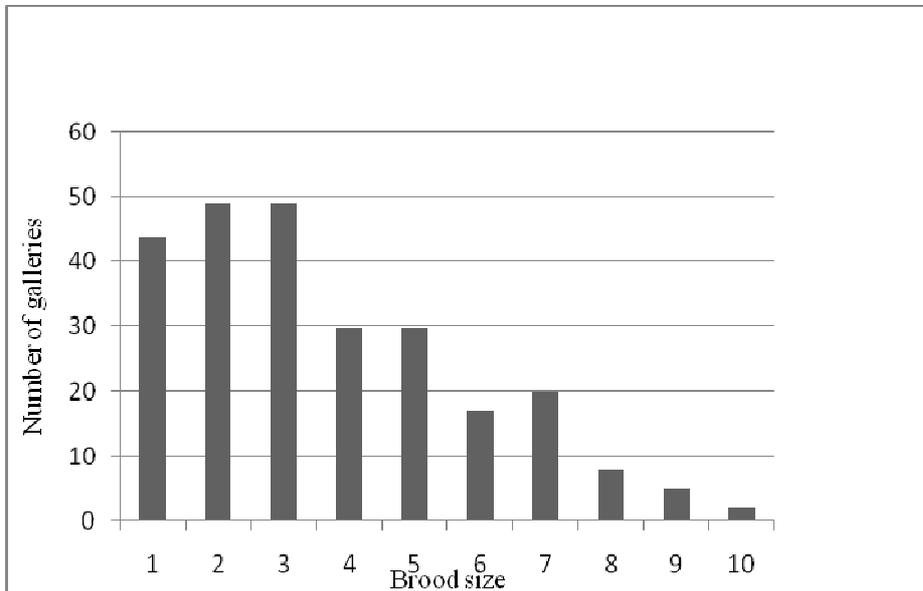
After 15 days 19.5 percent of the galleries starting at the eggs stage had no hatched eggs, while 36.6 percent of the galleries had a combination of unhatched eggs and larvae, and lastly 41.5 percent of the galleries were containing only larvae (Table 8). The larval stage is longer in duration than the egg stage; of the galleries starting at the larval stage 35 percent of the galleries had only larvae after 15 days in the experiment, 31 percent of the galleries were containing larvae and pupae, and 31 percent were only containing pupae (Table 8).

During the field work offspring of different stages were kept in Petri dishes, and I was able to hatch eggs and to get pupae to enclose without petiole pieces. The mortality was high for larvae kept under these conditions, and when I later provided them with small petiole pieces the survival increased.

## Results

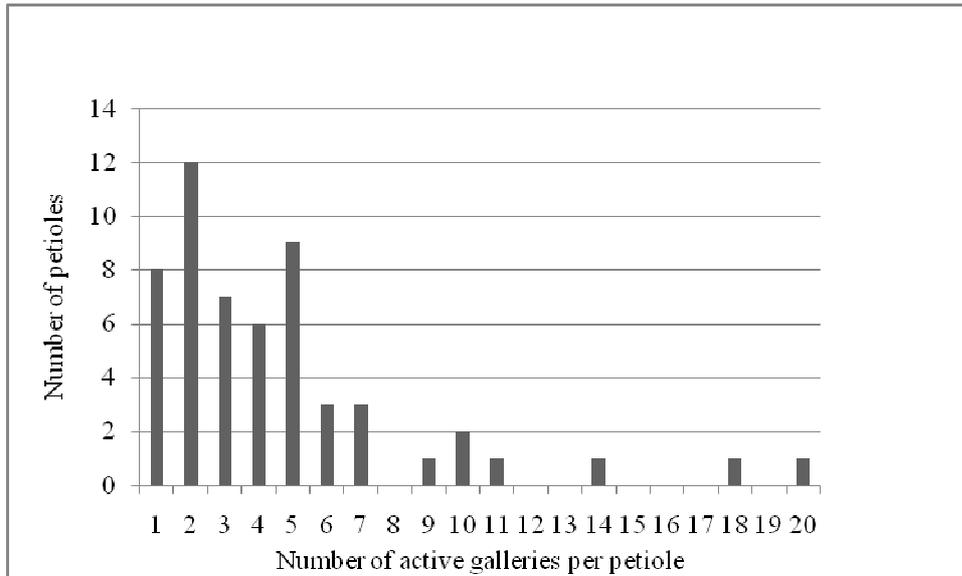
**Table 4.** Mean number of offspring per stage(s) and Standard Error ( $\pm$  SE). Based on dissected petioles.

Stage	Mean $\pm$ SE
Eggs	3.98 $\pm$ 0.23
Eggs + larvae	5.46 $\pm$ 0.40
Larvae	2.94 $\pm$ 0.19
Larvae + pupae	3.69 $\pm$ 0.39
Pupae	2.43 $\pm$ 0.23
Teneral adults	2.22 $\pm$ 0.42
Egg + larvae - pupae	3.51 $\pm$ 0.17
Total offspring (all stages)	3.66 $\pm$ 0.13



**Figure 3.** Number of galleries with given brood sizes, from one to ten. Ten is the highest number of offspring recorded in a gallery. Based on the results from the dissection of petioles.

## Results



**Figure 4.** The frequency distribution of active galleries for dissected petioles, with 1 to 20 galleries per petiole. 20 galleries was the highest number of active galleries found on a petiole.

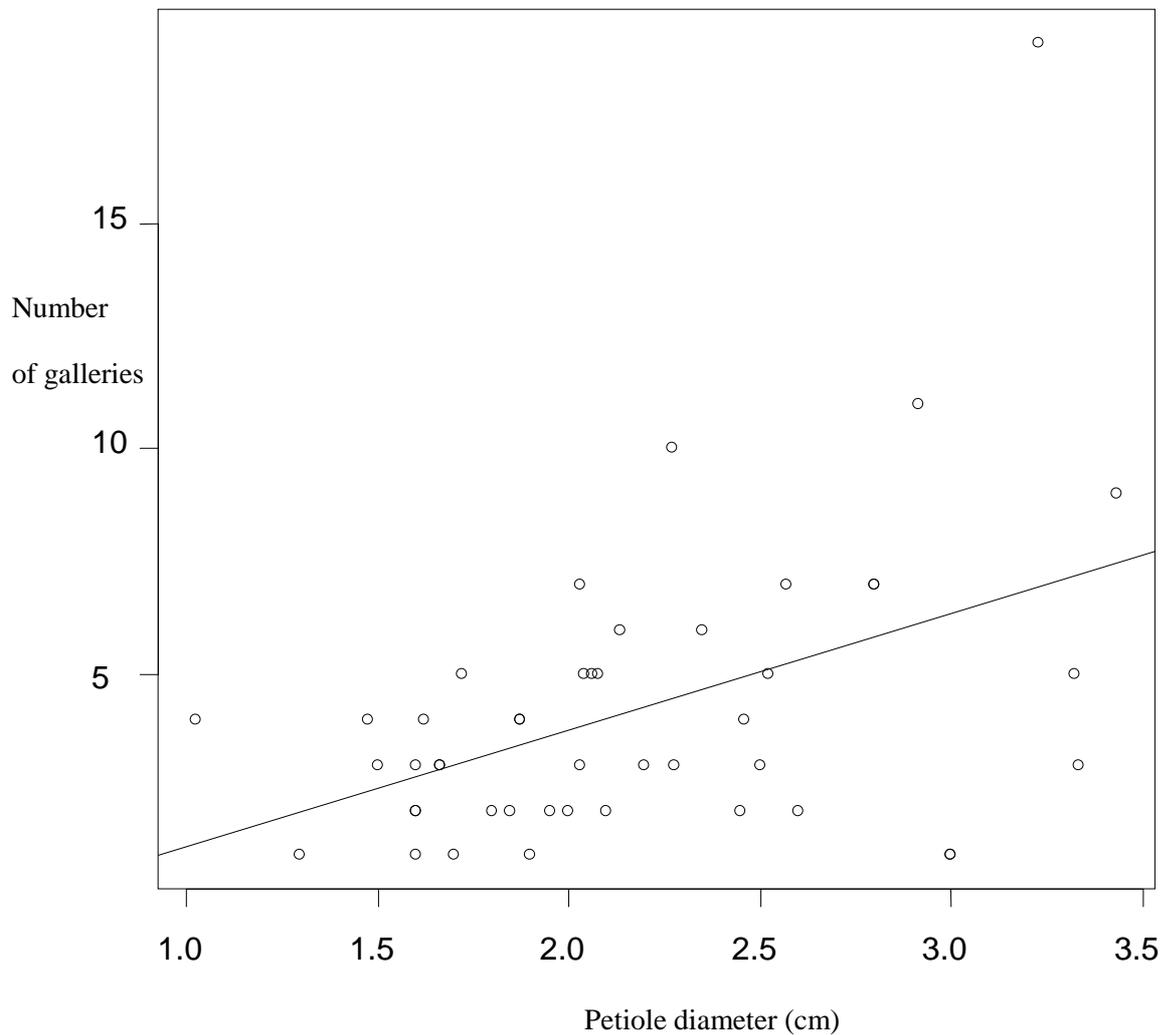
**Table 5.** The range and mean  $\pm$  SE of the plant measures from dissected galleries.

	Range	Mean $\pm$ SE
Leaf length (cm)	15 - 90	55.53 $\pm$ 1.4
Leaf diameter (cm)	28 - 160	104.45 $\pm$ 2.67
Petiole length (cm)	36 - 140	87.67 $\pm$ 1.7
Petiole diameter (cm)	0.9 - 4.1	2.37 $\pm$ 0.05
Gallery length (cm)	0.4 - 2.1	1.19 $\pm$ 0.02
Gallery width (cm)	0.3 - 1.5	0.54 $\pm$ 0.01
Number of galleries per petiole	1 - 20	4.6 $\pm$ 0.54

**Table 6.** Number of galleries at different stages and the percentage distribution of the different stages for all dissected galleries.

Stage	Number of galleries	Percent of total
Empty	314	55.28
Eggs	107	18.83
Eggs + larvae	28	4.93
Larvae	72	12.68
Larvae + pupae	16	2.82
Pupae	15	2.64
Pupae + teneral adults	2	0.35
Teneral adults	13	2.29
Larvae + teneral adults	1	0.18
Total colonized	254	44.72

## Results



**Figure 5.** The relationship between number of galleries on a petiole, and petiole diameter for dissected petioles. The regression line is described by the equation  $y = -1.395 + 2.586(x)$ .

**Table 7.** Parental presence depending on offspring stage for dissected galleries. Abbreviations: Eggs and larvae (E + L), larvae and pupae (L + P), and pupae and teneral adults (P + T). One gallery had larvae and teneral adults, but no pupae\*.

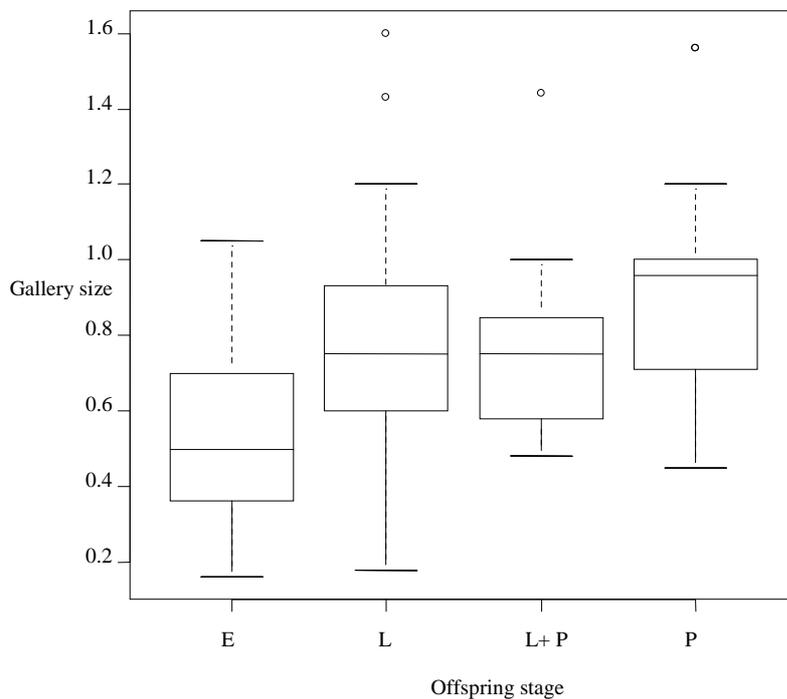
	Egg	E + L	Larvae	L + P	Pupae	P + T	Teneral adults	Total
Male	0	1	0	0	0	0	0	1
Female*	73	22	54	12	13	1	7	182
Male and female	23	1	5	0	0	0	0	29
No parents	12	3	14	3	2	1	6	41

## Results

**Table 8.** Number of galleries at different stages (eggs to teneral adults) and their percentage distribution of the total after 15 days, depending on start stage (egg or larvae). Data based on the mother removal bag experiment.

	Eggs	Eggs + larvae	Larvae	Larvae + pupae	Pupae	Teneral adults
Egg	8 (19.5 %)	15 (36.6 %)	17 (41.5 %)	1 (2.4 %)	0	0
Larvae	0	0	10 (35 %)	9 (31 %)	9 (31 %)	1 (3 %)

There is a difference in gallery size for the different offspring stages (Figure 6), with galleries containing pupae being the largest, while galleries containing eggs had the smallest galleries (Figure 6). The difference in gallery size between the offspring stages was found to be significant (Anova-test:  $f = 18.35$ ,  $df = 160$ ,  $p < 0.001$ ), there is a significant difference in gallery size between galleries of the following stages: larvae and eggs, between larvae + pupae and eggs, and lastly between pupae and eggs (Table 9).



**Figure 6.** Gallery size (gallery length (cm) multiplied with gallery width (cm)) depending on offspring stage. From eggs (E), to galleries containing larvae (L), larvae and pupae (L + P) and pupae (P). Data from unmanipulated galleries from the mother removal experiments and from dissected petioles.

## Results

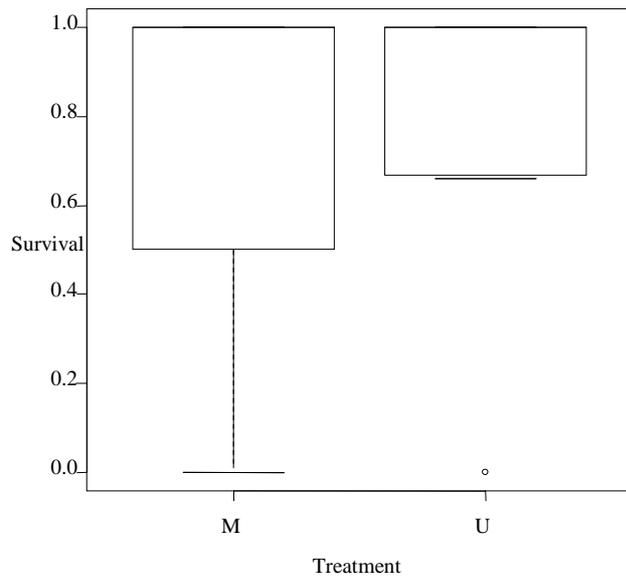
**Table 9.** Result from the multiple comparison test with gallery size (gallery length (cm) multiplied with gallery width (cm)) as a response variable for offspring stage. Data from both dissected petioles and from unmanipulated galleries from the mother removal experiments.

Stages	Estimate	Std. error	T-value	P-value
Larvae - egg	0.258	0.044	5.772	0.001
Larvae + pupae - egg	0.248	0.081	3.061	0.012
Pupae - egg	0.397	0.069	5.755	0.001
Larvae + pupae - larvae	0.005	0.083	-0.07	0.99
Pupae - larvae	0.143	0.071	1.996	0.182
Pupae + larvae - pupae	0.149	0.098	1.506	0.419

### Frass removal experiment

There was some difference in survival between the treatments, with the survival being slightly higher for the unmanipulated group (Figure 7). However the difference in survival was not significantly different between the two treatments (Fisher exact test:  $p = 0.7131$ ). The amount of frass seemed to be related to the stage of the offspring, and not treatment (Table 10). The three galleries containing large amount of frass contained larvae, while four out of six galleries that had no frass contained pupae (Table 10).

## Results



**Figure 7.** Offspring survival after 11 days depending on treatment for the frass removal experiment. Manipulated (M = frass removed) and unmanipulated galleries (U = Frass not removed). For both manipulated and unmanipulated galleries the female was removed from the gallery. All galleries started at the larval stage.

**Table 10.** The amount of frass (no, some or much), in galleries depending on stage and treatment. Frass removed from gallery (M), frass not removed (U). Parents were removed from both manipulated and unmanipulated galleries in this experiment. To have equal number of offspring of the same stages, some offspring were removed from their natal gallery.

Stage / treatment	No	Some	Much
Larvae / U	1	3	1
Larvae / M	0	1	1
Pupae / U	2	0	0
Pupae / M	2	2	0
Pupae + larvae / U	0	0	1
Pupae + larvae / M	0	1	0
Empty	1	0	0

## Establishment in petioles

This experiment failed since I was only able to get a few beetles to establish galleries in both dead and live petioles, and none of them attracted a mate when the petioles were placed in the field (Table 11). Both males and females established themselves in the petioles, but the success rate was slightly higher for females (Table 11).

**Table 11.** The number of successful and failed establishment for males and females in both live, and dead petioles. The success rate is the number of successful establishments divided by the total number of attempts (failed + successes).

	Success	Failed	Success rate (%)
Live petiole, male	0	2	0
Live petiole, female	1	3	25
Dead petiole, male	2	17	10.5
Dead petiole, female	2	4	33

## Body size measurements

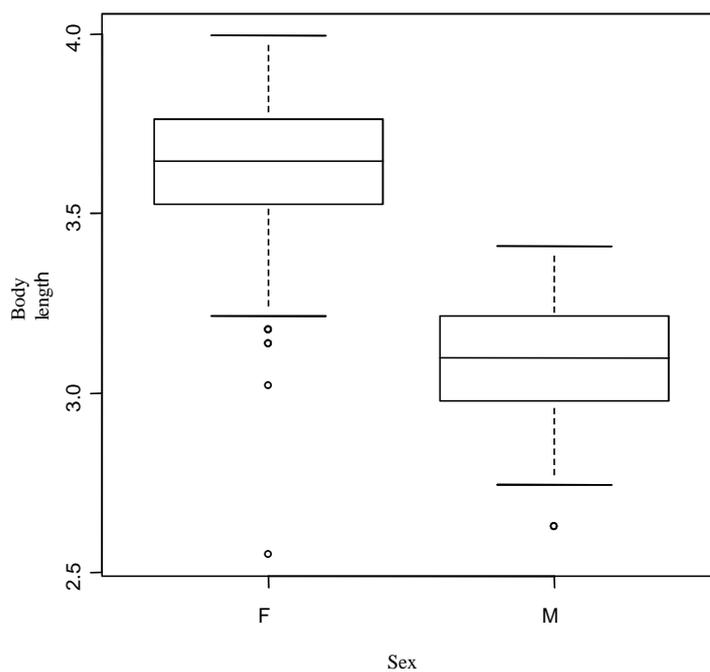
Females were consistently larger than males for all the measured traits, and for pronotum width/ pronotum length and elytra length/ elytral width (Table 12). Figure 8 shows the mean total body length (pronotum length + elytra length) for males and females. There was a large difference in total body length between males and females (Figure 8 and Table 12) (T-test:  $t = 19.08$ ,  $df = 136$ ,  $p < 0.001$ ).

There was no significant difference in total body length between individuals found at 1500 m, and individuals found above 2500 m (T-test with 95 percent confidence interval: males  $t = -1.4255$ ,  $df = 18.327$ ,  $p = 0.1708$ ; females  $t = -0.745$ ,  $df = 83.204$ ,  $p = 0.4584$ ). There was also no difference in pronotum width between beetles collected at the two altitudes, neither for males ( $t = 1.1817$ ,  $df = 20.65$ ,  $p = 0.2507$ ) nor for females ( $t = 1.1056$ ,  $df = 130.523$ ,  $p = 0.270$ ).

## Results

**Table 12.** Mean length measures in mm ( $\pm$  SE) for the measured traits for males and females. Total body length is pronotum length + elytra length. Sample size: Male (n = 43), female (n= 149), teneral adult males (n = 20) and teneral adult females (n=18). All traits measured with 25 times enlargement.

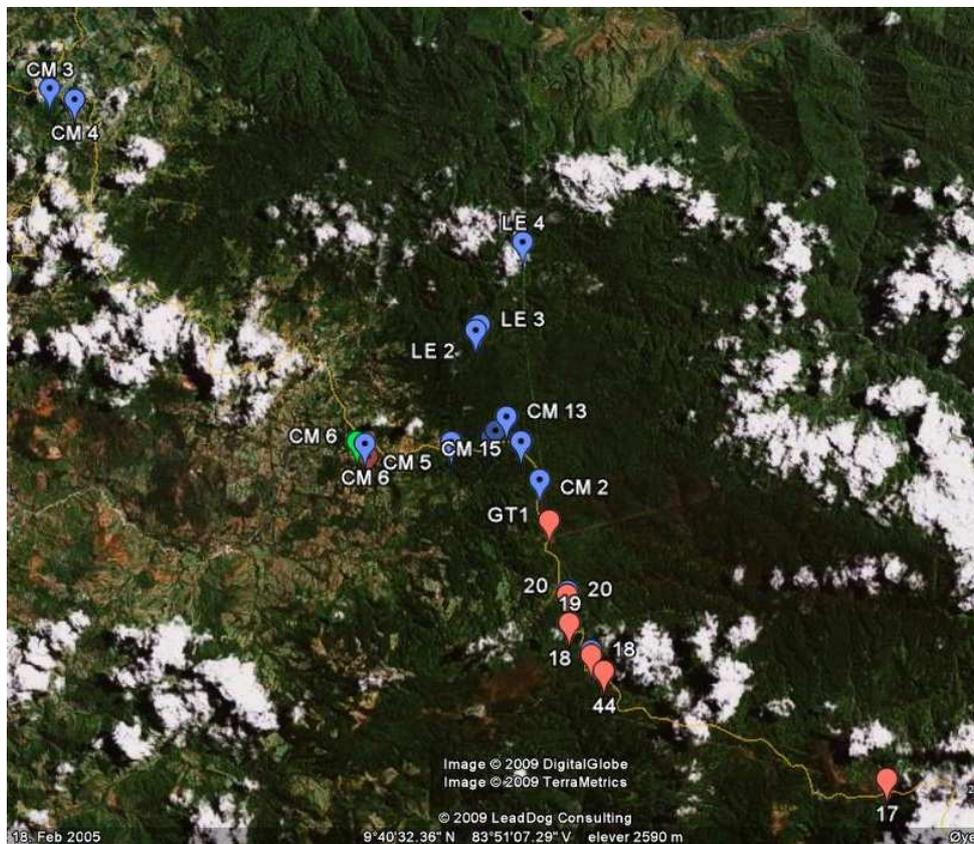
	Pronotum length	Elytra length	Pronotum width	Elytral width
Male	1.03 $\pm$ 0.008	2.05 $\pm$ 0.017	1.06 $\pm$ 0.009	1.33 $\pm$ 0.009
Female	1.21 $\pm$ 0.006	2.39 $\pm$ 0.011	1.19 $\pm$ 0.006	1.50 $\pm$ 0.007
	Total body length*	El:Ew	Pl:Pw	
Male	3.08 $\pm$ 0.022	1.54 $\pm$ 0.008	0.97 $\pm$ 0.0054	
Female	3.61 $\pm$ 0.017	1.59 $\pm$ 0.004	1.01 $\pm$ 0.003	



**Figure 8.** Total body length (mm) depending on sex, male (M) and female (F).

## Distribution of plants and beetles in the Cerro de la Muerte

Population number 6 on Cerro de la Muerte (CM 6) had all three *Gunnera* species present, and all three of them were colonized with beetles as shown on the map (Figure 9). The beetles were found in an altitudinal gradient from 2035 meters at CM population 3 to 2703 m at CM population 13 in the Cerro de la Muerte. After Tres de Junio there were populations with *G. insignis* and with *G. talamancana* but they were not colonized by beetles. At La Esperanza beetles were found at 2831 m (LE population 2), which is the population recorded at the highest altitude.



**Figure 9.** Map over the locations in the Cerro de la Muerte (CM) and La Esperanza (LE), beetle presence in the population are indicated with a black circle within the marker. The different colors on the marker indicates the three different species: *G. insignis* (Blue), *G. talamancana* (Red) and lastly the hybrid (Green). The coordinates for the different locations follows as an appendix and are plotted into google earth through the webpage [www.boulter.com](http://www.boulter.com) and google maps ([www.maps.google.com](http://www.maps.google.com))

## Discussion

I found no consistent effects of female presence on the number of offspring in galleries, or on offspring survival. The female removal bag experiment showed a higher survival for unmanipulated galleries, but the difference in survival was not significant. I therefore had to reject the hypothesis that maternal care enhances offspring survival. The female removal bag experiment did however not test for maternal care as protection from predators or parasites, but data from two other female removal experiments did. The female removal probe experiment had no significant difference in the total number of offspring between the treatments. The mother removal by opening and closing of petioles on the other hand had a significant difference in the total number of offspring, with unmanipulated galleries having a higher number of offspring than manipulated galleries. For the latter experiment it had the lowest sample size of the three female removal experiments, and the contrasting result from this experiment could be due to a combination of small sample size and unequal number of offspring between the treatments at initiation. Both predation and parasitism are known to have profound effects on offspring survival, and protection is the most common form for parental care provided by insects (Mas and Kölliker 2008). For this species predation and parasitism are unlikely to affect offspring mortality to a large extent, firstly because in dissected galleries neither predators nor parasites were found. Secondly the low brood size could only be adaptive if egg to adult mortality is very low, and lastly if predators or parasites affected offspring survival to a large extent then you should have consistently higher number of offspring in unmanipulated galleries for both the probe and the opening and closing of petiole experiment. Lastly the frass removal experiment showed no significant difference in survival between manipulated and unmanipulated galleries.

For both the female removal experiments and the frass removal experiment I used the same petioles for both manipulated and unmanipulated galleries, which removed potential plant or petiole-specific effects influencing the results. The durations of the experiments were 15 days and 11 days respectively for the mother removal experiments and the frass removal experiment. This should be sufficient to detect a difference between the treatments. However the temperature is relatively low at these higher altitudes and secondly there could be a small effect of maternal care over the entire period from egg to teneral adult, which would not be detected in my experiments with short duration. It could therefore be beneficial to increase the

duration of the experiment to test this possibility. For the experiments with exception of the mother removal bag experiment I should also have increased the sample size.

For the female removal experiments with probe and the opening and closing of petioles, I used the number of offspring at the end of the experiment as an indication of offspring mortality. These two experiments were meant to function as back up experiments in case the mother removal bag experiment failed, which was more destructive on the petioles and to detect the possibility of maternal care as protection against parasites and predators. The results from these two experiments were not congruent, and the number of offspring was not a good measure to use for offspring mortality.

In the female removal bag experiment the petioles with galleries were kept in sealed sandwich bags. This was done to standardize experimental conditions, and to enable me find offspring, or adults that had deserted the gallery during the experiment. Some galleries had a survival rate higher than 100 percent, because the number of offspring was higher in the end of the experiment than when it was initiated. This could result from either that the number of offspring at initiation was underestimated, or for the unmanipulated galleries additional offspring could have been laid after initiation of the experiment. In order to record the number of offspring more accurately at initiation, the galleries would have to be divided into several pieces, which could have adverse effects on the petioles.

### **Female presence**

Studies on brooding insects have found significant reduction in survival for broods where the parents are experimentally removed. A study on the burying beetle *Nicrophorus vespilloides* found reduced growth and much lower survival for broods receiving no parental care (Eggert et al. 1998). Reduced survival in the absence of parental care was also found in a study on *N. mexicanus* (Anduaga and Huerta 2001).

Two other hypotheses besides maternal care have been proposed to explain maternal presence in the gallery for bark beetles. Firstly the female can remain in the gallery after she has ceased laying eggs to feed and regenerate flight muscles (Kirkendall et al. 1997), this seems unlikely for this species because females were found with offspring of all stages. Studies on duration of flight muscle degeneration in bark beetles shows that it varies between

5 and 12 days, ( Bhakthan et al. 1970, Langor 1987, Robertson 1998b), and for *Ips pini* which was the only species where data on the duration of flight muscle regeneration was available it took only 5 days, as did the flight muscle degeneration (Robertson 1998b). To regenerate flight muscle is therefore unlikely to explain such a prolonged residency in the gallery. The second explanation for maternal presence is overwintering in the gallery (Kirkendall et al. 1997), and this is not relevant for this species because there is little seasonal variation in climate at these latitudes.

Maternal care is considered to be rare among insects (Reid and Roitberg 1994). This is probably due to the cost of providing maternal care in terms of future reproduction is being higher than the benefit measured in increased offspring fitness (Clutton-Brock 1991). Some of the “prime movers” that Wilson (1975) believed could explain evolution of parental care among animals is present for this species, they live in a physically demanding environment (live petioles) which is stable in structure, and are feeding on a specialized food resource (plant tissue from two species of *Gunnera*). This species should therefore be a good candidate for studies on parental care.

Bark beetles are believed to lay all or most of their eggs in one gallery, one gallery therefore probably represents much of the lifetime reproduction of that female (Kirkendall et al. 1997). This could have implications for the interpretation of the results, since the female would in terms of fitness have nothing or little to gain by deserting the gallery and establish a new brood. This could explain maternal care even though the benefit in terms of increased fitness is small. Tallamy and Brown (1999) defined this as functional semelparity, which occurs when there is a low probability for future reproduction and then; maternal care can evolve not because the benefits are high, but rather because the cost in terms of future reproduction is low (Tallamy and Brown 1999). The most likely explanations for maternal presence in the galleries, seems therefore to be that the female is providing some type of maternal care for the offspring. However the benefit of maternal care is small for this species. This could explain why the difference in survival is not significant between the treatments for the mother removal bag experiment, and the contrasting results between the two other female removal experiments conducted. This is in agreement with the hypothesis proposed by Tallamy and Brown (1999).

## Male desertion

In *S. gunnerae* the male left the gallery a short time after mating, and were therefore predominately found with offspring at the egg stage. This is uncommon behavior for bark beetles (Kirkendall 1983). The male could therefore not be providing paternal care for his offspring, and based on the female removal experiments maternal presence did not enhance offspring survival to a large extent either. The male has therefore more to gain in terms of fitness, by leaving the gallery and searching for a new mate.

The lack of paternal care could be related to the host transition to live plants. The normal breeding material for bark beetles is dead trees which are a scarce and ephemeral resource. Live *Gunnera* leaves on the other hand are quite widespread in the cloud forest, which could give the males good chances for re-mating, and thus increasing the benefit of deserting the gallery. This is in agreement with a study on the bark beetle *Ips pini*, which found that large males deserted the gallery earlier than small males (Robertson and Roitberg 1998). The authors believe that this is because larger males have higher prospects of future reproduction, and therefore have more to gain by deserting than small males. Why males leave and females remains in the gallery is in accordance with Bateman's theory about asymmetry in fitness maximizing between the sexes (Bateman 1948). Since male fitness is believed to be more closely related to mating frequency than female fitness, the male have more to gain by deserting the gallery. The predominant occurrence of males in galleries containing eggs could also be explained by mate guarding to ensure paternity of the offspring, and three dissected galleries had two males and a female where competition for mating with the female is likely to occur. Mate guarding is not uncommon behavior for bark beetles (Kirkendall 1983).

## Gallery dissection and brood size

The brood size recorded for this species is extremely small for any animal, especially since bark beetles are believed to lay most or all of their eggs in one gallery (Kirkendall et al. 1997). Life history theory and optimal clutch size theory are based on the assumption that individuals should be selected to maximize lifetime reproduction. The optimal clutch size is defined as the clutch size that gives the highest expected fitness per offspring (Brockelman

1975), and this theory is based on the assumption that brood size is adaptive. For the extremely small brood size recorded for this species to be adaptive, the egg to adult mortality must be small. Parasites and predators are therefore unlikely to lead to high offspring mortality, and this is supported by the fact that neither predators nor parasites were found in dissected galleries. This is in agreement with the view presented by Jordal and Kirkendall (1998), to explain the low brood size for bark beetles breeding in *Cecropia* leafstalks. The factors found to be associated with brood size in the analysis were petiole and gallery size; the other predictor variables did not improve this model and had to be rejected as explanatory variables.

Small brood size could be explained by that the female can physiologically not be able to lay more eggs (Godfray et al. 1991), this is unlikely because most bark beetles have much larger brood sizes and *S. gunnerae* egg size is not unusually large for a bark beetle (L. R. Kirkendall, personal comment). Secondly if the female is providing the offspring with some type of maternal care, an increase in the number of offspring could lead to a decrease in investment per offspring. Then the fitness gain by increasing the number of offspring will reach a peak at the optimal clutch size. For this species I was however not able to detect a difference in survival between galleries with female present, and galleries where she was experimentally removed. The kind of maternal care she then possibly is providing must give small benefits to the offspring, and should thus not lead to the evolution of such small brood sizes.

Competition between offspring is also believed to affect optimal clutch size decision among animals, especially when offspring are laid in clutches (Fox et al. 1996). In *S. gunnerae* the offspring were laid in relatively small galleries, and since gallery size increased with the number of offspring and offspring stage they are likely feeding directly on the plant tissue. It seems therefore highly likely that intra brood competition for food occur in the galleries, probably especially at the larval stage. Competition could explain the small brood sizes recorded for this species, because it would not be beneficial for the female in terms of fitness to lay larger broods, if the intra brood competition then increased. Intra brood competition has been confirmed in studies that showed a decrease in mean weight per offspring with increasing brood size, which for insects could have implications for the future fitness of the offspring (Godfray et al. 1991, Beaver 1976).

Small brood sizes were also recorded for bark beetles breeding in *Cecropia* leafstalks (Jordal 1998). Jordal suggested that the low brood size could be associated with the host shift

## Discussion

to a less productive tissue (Jordal 1998). The shift to a live host could explain the low brood size for *S. gunnerae*, not because the live tissue is lower in quality, but rather because there could be secondary compounds present, and large broods could potentially have adverse effects on the internal environment of the petioles. Plant chemistry is believed to be highly important for phytophagous insects and most of them are therefore highly host specific (Jaenike 1990). Differences in plant chemistry or other features varying between petioles like age and the amount of nutrients can explain why brood size is dependent on petiole, when petiole length and diameter per se was not important. The beetles live in symbiosis with live plants, and the host plant could therefore represent a selective force on brood size for this species. The plant tissue per se should not be particularly low in nutrients, since the plants are alive and moreover are in symbiosis with nitrogen-fixating cyanobacteria. This is in contrast to *Cecropia* leafstalks, which have a very low amount of nitrogen (Brueland and Nygård 1997).

There was a correlation between petiole diameter and length and between petiole diameter and the number of galleries on a petiole, but no correlation was found between the number of galleries and petiole length. The positive relationship between petiole diameter and the number of galleries is probably to avoid overcrowding of the resource. A petiole with large diameter has more available space than petioles with smaller diameter and can support more galleries without having adverse effects due to competition, damaging of conductive tissue and reduction of resource quality in the petiole. Petiole diameter is probably more important than petiole length, because galleries are constructed inward into the petiole, in a petiole with small diameter interference and competition is therefore more likely to occur. The beetles were able to utilize both small and large petioles, and I found galleries in a wide range of petiole lengths, diameters and even in leaf veins. The number of active galleries varied widely, from 1 and up to 20 per petiole. This shows that resource size is not crucial for successful establishment and breeding in the petioles, but still the positive relationship between the number of galleries and petiole diameter shows that larger resources can sustain a higher number of galleries. *Gunnera* leaves were abundant at the locations, and the competition for suitable habitat should therefore be lower, than for species breeding in dead trees which are scarce and ephemeral resource.

## **Frass removal experiment**

The mortality was slightly higher for the manipulated galleries (frass removed) however there was no statistically significant difference in mortality between the treatments. I therefore have to reject the hypothesis that the mother is providing frass as a food source for the larvae. The amount of frass seemed however to be associated with offspring stage, and large amount of frass was found mostly in galleries containing larvae, while galleries containing no frass were in four out of six galleries containing pupae. The frass consists probably of a mixture of plant material from the petioles and excrement, as for most bark beetles (Byers 1981), and since insects are mostly feeding at the larvae stage this explains why large amounts of frass is associated with this stage. Gallery size was found to be dependent on the stage and the number of the offspring in the gallery (Table 9 and Figure 6), which indicate that the offspring are feeding directly on the plant tissue. The plants are also in facultative symbiosis with nitrogen-fixing cyanobacteria (Bergman et al. 1992), and the plant should therefore have good reserves of nitrogen, a nutrient that is often in short demand for phytophagous insects.

## **Establishment in petioles**

The experiment failed, and I observed very few beetles establishing galleries in the field. The establishment success rate was slightly higher for females than for males, but a few individuals of both sexes made galleries during the experiment. Pheromone to attract conspecifics is recorded for many species of bark beetles (Byers 1981, Byers 1989), but since none of the beetles attracted mates I can not say if pheromones are used for mate attraction.

For the dissected petioles I found a slightly higher proportion of single males in galleries without offspring and a mate, than for females. This could indicate male initiated galleries. For most monogynous bark beetles females are the colonizing sex (Kirkendall 1989), but in the genus *Scolytodes* galleries are male-initiated. For *S. gunnerae* the form of the frons of the males are flattened while the female frons is concave, and has long setae which most likely is used somehow for courting the male. Based on these traits the male is expected to be the pioneering sex for this species. This is the same as with the species *S. cecropiavorus* and *S. atratus* which both are male-initiated (Brueland 1997).

I tried to make beetles establish themselves mostly in dead petioles, which could be less suitable for the beetles as breeding material if the physical and/or chemical environment within the petiole is altered. In the field I did not find beetles breeding in dead petioles, which could indicate that dead petioles are less suitable as habitat. Beetles already established in the petioles were, however doing well after 15 days in the mother removal experiment, which shows that breeding was possible even in dead petioles. The cause is probably that already established beetles have invested heavily in the gallery, and to desert and establish a new gallery could be very costly in terms of fitness. On the other hand, for beetles that have not yet established themselves, it could possibly pay off to search for live breeding material with higher resource quality.

### **Body size measurements**

The measures taken at the laboratory are mostly in agreement with the species description given by Wood (2007). My measures of total body length are for females in the upper part of the size range recorded by Wood (2007). This discrepancy is probably because his data is based on a small sample size; the female holotype, a male allotype and 27 paratypes, while I measured 230 individuals.

There is a clear female-biased body size dimorphism for *S. gunnerae*, and the difference in total body length was significantly different between the sexes. These results agree with studies on *Scolytodes* associated with *Cecropia* leafstalks (Jordal 1998), and is the trend for most monogynous bark beetles (Kirkendall 1983). Female-biased body size dimorphism was found for most of the *Cecropia* associated species, but was especially pronounced for *Scolytodes cecropiavorus* and *S. blandford* (Jordal 1998).

Sexual size dimorphism is wide spread among animals (Shine 1989), and female-biased body size dimorphism in insects is believed to be adaptive because there is a positive correlation between size and fecundity for females (Hönek 1993). A study of the beetle *Dineutus nigrior* found a female-biased body size dimorphism, and they believed that the cause of the body size dimorphism was due to fecundity selection (Fairn et al. 2007). Female body size could therefore be under strong selection for *S. gunnerae*, because of the relationship between size and fitness and that could result in the female-biased size

dimorphism recorded for the species. For males there is probably no benefits related to large body size, and they are not under selection for this trait.

No difference in total length or pronotum width was detected between beetles collected at 1500 m, and beetles collected at populations located above 2500 m. This is quite a wide altitudinal gradient and it should detect a difference, if the measured traits varied with altitude. This contrasts findings by Jordal, who found a positive relationship between body size and altitude for seven *Cecropia*-associated *Scolytodes* species (Jordal 1998). Jordal believed that this could be common for the genus (Jordal 1998), which is proven not to be correct for this species.

## **Distribution of plants and beetles in the Cerro de la Muerte**

The beetles were predominately found in *G. insignis* plants, but also in the hybrids at several populations. I found beetles in several plants of *G. talamancana*, but only at population 6 in the Cerro de la Muerte. However it shows that *G. talamancana* is suitable breeding habitat for the beetles. One explanation is that here all the three plant species were located in close proximity to each other, which could give the beetles a good chance to colonize *G. talamancana* plants. In the field I observed an ecological difference between the two gunnera species; the *G. talamancana* plants were predominately found in more shaded areas than *G. insignis*. This difference in habitat choice could potentially affect colonizing by the beetles. The absence of beetles in *G. talamancana* at other populations is therefore because they have not been able to colonize these plants, and not because the plants themselves are not suitable.

The beetles also showed an interesting distributional pattern in the Cerro de la Muerte, where they had not colonized plants above 2703 m even though there were large host populations readily available above this altitude. At La Esperanza I found beetles up to 2831 m, which was the highest altitude in the park.

The beetles are only found in *G. insignis* and *G. talamancana* plants and should thus not be affected by reduced resource diversity which normally occurs at higher altitudes. The lack of beetles above 2831 m altitude could therefore be due to lack of the ecological opportunity to colonize plant populations above this altitude, or that with higher altitude the environment is not suitable for colonization. This first hypothesis seems not very likely,

## Discussion

because there were many populations with suitable host plants, above this altitude and quite close to colonized populations at lower altitude, thus chances for colonization should be good. I believe that the lack of beetles above this altitude therefore is most likely due to harder and more variable environmental conditions, because even in the Neotropics the temperature is quite low at high altitudes. The normal change is a decrease in temperature of 6.5 Celsius degrees per 1000 m increase in altitude (Britannica encyclopedia 2007). The decrease in temperature could therefore affect physiological processes and increase the developmental time of the offspring, and therefore possibly restrict the distribution of beetles above this altitude.

It would be very interesting to test experimentally if the lack of beetles above 2831 m is due to lack of possibility for colonialization or that the temperature is posing a limitation for the distribution of the species above this altitude.

## Summary

*Scolytodes gunnerae* was found breeding in live petioles and veins of two species of *Gunnera* (*G. insignis* and *G. talamancana*) and in a hybrid between the two species. The beetles were not found in plant populations located above 2831 m altitude, and are most likely restricted by the lower temperatures. There was no difference found in either total body length or pronotum width, between beetles collected at 1500 m and those collected at populations above 2500 m altitude. There is a clear female-biased body size dimorphism for this species, and this is probably due to fecundity selection on female body size.

Males are the pioneering sex, and initiate gallery construction. The female remains in the gallery throughout most of the offspring developmental stages, while the males desert early. This is probably because the male can not assist the female and offspring effectively, and has more to gain fitness wise by deserting the gallery.

I found no strong effect of maternal care in my removal experiments. Female presence in the galleries could not be explained by either flight muscle regeneration or overwintering, so some type of maternal care is therefore the most likely explanation for her presence. The benefit of maternal care is however small for this species. Maternal presence could therefore

## Discussion

be a case of functional semelparity, since bark beetles are believed to lay most or all eggs in one gallery. The female could then remain in the gallery to provide maternal care not because the benefits are high, but rather because the costs are low.

The brood size recorded for this species is extremely small, and was found to be related to petiole and gallery size in the forward selection model. The small brood size could result either from in-brood competition and/or the transition to a live host plant. These are possibly strong selective agents on brood size, because the offspring are restricted to small galleries and they breed in live plant petioles. The small brood size found for this species can only be adaptive if egg to adult mortality is extremely small. This seems to be the case since neither predators nor parasites were found in the dissected petioles.

The frass removal experiment showed no significant difference in survival between the treatments; the amount of frass seems however to be related to offspring stage. Galleries containing large amount of frass were containing offspring at the larval stage, and since insects are mostly feeding at the larval stage this probably explains why large amount of frass is related to this stage.

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# Appendix

## Female removal R-syntax

Female removal bag (All stages):

```
> sol.df<-read.table("clipboard", header=T)
```

```
> attach(sol.df)
```

```
> names(sol.df)
```

```
[1] "Treatment" "Survived" "Dead" "Petiole"
```

```
> library(MASS)
```

```
> fit1.lm<-glmmPQL(cbind(Survived, Dead)~Treatment,random=~+1|Petiole,binomial)
```

Loading required package: nlme

```
> fit1.lm<-glmmPQL(cbind(Survived, Dead)~Treatment,random=~+1|Petiole,binomial)
```

```
iteration 1
```

```
iteration 2
```

```
iteration 3
```

```
iteration 4
```

```
> summary(fit1.lm)
```

Linear mixed-effects model fit by maximum likelihood

Data: NULL

AIC BIC logLik

NA NA NA

Random effects:

Formula: ~+1 | Petiole

(Intercept) Residual

## Appendix

StdDev: 0.7049596 1.393726

Variance function:

Structure: fixed weights

Formula: ~invwt

Fixed effects: cbind(Survived, Dead) ~ Treatment

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.7390337	0.2610889	67	2.830582	0.0061
TreatmentU	0.4051847	0.3309547	67	1.224290	0.2251

Correlation:

	(Intr)
TreatmentU	-0.51

Female probe removal (Opening and closing of petioles was analyzed with the same syntax):

```
> fit.glm<-glmmPQL(Offspring~Treatment,random=~+1|Petiole,poisson)
```

```
iteration 1
```

```
iteration 2
```

```
iteration 3
```

```
iteration 4
```

```
iteration 5
```

```
> summary(fit.glm)
```

Linear mixed-effects model fit by maximum likelihood

Data: NULL

AIC BIC logLik

NA NA NA

Random effects:

## Appendix

Formula: ~+1 | Petiole

(Intercept) Residual

StdDev: 0.70711 1.349019

Variance function:

Structure: fixed weights

Formula: ~invwt

Fixed effects: Offspring ~ Treatment

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.5315309	0.3661541	46	1.451659	0.1534
TreatmentU	-0.5119962	0.3431533	46	-1.492034	0.1425

Correlation:

(Intr)

TreatmentU -0.42

Standardized Within-Group Residuals:

Min	Q1	Med	Q3	Max
-1.1977522	-0.6881951	-0.5327634	0.3307971	2.6284774

Number of Observations: 54

Number of Groups: 7

## Forward selection model (Factors affecting brood size)

```
> sol.df<-read.table("clipboard", header=T,na.string="omit")
> attach(sol.df)
> names(sol.df)
[1] "Species"    "Date"       "Location"   "Population"
[5] "plant"     "Leaf"       "gallery"    "Adults"
[9] "Parents.m.f." "Female"     "Male"       "SEX."
[13] "Teneraladults" "eggs"      "Larvae"     "Puppa"
[17] "Totoffspring" "Stadium"   "petLength"  "Petdiameter"
[21] "Gallength"   "Galwidth"  "Gallerysize" "Gallsizedde"
> fit0.lm<-lm(Totoffspring~1)
> fit1.lm<-lm(Totoffspring~Location)
> anova(fit0.lm,fit1.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ Location

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	253	1214.88				
2	250	1159.72	3	55.16	3.9635	0.008725 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```
> fit1.lm<-lm(Totoffspring~Population)
> fit2.lm<-lm(Totoffspring~Population)
> anova(fit0.lm,fit2.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ Population

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	253	1214.88				
2	236	1109.14	17	105.75	1.3236	0.1785

```
> fit3.lm<-lm(Totoffspring~plant)
```

```
> anova(fit0.lm,fit3.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ plant

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	253	1214.88				
2	200	834.22	53	380.67	1.7219	0.004034 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```
> fit4.lm<-lm(Totoffspring~Leaf)
```

```
> anova(fit0.lm,fit4.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ Leaf

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	253	1214.88				
2	199	819.71	54	395.17	1.7766	0.002387 **

---

## Appendix

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```
> fit5.lm<-lm(Totoffspring~Petdiameter)
```

```
> anova(fit0.lm,fit5.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ Petdiameter

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	253	1214.88				
2	221	965.29	32	249.60	1.7858	0.008495 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```
> fit6.lm<-lm(Totoffspring~petLength)
```

```
> anova(fit0.lm,fit6.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ petLength

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	253	1214.88				
2	214	965.35	39	249.53	1.4184	0.06307 .

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

```
> fit7.lm<-lm(Totoffspring~Gallerysize)
```

```
> anova(fit0.lm,fit7.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ 1

Model 2: Totoffspring ~ Galleriesize

```

Res.Df  RSS  Df Sum of Sq  F Pr(>F)
1  253 1214.88
2  199  910.28  54  304.61 1.2332 0.1529
> fit8.lm<-lm(Totoffspring~Leaf+Location)
> anova(fit4.lm,fit8.lm,test="F")

```

Analysis of Variance Table

Model 1: Totoffspring ~ Leaf

Model 2: Totoffspring ~ Leaf + Location

```

Res.Df  RSS  Df Sum of Sq F Pr(>F)
1  199 819.71
2  199 819.71  0  0.00
> fit9.lm<-lm(Totoffspring~Leaf+Population)
> anova(fit4.lm,fit9.lm,test="F")

```

Analysis of Variance Table

Model 1: Totoffspring ~ Leaf

Model 2: Totoffspring ~ Leaf + Population

```

Res.Df  RSS  Df Sum of Sq F Pr(>F)
1  199 819.71
2  199 819.71  0  0.00
> fit10.lm<-lm(Totoffspring~Leaf+Plant)
Error in eval(expr, envir, enclos) : object "Plant" not found
> fit10.lm<-lm(Totoffspring~Leaf+plant)
> anova(fit4.lm,fit10.lm,test="F")

```

## Analysis of Variance Table

Model 1: Totoffspring ~ Leaf

Model 2: Totoffspring ~ Leaf + plant

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	199	819.71				
2	199	819.71	0	0.00		

```
> fit11.lm<-lm(Totoffspring~Leaf+petLength)
```

```
> anova(fit4.lm,fit11.lm,test="F")
```

## Analysis of Variance Table

Model 1: Totoffspring ~ Leaf

Model 2: Totoffspring ~ Leaf + petLength

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	199	819.71				
2	199	819.71	0	0.00		

```
> fit12.lm<-lm(Totoffspring~Leaf+Petdiameter)
```

```
> anova(fit4.lm,fit12.lm,test="F")
```

## Analysis of Variance Table

Model 1: Totoffspring ~ Leaf

Model 2: Totoffspring ~ Leaf + Petdiameter

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	199	819.71				
2	168	668.71	31	151.00	1.2237	0.2095

```
> fit13.lm<-lm(Totoffspring~Leaf+Gallerysize)
```

```
> anova(fit4.lm,fit13.lm,test="F")
```

## Analysis of Variance Table

Model 1: Totoffspring ~ Leaf

Model 2: Totoffspring ~ Leaf + Gallerysize

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	199	819.71				
2	147	535.63	52	284.08	1.4993	0.03146 *

---

Signif. codes: 0 '\*\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

&gt; fit14.lm&lt;-lm(Totoffspring~Leaf+Gallerysize+Location)

&gt; anova(fit13.lm,fit14.lm,test="F")

## Analysis of Variance Table

Model 1: Totoffspring ~ Leaf + Gallerysize

Model 2: Totoffspring ~ Leaf + Gallerysize + Location

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	147	535.63				
2	147	535.63	0	0.00		

&gt; fit15.lm&lt;-lm(Totoffspring~Leaf+Gallerysize+Population)

&gt; anova(fit13.lm,fit15.lm,test="F")

## Analysis of Variance Table

Model 1: Totoffspring ~ Leaf + Gallerysize

Model 2: Totoffspring ~ Leaf + Gallerysize + Population

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	147	535.63				
2	147	535.63	0	0.00		

## Appendix

```
> fit16.lm<-lm(Totoffspring~Leaf+Galleriesize+plant)
```

```
> anova(fit13.lm,fit16.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ Leaf + Galleriesize

Model 2: Totoffspring ~ Leaf + Galleriesize + plant

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
--	--------	-----	----	-----------	---	--------

1	147	535.63				
---	-----	--------	--	--	--	--

2	147	535.63	0		0.00	
---	-----	--------	---	--	------	--

```
> fit17.lm<-lm(Totoffspring~Leaf+Galleriesize+petLength)
```

```
> anova(fit13.lm,fit17.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ Leaf + Galleriesize

Model 2: Totoffspring ~ Leaf + Galleriesize + petLength

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
--	--------	-----	----	-----------	---	--------

1	147	535.63				
---	-----	--------	--	--	--	--

2	147	535.63	0		0.00	
---	-----	--------	---	--	------	--

```
> fit18.lm<-lm(Totoffspring~Leaf+Galleriesize+Petdiameter)
```

```
> anova(fit13.lm,fit18.lm,test="F")
```

Analysis of Variance Table

Model 1: Totoffspring ~ Leaf + Galleriesize

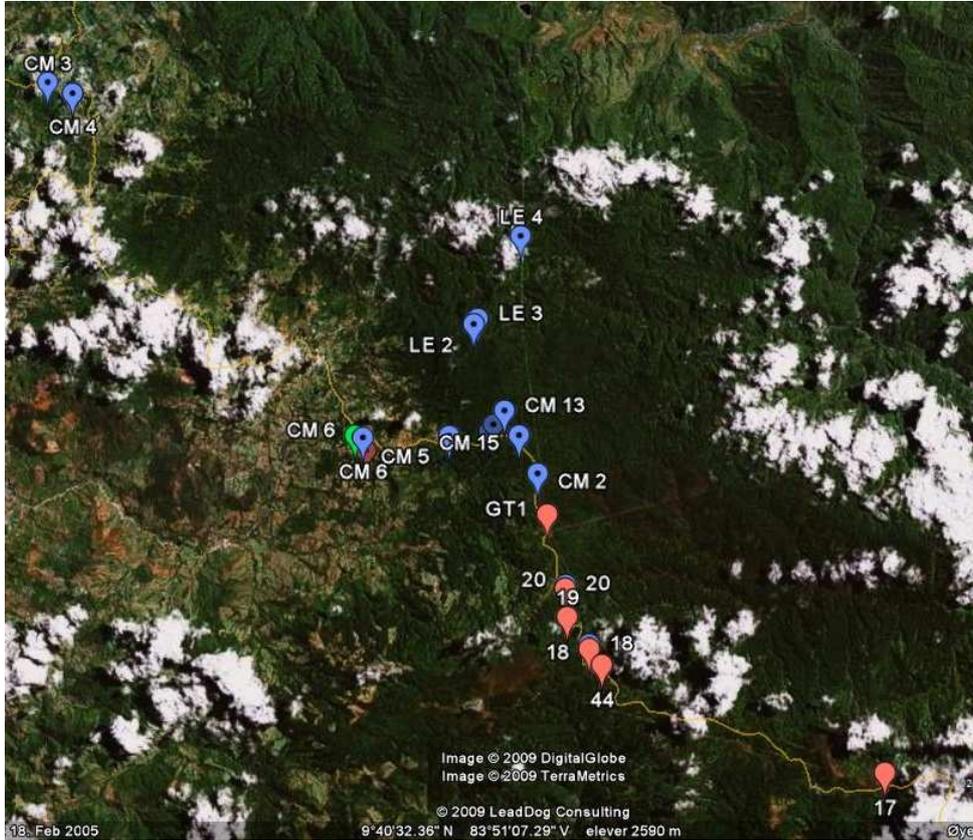
Model 2: Totoffspring ~ Leaf + Galleriesize + Petdiameter

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
--	--------	-----	----	-----------	---	--------

1	147	535.63				
---	-----	--------	--	--	--	--

2	117	418.87	30	116.77	1.0872	0.3642
---	-----	--------	----	--------	--------	--------

## Coordinates and map



Cerro de la Muerte (CM), and La Esperanza (LE). The numbers refers to recording on the GPS for the different populations that were not named.

CM population 1

Coordinates: 9° 40' 16.56, -83° 51' 40.02

*G. insignis* and *Scolytodes*

Altitude: 2650 m

CM population 2

Coordinates: 9° 39' 46.92, -83° 51' 1.44

*G. Insignis* and *Scolytodes*

Appendix

Altitude: 2682 m

CM population 3

Coordinates: 9° 44' 26.70, -83° 57' 39.96

*G. insignis* and *Scolytodes*

Altitude: 2035 m

CM population 4

Coordinates: 9° 44' 18.12, -83° 57' 18.42

*G. insignis* and a few *Scolytodes*

Altitude: 2164 m

CM population 5

Coordinates: 9° 40' 11.40, -83° 52' 10.32

*G. Insignis* and *Scolytodes*

Altitude: 2602 m

CM population 6

Coordinates: 9° 40' 11.52, -83° 53' 18.60

*G. insignis*, hybrid and *G. Talamancana* all with *Scolytodes*

Altitude: 2593 m

CM population 9

Coordinates: 9° 40' 21.90, -83° 51' 37.08

*G. insignis* and *Scolytodes*

Altitude: 2693 m

CM population 13

Coordinates: 9° 40' 32.82, -83° 51' 29.28

*G. insignis* and *Scolytodes*

Altitude: 2703 m

CM population 15

Coordinates: 9° 40' 14.88, -83° 51' 17.22

*G. insignis* and *Scolytodes*

Altitude: 2661 m

LE population 2

Coordinates: 9° 41' 36.12, -83° 51' 56.04

*G. Insignis* and *Scolytodes*

Altitude: 2831 m

LE population 3

Coordinates: 9° 41' 40.38, -83° 51' 53.28

*G. insignis* and *Scolytodes*

Altitude: 2809 m

LE population 4

Coordinates: 9° 42' 47.10, -83° 51' 24.84

*G. insignis* and *Scolytodes*

Altitude: 2266 m

Number 17

Coordinates: 9° 36' 20.88, -83° 46' 30.42

*G. talamancana*, no *Scolytodes*

Altitude: 3121 m

Number 18

Appendix

Coordinates: 9° 37' 42.42, -83° 50' 15.66

*G. insignis* and *G. talamancana*, no *Scolytodes*

Altitude: 2944 m

Number 19

Coordinates: 9° 38' 1.68, -83° 50' 33.18

*G. talamancana*, no *Scolytodes*

Number 20

*G. insignis* and *G. talamancana*, no *Scolytodes*

Coordinates: 9° 38' 24.90, -83° 50' 35.46

Altitude: 2816 m

GT1

Coordinates: 9° 39' 16.80, -83° 50' 52.44

*G. talamancana*, no *Scolytodes*

Altitude: 2725 m

Number 44

Coordinates: 9° 37' 28.20, -83° 50' 5.34

*G. talamancana*, no *Scolytodes*

Altitude: 2917 m