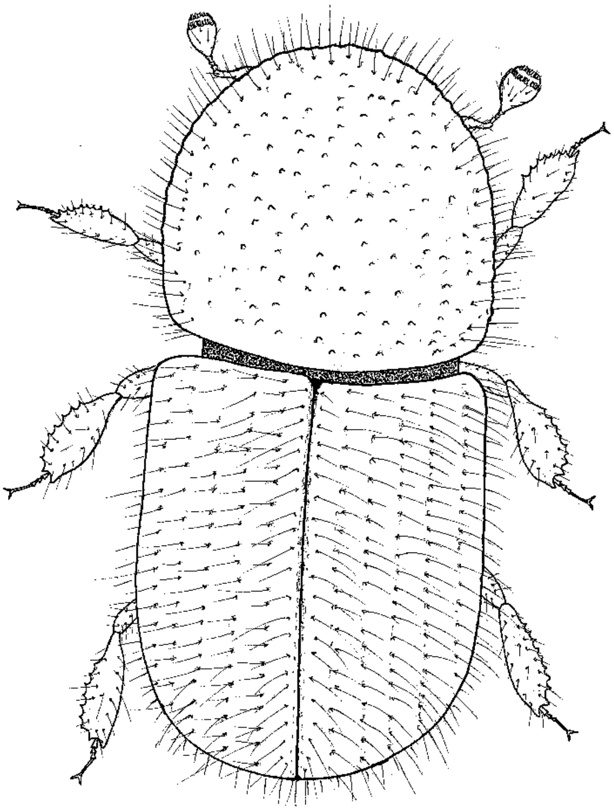
A peek into the private life of a seed-breeding bark beetle

Part 1: Sexual size dimorphism, mate choice and body size evolution in a seed-breeding bark beetle

Part 2: An overview of *Dactylotrypes longicollis* based on literature review and own laboratory observations

by

Anders Isaksen



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A peek into the private life of a seed-breeding bark beetle
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Front-page picture: Dorsal habitus of *Dactylotrypes longicollis*. Drawing by Anders Isaksen.

Preface & Acknowledgements

At first, I found it hard to choose a topic for my master thesis. I was left open with a wide range of possibilities with the necessarily cost of eliminating the interesting aspect of something else. It was by no means obvious that size dimorphisms and mate choice in a bark beetle species inhabiting the Canary Islands would be the topic for my thesis. At last, I decided to *choose* a topic that involved studying *choices*, as it turned out to be an inspiring self-fulfilling prophecy.

My approach when starting to work with the thesis was to gather as much information on the model organism as possible, which I soon found out to be close to nothing. I could almost count all papers written about it with my bare two hands. I found this to be both inspiring and challenging. Throughout approximately a year of rearing and regular observations, I had the impression that I possessed more information than previous work had conveyed. After wrapping up the experiments, I felt lucky to have had the chance to observe the beetles for such a long time, it had been clandestine and exclusive. As this feeling stuck with me, it also shaped my thesis. I decided to separate it into two parts; the first concerns the original topic of the thesis, which comprises field observations and a series of experiments related to body size, mate choice, and intrasexual competition. Part two is a mixture of a literature review integrated with own observations, where I have aimed at making a general, but comprehensive overview of the model organism.

Although working with bark beetles have been a pleasurable ride, it has also had its costs. One night I dreamt, almost as taken from a scene in a Luis Buñuel film, that the bark beetles bored into my right arm, and made it completely rot from the inside out. The brief second before I woke up, it was porous and disintegrated. Frass of rotten flesh and powdered bones fell to the ground. It was a relief to wake up only to realize that I had slept on my arm to complete numbness. However, that is the karma for working with boring beetles. As if the concern for them in real life is not enough, they even bore tunnels into your dreams while asleep.

I feel privileged to have had the opportunity to do fieldwork on the Canary Islands. I was clear from the beginning that I wanted to conduct fieldwork and generate my own data, which was made possible mainly due to the open-mindedness of my supervisor, Lawrence Kirkendall. The accomplishment of this thesis would not have been the same without the help from several people, which I am forever grateful. First, thanks to my supervisor Lawrence Kirkendall for excessive guidance throughout the year and motivating discussions, be it inspiring digressions or profound knowledge on bark beetles and biological theory. Thanks to both Lawrence Kirkendall and Torild Wardenær for traveling with me to La Gomera and helping me during the fieldwork. Thanks also to Lawrence Kirkendall for all the assistance with importing the seeds needed for the rearing of the beetles.

Thanks to Kenneth Meland and Jonathan Soulé for providing me with all the needed equipment for the field trip, as well as helping me finding the needed equipment in the lab. Thanks to Knut Helge Jensen for helping me with finding a proper climate chamber for rearing the bark beetles. I am also very grateful for statistical considerations. Thanks to Adéle Mennerat for giving a brief introduction on how to use the Leica Z16 APO A (Type DFC295) trinocular stereomicroscope, as well as showing me how the software LAS (Leica Application Suite) Version 3.6.0 for measuring the body sizes of the beetles worked out.

Thanks to Carol Bedoya for insightful information on stridulations in bark beetles. I am also grateful for the help with the easy tests during the pilot studies on whether *Dactylotrypes longicollis* stridulate or not, making me discard my ambitious plan on sound recordings of the beetles. Thanks to Christopher Foelker for mail correspondence and helpful insight in how to conduct the statistics in estimating the narrow-sense heritability.

I would also expel a big thank you to the University Library for Mathematics and Natural Sciences in Bergen for all their help with tracing and obtaining some of the more obscure papers. The completion of the review would not have been possible without their effort.

Last but not least, thanks to friends and family. Thanks to Hege, Sunniva and Sigrid at BIO. It has been a pleasure to share reading room with you. Thanks to Gjert, Katrine and Shiny Shainett Nødtmansen for housing me throughout the last month of this thesis. Finally, a special thanks to my mom, dad and brothers for proofreading and giving me insightful feedback. I would also thank them for inspiring me by expelling commitment to my thesis throughout the year.

Your contributions are all invaluable. Thank you so much!
Anders Isaksen
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Part 1:

Sexual size dimorphism, mate choice and body size evolution in a seed-breeding bark beetle

Two males competing at the tunnel entrance. The male to the right has a greater proportion of his body inside the tunnel. The other male is rubbing his frons towards the other male’s elytra. Photo: Anders Isaksen.

ABSTRACT

Mate choice and intrasexual selection is a key component of sexual selection and may be important for the evolution of dimorphic traits. Although, sexual selection theory predicts that females should be choosy and males competitive, exceptions to this pattern occur. Males can also be choosy, especially in monogamous species with biparental care. I conducted a series of experiments in a monogamous bark beetle species related to body size. I tested for sexual size dimorphisms between the sexes, and further tested patterns of assortative mating, both among pairs in natural populations and in mate choice experiments. Two different mate choice experiments were conducted in order to elucidate the importance of body size in males and females for pair formation. As a proxy for the ability to respond to selection, I estimated the narrow-sense heritability of body size. Results showed that females were on average slightly larger than males. There was a lack of assortative mating, both among pairs in natural populations and pairs formed during mate choice experiments. Body size was not associated with greater chance for pair formation, neither for females nor males. The mating outcome was random in relation to body size, meaning that both males and females paired up with the first encounter. The estimated narrow-sense heritability was non-significant, suggesting that environmental factors masks the effects of genes. Intraclass correlation between siblings were low, indicating high variance in body size among siblings within single broods, and thereby also low resemblance between siblings and offspring. The findings suggests that the underpinning mechanisms of sexual selection do not currently operate on body size in *Dactylotrypes longicollis*.

INTRODUCTION

Sexual size dimorphism

Sexual size dimorphisms; differences in body size between sexes within same species is common throughout the animal kingdom (Andersson, 1994; Fairbairn, 2013). Except from mammals and birds, females tend to be larger than males in all animal taxa (Shine, 1988; Fairbairn, 2013), which is also the general rule in insects (Darwin, 1871; Richards, 1927; Honěk, 1993; Stillwell *et.al.*, 2010). In species where males are the larger sex, sexual selection through female choice and male-male competition have been ascribed as the underlying evolutionary mechanism (Darwin, 1871). On the other hand, larger size in females are usually not attributed to sexual selection, but is rather thought to be invoked by fecundity selection (Darwin, 1871; Williams, 1966; Honěk, 1993; Andersson, 1994; Beukeboom, 2018). However, the fecundity selective approach has received critique, and probably cannot alone explain all of the observed female biased sexual size dimorphisms across all taxa (Shine, 1988, 1989; Pincheira-Donoso & Hunt 2017).

According to the conventional sexual selection theory, female should be the choosy sex, and males should compete with other males for access to females (Darwin, 1871; Bateman, 1948; Williams, 1975). More recent research shows that larger body size in females also can be selected through male choice (Amundsen, 2000; LeBas, 2006; Clutton-Brock, 2009), it may be more common than previously thought (Amundsen, 2000; Bonduriansky, 2001; Edward & Chapman, 2011; Fitzpatrick & Servedio, 2018). So called sex-role reversals, where males chooses mates are now a well-known phenomenon (Andersson, 1994; Andersson & Iwasa, 1996; Rosenthal, 2017; Zuk & Simmons, 2018), and have been reported in pipefish (Berglund & Rosenqvist, 2001), arachnids (Aisenberg *et.al.*, 2010), and insects (Jiggins *et.al*, 2000; Krupke *et.al*, 2008; Hopkins *et al.*, 2015), to mention some.

Although larger males traditionally have been thought to be important for winning male-male competitions (Darwin, 1871), there are little evidence that male size confers any advantage for fecundity in insects (Partridge, 1983). Evidence for disadvantages of large body size in general are scarce, not necessarily because they are rare, but may be due to research bias (Blanckenhorn, 2005). Advantages of small body size in males have received little attention (Blanckenhorn, 2000), although selective mechanisms for smaller males potentially could create the common pattern of female biased sexual size dimorphism (Pincheira-Donoso & Hunt, 2017). Smaller size in males may be beneficial as a result of increased agility and maneuverability during mate location or copulation (Steele & Partridge, 1988; Blanckenhorn, 2000).

The evolution of sexual size dimorphisms within a species may be a result of interacting forces of different types of selection mechanisms. Disentangling the different effects of natural, sexual and fecundity selection on body size evolution is beyond the scope of this thesis. I have conducted a series of experiments (see below), looking at the effect of body size in males and females in relation to mate choice and intrasexual selection.

Mate choice is an essential proximate mechanism of sexual selection, and can be defined as "any pattern of behavior, shown by members of one sex, that leads to their being more likely to mate with certain members of the opposite sex than with others." (Halliday, 1983, p. 4). Discrimination towards potential mates can be based on selective responses to both behavioral and morphological traits, and will typically result in some individuals being rejected while others are accepted (Andersson & Iwasa, 1996). One of the main prerequisites for mate choice to evolve is that there is a low costs related to mate search and mate assessment (Bonduriansky, 2001).

Although sexual size dimorphisms usually are more conspicuous among polygynous species (Arak, 1988), it is well documented that mate choice also occur in monogamous species (Mock, 1985; Kirkpatrick *et al.*, 1990; Andersson, 1994; Kvarnemo *et al.*, 2007; Kvarnemo, 2018). Even in strict monogamous species where no individuals will have more than one mate, mate choice can be generated if the operational sex ratio is biased (Emlen & Oring, 1977), if the sexes display different roles during the provision for the young (Clutton-Brock, 1991), or if there simply is a variation in quality among potential partners (Halliday, 1983; Parker, 1983; Owens & Thompson, 1994; Andersson & Iwasa, 1996).

The operational sex ratio (OSR), defined as the proportion of mature males to receptive females, is closely associated with mating systems, and can often influence the strength of intrasexual competition (Emlen & Oring, 1977; Kvarnemo & Ahnesjö, 1996). Although the adult sex ratio may be even, there may be differences in the 'time out' period where females (or males) are non-receptive for further copulation, by for example devoting time and energy to egg production, oviposition and parental care. If one of the sexes are non-receptive for a significant period, this will alter the potential reproductive rate (PRR), which may generate temporarily biased sex ratio, even in monogamous mating systems (Kvarnemo & Ahnesjö, 1996). In monogamous mating systems with initial even sex ratios, female receptivity for new fertilizations may therefore be a limiting resource for male reproduction, and competition among males can be prominent (Kvarnemo & Ahnesjö, 1996).

In most species, females tend to care more for the offspring than males, thereby limiting their possibilities of mating with subsequent males (Bateman, 1948; Trivers, 1972; Clutton-Brock, 1991). If both sexes invest equally in offspring, given an unbiased sex ratio, both parents would sacrifice the same potential of future mating opportunities for the prolonged partnership (Kvarnemo, 2006). Therefore, in species with biparental care, both sexes should be interested in discriminating among potential partners (Trivers, 1972; Clutton-Brock, 2007). Consequently, mutual mate choice may operate in monogamous species with biparental care (Trivers, 1972; Thornhill & Alcock, 1983; Owens & Thompson, 1994; Johnstone *et al.*, 1996; Amundsen, 2000; Kokko & Johnstone, 2002; Rosenthal, 2017).

Mutual mate choice has been reported in most animal taxa (Johnstone *et al.*, 1996; Kokko & Johnstone, 2002; Rosenthal, 2017), and may shape patterns of positive assortative mating, a mating pattern where individuals of similar phenotypes mate more frequently than under a random mating pattern (Crespi, 1989). Assortative mating by body size is a common mating pattern in natural populations, and can be an indicator of mate choice and intrasexual competition (Crespi, 1989).

Sexual size dimorphisms in bark beetles

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a diverse subfamily of weevils that breed and feed in woody tissue, most commonly in the dead inner bark or wood (Kirkendall *et al.*, 1997). Approximately 6000 species are described, but many probably still remain unknown to science and await description (Wood, 1982). Bark beetles show a remarkable diversity in mating systems, mating strategies, life-histories, feeding strategies, and host preferences (see Kirkendall, 1983; Wood, 1982; 1986). While female-initiated outbreeding monogyny is the ancestral mating system among bark beetles (Kirkendall *et al.*, 2015), bigyny, harem polygyny, colonial polygyny and inbreeding are well represented (Kirkendall, 1983). Given the striking variety of mating systems, which have arisen independently several times among phylogenetic bark beetle lineages, they represents an epitome for studies on sexual size dimorphisms and mate choice (Kirkendall, 1983).

Size differences between the sexes are common in bark beetles and where they exist, the pioneering sex is usually the larger of the two (Kirkendall *et al.*, 2015; see also Foelker & Hofstetter, 2014). Females tend to be larger in monogynous species, while males are usually larger in harem polygynous species (Kirkendall *et al.*, 2015). Size dimorphism tends to be greater in species where inbreeding polygyny occurs. For example, in *Araptus* and *Coccotrypes*, males are both flightless and dwarfed and are substantially smaller than females (Beeson, 1941; Wood, 1982). In monogamous species, size dimorphisms can be less conspicuous, or even totally absent (Balachowsky, 1949). Size monomorphism has for example been reported in the monogamous bark beetle *Dendroctonus rufipennis* (Safranyik, 2011). In summary, bark beetles differ strongly in appearance and strength of their dimorphic traits, but the selective processes shaping such size differences remain poorly known.

Similar to most animal taxa, fecundity selection has been suggested as an explanation for the female biased sexual size dimorphism that is apparent in most monogynous bark beetle species (Kirkendall *et al.*, 2015; Raffa *et al.*, 2015). However, male mate choice has been reported in monogynous bark beetle species such as *Dendroctonus ponderosae*, *D. valens* and *Phloesinus armatus* (Reid & Baruch, 2010; Chen *et al.*, 2012; Liu *et al.*, 2017). Although rare in insects (Zeh & Smith, 1985), biparental care is common in monogamous bark beetle species (Kirkendall, 1983), suggesting that male mate choice may be more common than previously thought.

For mate choice to evolve, there must be a significant variation in quality of potential mates (Parker, 1983). Body size is a key trait for fitness in insects, which is positively correlated with fecundity in females (Honěk, 1993). In bark beetles, larger size in females are associated with increased egg production (Amman, 1972), increased numbers of offspring (Anderbrant *et al.*, 1985; Reid & Roitberg, 1995), greater flight capacity and dispersal (Evenden *et al.*, 2014), and greater pheromone production (Schlyter & Birgersson, 1989). On the other hand, the advantages of larger size in male bark beetles remains vague or even absent (Lissemore, 1997; Robertson & Roitberg, 1998; Pureswaran & Borden, 2003).

Few studies on bark beetles have tested the advantage of smaller males in monogynous species, but there are some findings pointing towards increased maneuverability for smaller males within tunnels (Liu *et al.*, 2017). If tunnel diameters, which is often determined by the size of the pioneering sex, may have implications for which individuals who will fit into excavated tunnels (Pureswaran & Borden, 2003; Foelker & Hofstetter, 2014; Liu *et al.*, 2017), this may be a potential selective agent favoring smaller males. Selection for smaller males could be a proximate mechanism resulting in the common female biased sexual size dimorphism in monogynous bark beetles.

The mating strategies of males and females in the bark beetle *Dactylotrypes longicollis* are poorly known, and no studies have been conducted on mate choice and intrasexual competition with regards to body size (but see Eggers, 1927; Uyttenboogaart, 1927; Enderlein, 1929; Sampò & Olmi, 1975a, 1975b; Bernabò, 1991; Longo *et al.*, 1991 for general treatises). The phenotypic variation in body size within each sex has never been empirically investigated in *Dactylotrypes longicollis*, although claims from previous theses report that females are the larger sex (Halvorsen, 2006; Hestvik, 2002). I tested whether females were larger than males, and also whether females exhibited greater variation in body size. I questioned whether an eventual sexual size dimorphism in *Dactylotrypes* could be explained by male mate choice for larger females and eventual selective mechanisms of males being small by increased agility during mate location and copulation.

Being a monogamous species with biparental care of the young, male mate choice and male-male competition may be present in *Dactylotrypes longicollis* (Trivers, 1972; Reid & Baruch, 2010; Rosenthal, 2017), which can generate patterns of assortative mating (Crespi, 1989). It is hard to disentangle mate choice from intrasexual competition. However, in order to understand the selective potential through mate choice experiments, one does not necessarily need to disentangle selectivity and male-male competition, but simply observe the realized outcome of mating events (Arnold, 1983; Rosenthal, 2017).

Individual variation in body size is a result of both genes and environment, and the interaction between the two (Roff, 1997). While both biotic and abiotic effects on body size in bark beetles is well studied, the genetic variation underlying body size variation remain poorly understood (see Anderbrant & Schlyter, 1989). I tested the narrow-sense heritability of body size as a proxy for the selective potential of body size. If body size is selected through mate choice and intrasexual competition, the trait must still be heritable in order for the trait to be passed on from parent to offspring (Hedrick & Temeles, 1989).

I tested five main hypotheses related to variation in body size in *Dactylotrypes longicollis*:

1. That average body size between the sexes is different, and that sexes exhibit difference variance in body size.
2. That *Dactylotrypes longicollis* exhibit a positive assortative mating pattern, shaped through mate choice and mate constraints of tunnel diameters.
3. That males and females discriminate among potential partners related to body size.
4. That body size in males is important for male-male competition and mate location.
5. That body size a heritable trait.

The hypotheses were tested with data obtained from pairs in natural populations and by a series of controlled experiments of reared beetles in the laboratory. The observations and experiments were broadly categorized into the three following general headings:

1) *Sexual Size Dimorphism and Assortative Mating in Natural Populations*
2) *Mate Choice Experiments*
3) *Narrow-sense heritability*

METHODS

Model organism

Dactylotrypes longicollis (Wollaston, 1864) is a bark beetle native to Madeira and the Canary Islands (Eggers, 1927; Uyttenboogaart, 1927; Enderlein, 1929; Liebmann, 1939; Lundblad, 1958; LaBonte & Takahashi, 2012). The beetles breed and feed in seeds from a variety of hosts, although seeds from the Canary date palm (*Phoenix canariensis*) and the Dragon tree (*Dracaena draco*) are considered to be the original ones (Enderlein, 1929; Schedl *et al.*, 1959). Based on the criteria in Kirkendall (1983), it can be considered a monogamous outbreeding species. A complete reproductive cycle takes place within one single seed. Females bore tunnels and remain within seeds, while mobile males are searching for single receptive females. Males usually encounter females sequentially. Upon encounter, the males rubs his frons against the posterior part of the female's elytra. The female backs out of the excavated tunnel, and copulation takes place. After copulation, males and females forms a prolonged relationship, where the male contributes significantly to rearing of the offspring by clearing of frass from the tunnels. Males usually blocks the tunnel during the whole oviposition period. Presumably, both males and females mate only once during their lifetime (see part 2 of this thesis).

Sampling

La Gomera comprises the largest native populations of the Canary Island date palm, *Phoenix canariensis* (Barrow, 1998; Morici, 1998; Obón *et al.*, 2018), the native host of *Dactylotrypes longicollis*, and was selected as an ideal site for sampling. Colonized fruits and seeds of the Canary Island date palm were collected from several locations on La Gomera and Tenerife between 21 July and 1 August 2018 (Appendix 1). Seeds were picked haphazardly from the ground beneath clustered and solitary palm trees. Old, porous seeds were not collected, since these assumingly neither contained living individuals nor were suited for new colonization in the lab. Geographic coordinates were recorded for each sampling site (Appendix 1). The collected seeds from each sampling site were put in sealed plastic bags and stored in a refrigerator during the fieldwork period. Cooler temperatures served to reduce the beetle’s activity, to halt the development (Lissemore, 1997), and to avoid excessive growth of mold. Lawrence Kirkendall carried out additional sampling in Montpellier on August 18, 2018.

To ensure that *Dactylotrypes longicollis* was present in the samples, a couple of colonized seeds were dissected for each sample site at the same day of sampling. *Dactylotrypes longicollis* is relatively similar to *Coccotrypes dactyliperda*, another scolytine beetle which is present on the Canary Islands and also inhabits seeds of *Phoenix canariensis* (Schedl *et al.*, 1959). *Dactylotrypes* can be distinguished from *Coccotrypes* by their steep declivity of the posterior part of the elytra (Eggers, 1927; Figure 1 A & B), and their different numbers of socketed teeth on their tibiae. *Dactylotrypes* have 8 or 9 teeth while *Coccotrypes* only have 4 or 5 (Balachowsky, 1949; Palacios, 1973; Longo *et al.*, 1991; Figure 1 C & D).

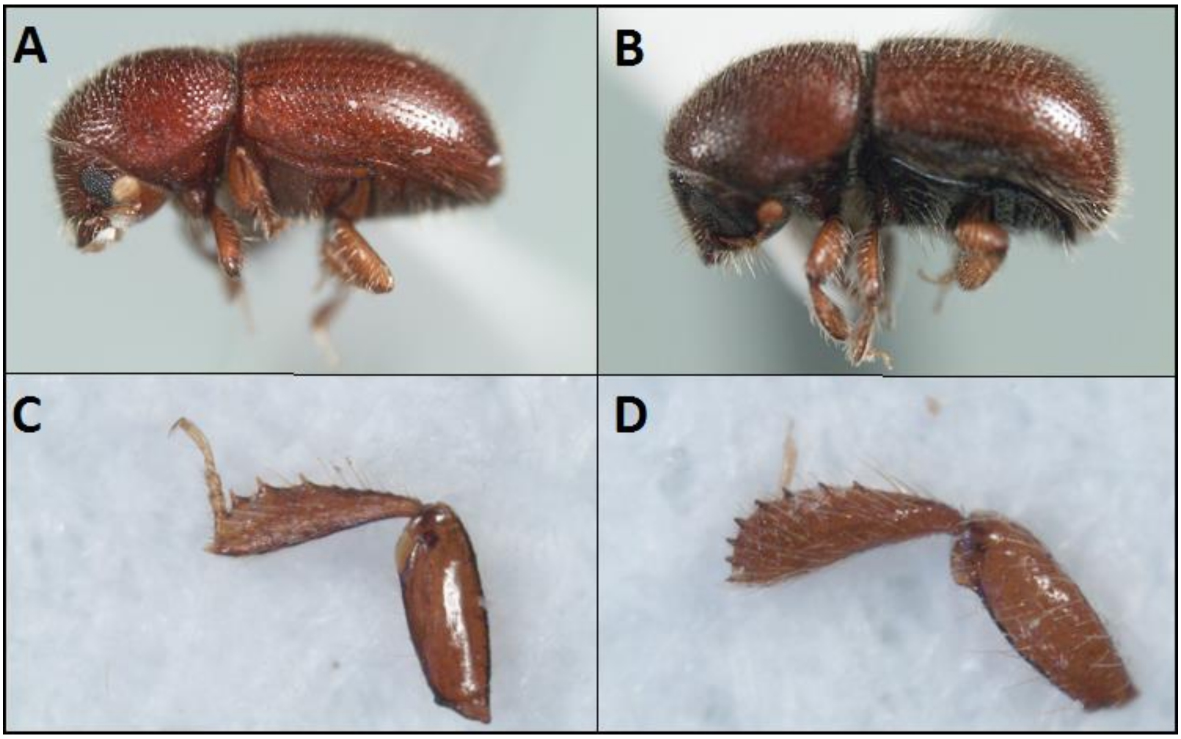

Figure 1: A) Differences in the elytral declivity of *Coccotrypes dactyliperda* (female) and B) *Dactylotrypes longicollis* (female). *Dactylotrypes* has a rounder appearance than *Coccotrypes*. Notice also the difference in the number of socketed teeth on the tibiae; C) *Coccotrypes* with 4 or 5, D) *Dactylotrypes* with 8 or 9. Photos: Anders Isaksen.

Measuring procedures

Body mass or body length is commonly measured for examination of sexual size dimorphisms. In insects, body mass may vary with time in single individuals due to differences in the effects of food availability, larval density, sperm storage and reproductive condition (Honěk, 1993; Edvardsson & Tregenza, 2005; Fairbairn, 2007). Body length in insects is fixed, as their exoskeleton does not grow after reached maturity and final molt (Nijhout, 2003; Fairbairn, 2007). Hence, length and width measurements, rather than body mass, were used as metric for body size in all experiments. Unless otherwise are stated, total length is used as equivalent to body size throughout the text.

Where size was of no importance prior to the experimental design, beetles were measured when they were dead. All dead individuals were pinned, as this was the most adequate way of positioning the beetles horizontally without lateral tilting. The beetles were mounted on points and placed under a trinocular stereomicroscope (Leica Z16 APO A, Type DFC295). Pictures were generated using the software Leica Application Suite (LAS), version 3.6.0, and measurements were made digitally using a two-point line measure. Elytra length was measured from the anterior margin of the scutellum to the posterior margin of the elytra along the medial plane. Similarly, pronotum length was measured from the posterior margin of the pronotum to the anterior margin along the medial plane. Total length was not measured directly due to differences in the gap between the elytra and the pronotum. I added the measurements of elytra length and pronotum length to generate total length. The width of the pronotum was measured at the widest part for each individual. Several two-point measurements were taken for each individual to ensure that the widest part was measured.

Sexual Size Dimorphism and Assortative Mating in Natural Populations

Sexual size dimorphism and assortative mating was tested from pairs in natural populations. A sub-sample of seeds was opened in the field. Seeds were cut in half with pruning shears, and further dissected with scalpel. Beetles within each seed were removed using scalpel and a brush, and put in individual plastic vials (1.5 mL) with 96% alcohol. In most seeds, single individual females resided. Occasionally, pairs consisting of one female and one male was found. Females and males inhabiting the same tunnel were defined as an established pair. At the University in Bergen, beetles from all pairs were pinned and measured as described above. Each individual was sexed under a microscope (Leica, series S-50) by their dimorphic frons. The elytra length, pronotum length and pronotum width were measured for each individual to the nearest 0.01 mm. In total, 67 pairs were measured and used for the analysis.

Due to differences in variance between males and females, I performed a Welch's two-sample *t*-test to examine the sexual size dimorphism between males and females independent of the pairs. The t-test was conducted on all measured size metrics, elytra length, pronotum length, pronotum width and total length. An F-test were performed to assess whether the variance in total length and pronotum width differed between the sexes. As pairs were obtained from three different sampling sites, I used a one-way ANOVA to test if the mean total length for each sex differed between sampling sites. As an estimate of difference in shape between the sexes, I examined the relationship between total length and pronotum width separately for both sexes with a Pearson's correlation test. I compared the magnitude of the correlation between males and females using the cocor-package in R (Diedenhofen & Musch, 2015). Pearson's correlation test was also used to test for size assortative mating between males and females for all size metrics. To examine whether the total length of females was greater than that of males within pairs I used a Wilcoxon signed-ranks test (Baruch *et al.*, 2017). For reasons discussed by Smith

(1999) and Fairbairn (2007), I calculated the size dimorphism index (SDI) as developed by Lovich & Gibbons (1992), by using the adjusted formulas by Blanckenhorn *et al*. (2007):

When males are the largest sex:

$$SDI = -\left(\left(\frac{male\ size}{female\ size}\right) - 1\right)$$

When females are the largest sex:

$$SDI = \left(\frac{female\ size}{male\ size}\right) - 1$$

SDI-values were calculated based on mean vales of total length of males and females irrespective of pairs, and between individuals within every pair. A positive SDI-value implies that females are the largest sex, while a negative value implies that males are the largest sex (Gibbons & Lovich, 1990; Lovich & Gibbons, 1992). In addition, the index produces symmetry around a central value of zero (Lovich & Gibbons, 1992).

Rearing

For the subsequent experiments, beetles were reared at the University in Bergen. For each sampled site a range of 30 – 150 seeds were put in plastic containers (length 18.5 cm, width 14.5 cm, depth 9 cm) with a cutout filter paper in the bottom. The populations from the different sample sites were kept in separate plastic boxes. To ensure that air could circulate, perforated aluminum was placed on top of each plastic box and tightened with rubber bands. The boxes were put in an incubator (Sanyo MIR-553) with temperatures of 25 ± 1^{0}C and relative humidity (RH) of 75 ± 10%. These regimes served to mimic the natural climatic conditions on La Gomera (see Fernandopullé, 1976), and has previously been successfully for rearing *Dactylotrypes longicollis* (Jacobsen, 2001; Hestvik, 2002; Hovda, 2005; Halvorsen, 2006). Concordant with Greenspan (1977), the RH was obtained by filling a container with a saturated sodium chloride-solution in the bottom of the climate chamber. An indoor thermometer and hygrometer (Co/Tech, model E0119TH) was placed inside the climate chamber. The temperature and humidity were checked regularly, at least once a week during the entire rearing period. Water was added occasionally to the bottom container in order to maintain a RH of approximately 75% within the incubator.

Over time, frass accumulated, and was removed from each box by replacing the filter paper in the bottom. In the beginning, mold was a slight problem in some of the boxes, but this problem was resolved by removing the pulp from all seeds by hand. As new brood started to emerge within the boxes, the perforated aluminum were not sufficient to avoid beetles from crawling out from the boxes. Hence, pin-holed plastic caps were replacing the aluminum for all the boxes similarly in the rearing process. Uncolonized seeds were added as new beetles emerged and old porous seeds were removed. Seeds of *Phoenix canariensis* used for rearing were sampled from Los Cristianos in Tenerife during the fieldwork. The pulp was removed from all of these seeds by hand, and stored in the refrigerator until added to the containers. Additional seeds of *Phoenix canariensis*, *P. sylvestris* and *P. roebelenii* were bought from external suppliers (Appendix 2). As the quality of the latter two species' seeds seemed to vary as judged by female willingness to bore tunnels in them, only seeds of *Phoenix canariensis* were used for the experiments.

Mate choice experiments

Two independent mate choice experiments were performed: (1) three females together with one male, and (2) one female with three males. To ensure that females and males were virgins, they were obtained from emerging offspring from single pairs of isolated seeds. The first step prior to both experiments was to establish individual females within separate single seeds. A female was put together with one seed in a plastic beaker with lid. As excavation of a tunnel is a prerequisite for copulation to take place, females were allowed up to 20 hours to bore an entrance tunnel. Those that had not bored a tunnel within 20 hours were excluded from further trials. For every trial in both of the mate choice experiments, the females and the male were reared non-siblings taken from the same population.

Mate choice experiment 1: Three females, one male

For each trial, three seeds with single established females were chosen randomly and put in a petri dish. The three seeds were put equidistantly apart along the edge. A single male was picked randomly and added to the center point of the dish. For each trial, I observed from the time of addition of each male until first encounter with a female was made. The time until first encounter was recorded. As *Dactylotrypes* often play dead (thanatopsis) (pers. obs.), the timing started when the male began to walk. After first encounter was made, continuous observations lasted for 10 more minutes, until each trial was terminated. Pair formation was defined as males that entered the tunnel and remained for 10 minutes, or by copulation that took place within 10 minutes, whereby the male followed the female inside the tunnel. The number of trials where males did not enter the tunnel within 10 minutes after an encounter were recorded. After 10 minutes, each seed was dissected with pruning shear, scalpel and brush. All individuals were put in separate vials with 96% alcohol in separate vials. Each individual was pinned. Total length, elytra length, pronotum length and pronotum width were measured for all beetles to the nearest 0.01 mm. In addition, the tunnel diameter excavated by every female was measured to the nearest 0.01 mm. Forty trials were performed.

To determine whether *Dactylotrypes* exhibited a random or comparative mating strategy (Janetos, 1980), I used a binomial test with '*males enter tunnel*' and '*males do not enter tunnel*' as categorical responses. Assortative mating was tested for total length in a binomial test against a probability of 0.5 were '*closest size*' and '*not-closest size*' were used as response variables. To test whether males paired up with the largest female and/or the widest tunnel diameter, I conducted a chi-square goodness of fit-test testing for both total length and pronotum width in females, and tunnel diameter. The females and tunnel diameters were categorized relative to size for each trial into small, intermediate and large, and tested against the null hypothesis of a 1:1:1 distribution according to pair formation. I tested the effect of tunnel diameter as a response variable of female pronotum width with a linear model. To test whether smaller males were better at mate locating, I compared the total length of males on time until encounter with a female by conducting a linear regression.

Mate choice experiment 2: One female, three males

Three virgin males of different sizes (large, intermediate, and small) were put in a petri dish with a randomly assigned seed-established virgin female. Categorizations of male size were based on the upper and lower quartiles from the data obtained in the assortative mating study (small males: ≤ 1.88 mm; medium males: 1.89-1.97 mm; large males: ≥ 1.98 mm; see Table 1 in result section). To measure male beetles alive, they had to remain still during the process of picture generation. The beetles were occasionally anaesthetized on ice (Harari *et al.*, 1999), or by blowing gently on them causing them to play dead. After measurement and assignment of males into different size classes, each male were marked with different color dots of enamel paint on the posterior part of the elytra (for same marking in beetles, see Harari *et al.*, 1999). Enamel paint has been shown to have retention time suited for the time lapse of the experiment, as well as having little or no confounding effect in other insects (Hagler & Jackson, 2001; Butler *et al.*, 2012). After applying the mark on each male, the paint were allowed to dry for 5-10 minutes until each trial started. Each male was checked that it could walk properly, unaffected by the paint, before each trial started.

For every trial, I observed continuously until the first encounter. I recorded the color of the first arriving male and started a countdown of 10 minutes. Eventual replacements of males were recorded. A chi-square goodness of fit-test with expected proportions of 1/3 was used to test whether larger, intermediate or smaller males tended to encounter the female first. After the first 10 minutes, I stopped the continuous observations. The set-up was left until the next day (approximately 15-20 hours) until the trial was ended. At the end, I recorded the color of the male in pair, and saw whether this corresponded to a new replacement. Similarly, a chi-square goodness of fit-test with expected proportions of 1/3 was used to test whether larger, intermediate or smaller males ended up in pairs more frequently. I compared the proportion of males not in tunnel after 10 minutes from the first mate choice experiment with the proportion of dislodged males after 10 minutes. I compared the two ratios against the null hypothesis of equal proportions. Finally, the fate of the two male individuals not with the female at the end were recorded. After each trial was ended, all seeds were dissected with pruning shear, scalpel and brush, and the individual beetles were put in separate vials with 96% alcohol. In total, 100 trials were performed. Seven trials were excluded from the analysis, as the female tunnel was too small to fit an additional male. In one of these trials, the tunnel was long enough to fit several individuals, but the female did not end up in a pair with any of the three males.

Narrow-sense heritability

To test for narrow-sense heritability, I used a two-factorial design with maternal and paternal total length as the categorical effects (Foelker & Hofstetter, 2014). I divided females and males into two size classes (small and large), giving four different parental size treatments (Large female + Large male, Large female + Small male, Small female + Large male, Small female + Small male). As above, I defined the size categories based on the lower and upper quartiles of male and female size obtained from the assortative mating study (large females $\geq$ 2.12 mm; small females $\leq$ 1.95 mm; large males $\geq$ 1.98 mm; small males $\leq$ 1.88 mm; see Table 1 in result section). The first step was to establish reared virgin females within separate seeds. Each female was measured alive (as described in mate choice experiment 2) and given a seed of known weight. They were given a maximum of 20 hours to excavate a tunnel. Females that had not initiated tunnels within 20 hours were excluded from the experiment. Similarly, the total lengths of reared virgin males were measured. For every female established within a seed, a single male was added. Each seed consisting of one female and one male was put in separate plastic vials with a lid. After 50-60 days, beetles started to emerge from the seeds. The emerging beetles were sexed continuously and were put in separate vials with 96% alcohol for each breeding pair. Eighty days after the male was put together with the female, each trial was terminated. The seeds were dissected and the remaining non-emerged offspring were removed and added to each respective vial. In total, 26 pairs were successfully bred, resulting in 958 individuals. All individuals were pinned and size measurements were recorded.

Narrow-sense heritability of total length was tested as a linear parent-offspring regression, but had to be done separately on fathers and mothers on sons and daughters due to differences in variability between the sexes (Falconer & Mackay, 1996). To assess the father-daughter and mother-son regressions, I adjusted for the difference in variation between sexes using the equation $b' = b*(\sigma_{♀}/\sigma_{♂})$ in the regression of daughters on fathers, and $b' = b*(\sigma_{♂}/\sigma_{♀})$ in the regression of sons on mothers, where b is the slope of the linear regression, and $\sigma_{♀}$ and $\sigma_{♂}$ is the standard deviations of total length in females an males, respectively (Falconer & Mackay, 1996). The coefficient of heritability (h^2) was obtained by doubling the values of the slope, since $b = ½h^2$ for the regression of offspring on one parent (Falconer & Mackay, 1996). I weighted the regression by brood size by taking mean values of full-sibs of each sex within each brood (Roff, 1997). To examine the resemblance between siblings within and across broods, I estimated the intraclass correlation coefficient (ICC) for each sex separately, using the ICC-package in R (Wolak *et al.*, 2012).

A two-tailed binomial test was performed to examine whether the sex ratio of the offspring differed from the null hypotheses of an even sex ratio, with the expected probability of 0.5 (Wilson & Hardy, 2002). I calculated the SDI-values for the mean values of males and females, and tested for sexual size dimorphism with a Welch's two-sample *t*-test. Due to the large sample size, I also examined the size distribution for each sex separately, disregarding broods.

All aforementioned experiments were performed in room conditions (temperature of 22 ± 2^0C and relative humidity of 40 ± 20%). A significance level of 0.05 were set prior to statistical analyses for all tests. Statistics have been conducted using RStudio, version 1.1.419 (RStudio Team, 2016). Videos of general observations and mate choice experiments were recorded (see Appendix 3 for download/streaming links).

RESULTS

Sexual Size Dimorphism and Assortative Mating in Natural Populations

Sexual size dimorphism

Disregarding pairs, females were larger than males for all size metrics from the individuals sampled from natural populations; total length ($p < 0.001$), elytra length ($p < 0.001$), pronotum length ($p < 0.001$), and pronotum width ($p < 0.001$) (Table 1). Females had greater variance than males for total length (F-test, $df_m = 66$, $df_f = 64$, $p = 0.001$), but not for pronotum width (F-test, $df_m = 66$, $df_f = 66$, $p = 0.48$). There was no difference in total length (males: $F = 0.88$, df = 2, $p = 0.42$; females: $F = 1.05$, df = 2, $p = 0.36$) and pronotum width (males: $F = 0.15$, df = 2, $p = 0.86$; females: $F = 0.68$, df = 2, $p = 0.51$) between the three sample sites.

Table 1. Size metrics obtained from males and females in pairs ($n = 67$) from natural populations.

Size metrics	**Total length**	**Elytra length**	**Pronotum length**	**Pronotum width**
Male smallest quartile (mm)	1.61-1.88	0.96-1.07	0.65-0.80	0.82-0.88
Female smallest quartile (mm)	1.81-1.95	1.00-1.13	0.70-0.83	0.83-0.92
Male largest quartile (mm)	1.98-2.05	1.13-1.22	0.86-0.93	0.92-0.99
Female largest quartile (mm)	2.12-2.32	1.22-1.31	0.91-1.03	0.97-1.03
Male size range (mm)	1.61-2.05	0.96-1.22	0.65-0.93	0.82-0.99
Female size range (mm)	1.81-2.32	1.00-1.31	0.70-1.03	0.83-1.03
Male mean value (mm ± SD)	1.92 ± 0.010	1.10 ± 0.006	0.82 ± 0.006	0.90 ± 0.004
Female mean value (mm ± SD)	2.04 ± 0.015	1.17 ± 0.008	0.87 ± 0.009	0.94 ± 0.005
t-value	6.67	6.68	4.64	6.86
df	117	127	121	131
p-value	<0.001	<0.001	<0.001	<0.001

There was a significant positive correlation between total length and pronotum width, both in males (Pearson R = 0.83, *n* = 67, *p* < 0.001) and females (Pearson R = 0.73, *n* = 65, *p* < 0.001) (Figure 2). However, the magnitude of the correlations did not differ between males and females (*z* = 1.456, *p* = 0.146).

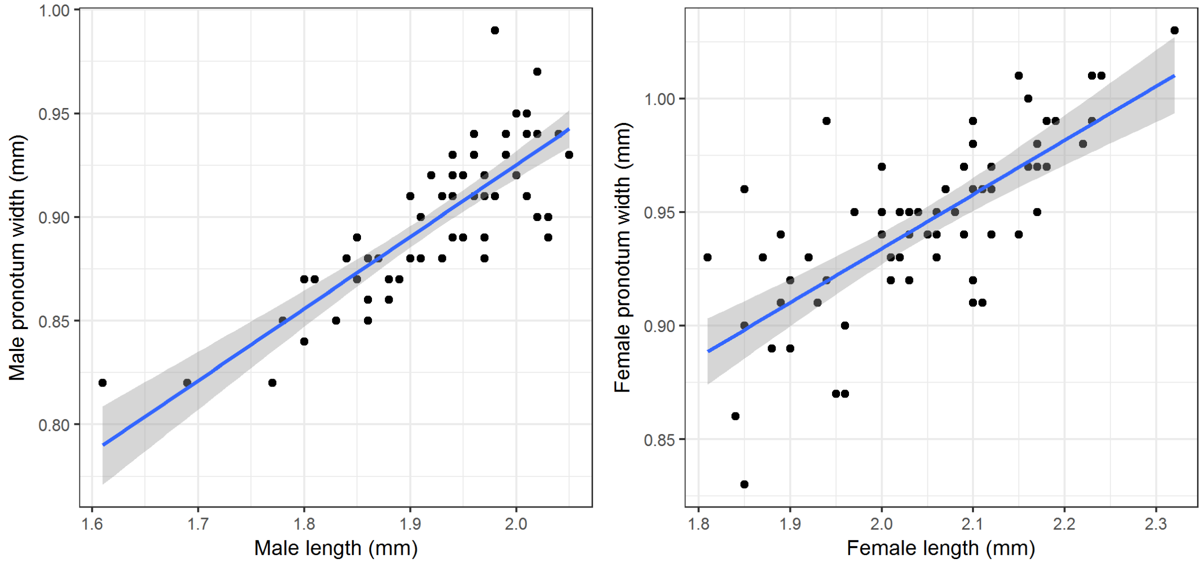

Figure 2: Correlation of total length and pronotum width in males (left) and females (right). Solid blue line indicates significant correlation. Grey area refers to the 95 % confidence interval.

Size Dimorphism Index

Disregarding pairs, females were on average 6% longer and 4% wider than the males. The smallest observed female was 12% larger than the smallest observed male, and the largest observed female was 13% larger than the largest observed male (Table 2).

Table 2: Quantification of body size dimorphisms based on the index developed by Lovich & Gibbons (1992). SDI = (female size/male size-1) when females are larger. SDI = - (male size/female size-1) when males are larger. SDI (mean) are calculated based on mean values for the whole sample. SDI (5max) and SDI (5min) are calculated based on mean values of the five largest and the five smallest individuals respectively. SDI (max) and SDI (min) are calculated based on the single largest and single smallest individuals, respectively.

Size Variable	**SDI (mean)**	**SDI (max)**	**SDI (5max)**	**SDI (min)**	**SDI (5min)**
Total length	0.063	0.132	0.105	0.124	0.067
Elytra length	0.064	0.074	0.068	0.042	0.051
Pronotum length	0.061	0.108	0.101	0.077	0.037
Pronotum width	0.044	0.040	0.054	0.012	0.041

Within pairs, females were longer (Wilcoxon signed rank test: Effect size = 0.624, *Z* = 5.03, *n* = 65, *p* < 0.001) and wider (Wilcoxon signed rank test: Effect size = 0.652, *Z* = 5.34, *n* = 67, *p* < 0.001) than males. However, calculations of the SDI from each pair showed that males tended to be longer than the respective female when in pair with small females (Figure 3). There was a positive association between the SDI and female total length (Pearson R = 0.79, *n* = 65, *p* < 0.001). Similar patterns emerged for pronotum width (see Appendix 4).

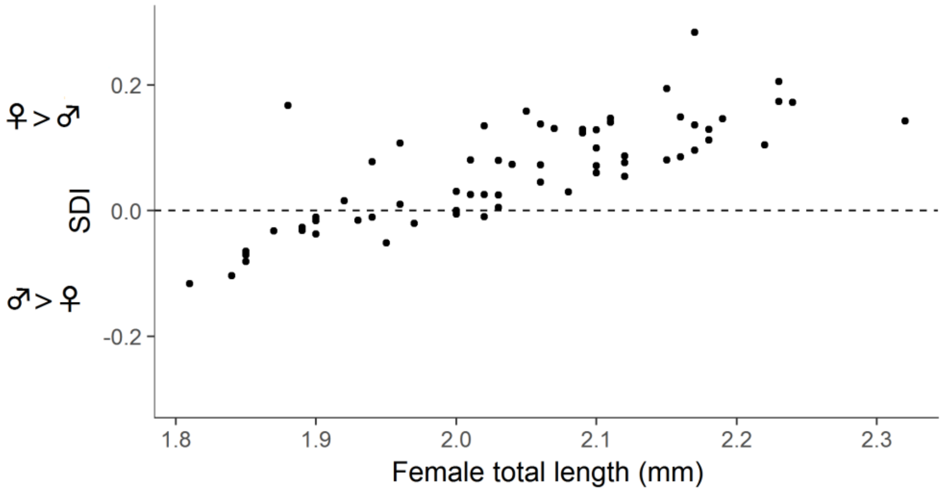

Figure 3: Relationship between calculated SDI values for each pair and the total length of females. SDI = (female size/male size-1) when females are larger. SDI = - (male size/female size-1) when males are larger. Every point refer to the size differences between the sexes of individual pairs. The horizontal shattered line (SDI = 0), refers to equal size of male and female. Dots under the line constitute negative SDI-values, which means that males were larger than their respective female. Contradictory, dots over the line constitute positive SDI-values, and refers to pairs where a female were larger than their respective male.

Assortative mating

The size of females and males in pairs did not correlate for any of the size metrics; total length (Pearson R = -0.079, *n* = 65, *p* = 0.532), elytra length (Pearson R = 0.037, *n* = 66, *p* = 0.771), pronotum length (Pearson R = -0.040, *n* = 66, *p* = 0.748), and pronotum width (Pearsons R < 0.001, *n* = 67, *p* = 0.999) (Figure 4).

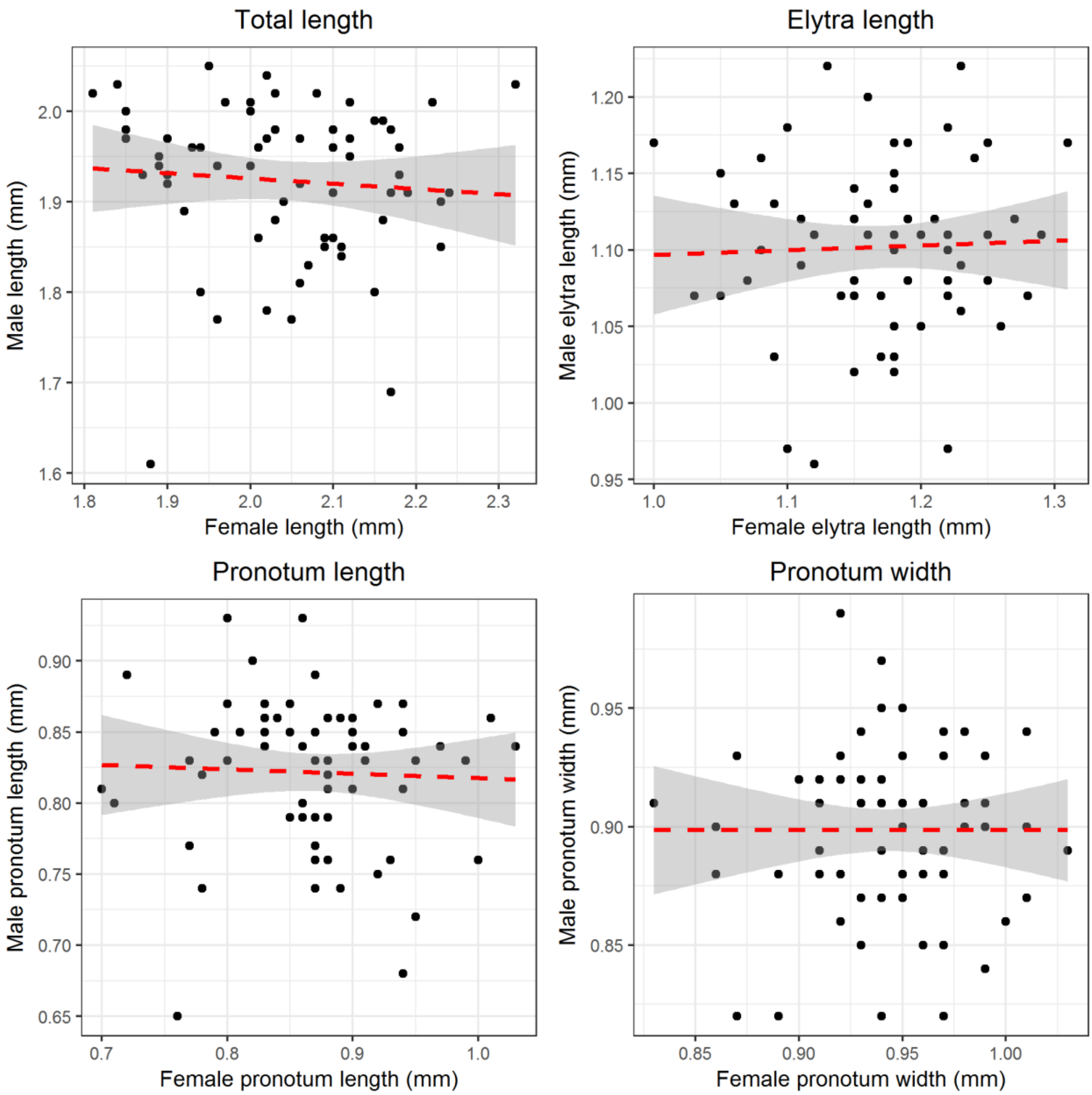

Figure 4: Association between female size and male size from pairs in natural environments. Each point represent a male and female from a single pair. Dashed red line indicate non-significant correlation. Grey area refer to the 95 % confidence interval.

Mate choice experiment 1: Three females, one male

Mating outcome and assortative mating in laboratory

Males did not end up in pair with larger females more often than with intermediate or small females (Chi-square goodness of fit-test: $x^2 = 0.8$, df = 2, $p = 0.67$). Neither did males tend to pair up with the closest sized female more often (two-sided binomial test, $n = 40$, $p = 0.43$).

There was a significant first encounter pair formation (two-sided binomial test, $n = 40$, $p < 0.001$). First encountered pair formation occurred in 90% of the trials ($n = 36$), while in 10% ($n = 4$), males did not enter the tunnel. Only in one of the four trials where male did not enter, the male visited another female. In the other three trials, the male remained at the tunnel entrance of the first encountered female. Dissection of all seeds after each trial revealed that the four occurrences of non-pair-formation were associated with a relative short tunnel, only deep enough to fit the female.

Tunnel diameter & time until encounter

Males did not show any consistent pattern towards choosing females with wider pronota (Chi-square goodness of fit-test: $x^2 = 3.05$, df = 2, $p = 0.22$) nor wider tunnel entrances (Chi-square goodness of fit-test: $x^2 = 3.65$, df = 2, $p = 0.16$). There was a significant relationship between female pronotum width and the tunnel diameter (linear regression, $t = 13.7$, df = 127, SE = 0.04, $p < 0.001$). There was no association between total length of males and time until first encounter (linear regression, $t = -0.75$, df = 38, SE = 0.01, $p = 0.46$).

Mate choice experiment 2: One female, three males

Smaller males did not tend to encounter females first compared to intermediate and larger males (Chi-square goodness of fit-test, $x^2 = 0.45$, df = 2, $p = 0.80$). After the end of experiment, there was no observed effect of male size on pair formation (Chi-square goodness of fit-test, $x^2 = 0.06$, df = 2, $p = 0.97$). During the first 10 minutes, male replacements occurred in 44% of the trials. Compared to mate choice experiment 1, the proportion of departed males compared to the number of males not entering tunnels at 10 minutes was greater in mate choice experiment 2 (C.I. = 0.186 – 0.496, $x^2 = 13.0$, $p < 0.001$). At end, replacements of males had occurred in 67% of all the trials. After ended trial, the most common behavior of the males not in pair was to bore a new tunnel in the seed (Table 3).

Table 3. Observations of male not in pair at the end of mate choice experiment number 2.

Observed male behaviors not in pair	**N=**	**%**
Started to bore new tunnel far away from pair (>1mm away)/Resided in groove	63	33.9
Started to bore new tunnel right next to pair (<1mm away)/Resided at the entrance	55	29.6
Walked around in petri dish	40	21.5
Walked on the seed	14	7.5
Resided within the same tunnel as the pair	10	5.4
Resided under the seed	4	2.2

Narrow-sense heritability

Estimated heritabilities were low for all parent-progeny regressions; none were significant ($p > 0.05$, Table 4). The estimated intraclass correlation coefficients (ICC) between the offspring were 0.13 (C.I.: 0.06 – 0.24) for sons and 0.09 (C.I.: 0.04 – 0.20) for daughters (Appendix 4). The variance in total length within broods (males: 0.008, females: 0.010) was greater than the variance among broods (males: 0.001, females: 0.001).

Table 4. Slopes (b ± SE) and the estimated heritability (h^2 ± SE) of total length of the parent-progeny regression. Degrees of freedom = 24 for all four regressions. Both regressions and standard errors were multiplied by two to obtain the given heritabilities (Falconer & Mackay, 1996).

Parent-progeny	Slope (b ± SE)	p-value	Heritability (h^2 ± SE)	Correction
Father-son	0.09 ± 0.04	0.14	0.17 ± 0.08	-
Father-daughter	0.02 ± 0.04	0.77	0.04 ± 0.09	b*(σ♀/σ♂)
Mother-son	- 0.06 ± 0.04	0.25	- 0.11 ± 0.08	b*(σ♂/σ♀)
Mother-daughter	0.01 ± 0.04	0.85	0.02 ± 0.09	-

Sexual size dimorphism and size distributions

Concordant with the findings from the pairs from the natural population, the sexual size dimorphism in average total length was significant between sons and daughters (Table 5). Daughters were on average longer than sons ($t = 23.0$, df = 916, $p < 0.001$). Quantification of the sexual size dimorphism by calculating the size dimorphic index (SDI) showed that total length of daughters on average were 7.7% larger than sons. Daughters also had a greater variation in total length than sons (F-test, $df_m = 496$, $df_f = 460$, $p = 0.003$). Both male and female size distribution of total length was left skewed. Few individuals were small, but the tail had a great range, especially in females (Figure 5).

During all experiments and rearing procedures, I measured an estimate of approximately 2,000 beetles. The two smallest measured beetles was both 1.50 mm. One of these was a female and the other was a male. The largest measured female was 2.32 mm, while the largest measured male was 2.15 mm.

Sex ratio

In total, 958 individuals were sexed, of which 497 were males and 461 were females. This ratio agrees with the expected 1:1 ratio (two-sided binomial test, probability of success = 0.519, C.I. = 0.487-0.551, $p = 0.26$).

Table 5. Size ranges for parental size treatments and size ranges and mean for progeny.

Size metrics	Female	Male
Parents: largest quartile (mm)	2.19 – 2.27	2.01 – 2.08
Parents: smallest quartile (mm)	1.81 – 1.92	1.53 – 1.80
Progeny size range (mm)	1.56 – 2.30	1.50 – 2.15
Progeny mean length (mm ± SD)	2.09 ± 0.11	1.94 ± 0.09
SDI total length (mean)	0.077	
Sex ratio of progeny (M:F)	497:461 (1.1 : 1)	
t-value	22.98	
Df	916	
p-value	< 0.001	

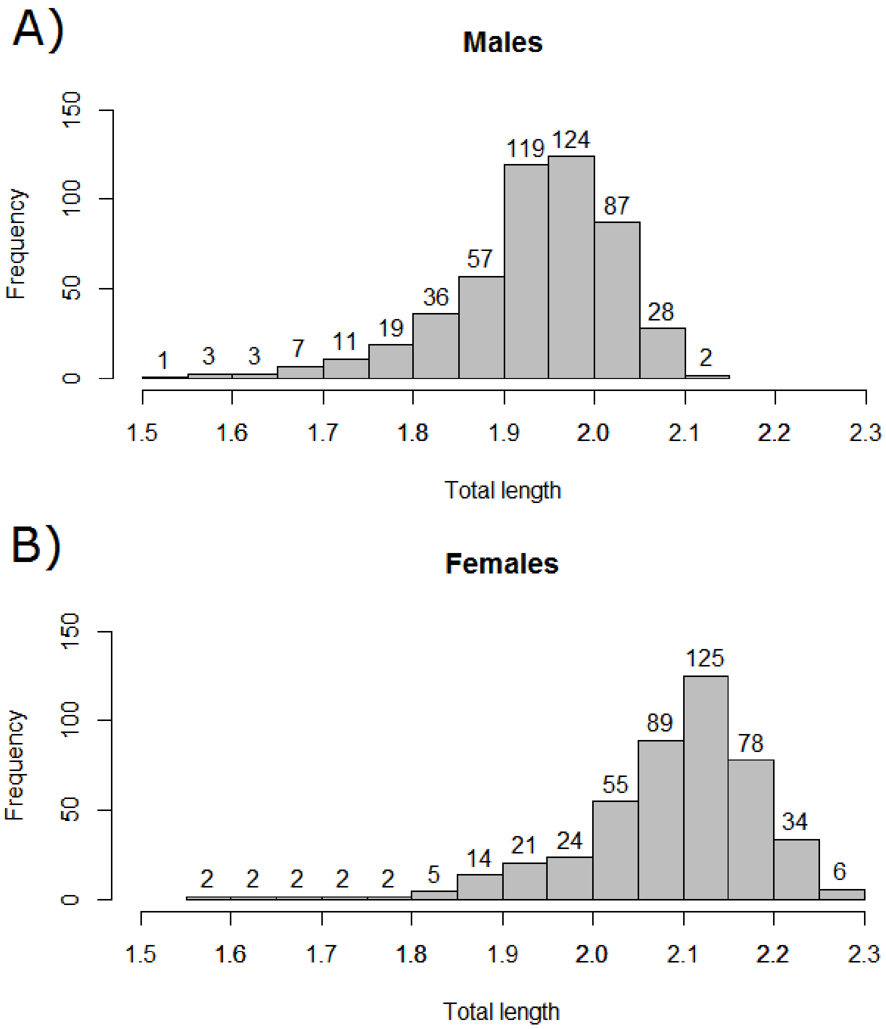

Figure 5. Size distribution of reared A) males (*n* = 497) and B) females (*n* = 461) from broods (*n* = 26). Total length is given in mm. Numbers above bars refers to frequencies of individuals.

DISCUSSION

Sexual Size Dimorphism and Assortative Mating in Natural Populations

Sexual size dimorphism & Sexual dimorphic index

Females were larger than males. They had longer elytra, longer pronotum and had greater total length. These findings are concordant with claims from Hestvik (2002) and Halvorsen (2006). Contradictory, all known taxonomical treatises states that males and females on average have the same size (Eggers, 1927; Palacios, 1973; Faccoli *et.al.*, 2012) and shape (Eggers, 1927; Lepesme, 1947; Balachowsky, 1949; Herfs, 1950). Females were also wider than males. The correlation between total length and pronotum width was significant for both males and females, indicating that the longest individuals also were the widest. The non-perfect correlation (0.83 in males, and 0.73 in females), indicates that there is individual variation in the relationship between total length and pronotum width. As the correlations between the sexes were insignificant, such disparities do apparently not differ between males and females, although individual variation occurs in both. Following, there is no evidence to claim that sexes differ in shape, where shape refers to variation in pronotum width in relation to total length.

Smaller size differences between the sexes are expected in monogamous mating systems compared to polygamous mating systems (Darwin, 1871; Maynard Smith, 1978; Andersson, 1994). Calculations of the size dimorphic index (SDI), showed that the mean total length of females was approximately 6% larger than the mean total length of males. Moderate sexual size dimorphisms of 10% or less are common and is predominant across most animal taxa (Fairbairn, 2007). Hence, there is no peculiarity to the observed sexual size dimorphism in *Dactylotrypes*. Although females on average were larger than males, the difference between sexes was small, and the size-ranges of the two sexes clearly overlapped (Table 1, see also Figure 5). While selection pressures may increase the magnitude of sexual size dimorphisms, resource availability may hamper it. Although living in seeds may restrict the evolution of size differences between the sexes, greater size dimorphisms exists in other seed-breeding bark beetles (Table 6), which implies that the sexual size dimorphisms in *Dactylotrypes* probably not are restricted by its seed inhabiting lifestyle.

Table 6. Calculated SDI-values for a selection of spermatophagous bark beetles. The table is not exhaustive. SDI are calculated based on mean values of total length given in references. Information on mating systems are found in Kirkendall (2015) and Wood (1982, 2007). Mean length values in mm. *Conophthorus terminalis* breeds in shoots and cones of *Pinus* spp., and are often are assigned to seed-breeding scolytines. The second SDI-value for *Dactylotrypes longicollis* were obtained from the Narrow-sense heritability experiment.

Species	**Total length male (mm)**	**Total length female (mm)**	**SDI (mean)**	**Mating system**	**References**
Dactylotrypes longicollis	1.92	2.04	0.063 0.077	Monogamous	Own data
Coccotrypes dactyliperda	1.83	2.26	0.235	Polygynous Inbreeding	Herfs, 1950
Coccotrypes graniceps	1.984	3.015	0.520	Polygynous Inbreeding	Ueda, 1997
Hypothenemus hampei	1.05	1.48	0.409	Polygynous Inbreeding	Ticheler, 1961
Conophthorus terminalis	2.06	2.36	0.146	Monogamous	Flores & Bright, 1987

Small mean size differences between the sexes have been reported from both polygamous (*Ips* ~ 1-2%) and monogamous (*Dendroctonus* ~ 2-4%; *Phloesinus armatus* ~ 2.5%) bark beetle taxa (Foelker & Hofstetter, 2014; Baruch *et al.*, 2017). In *Dactylotrypes*, variation in total length was greater in females than males. This is consistent with previous findings for *Dendroctonus* (Foelker & Hofstetter, 2014). It is a common pattern across scolytine taxa that the pioneering sex is the largest, and exhibits greater variability in body size (Kirkendall *et al.*, 2015).

Females were usually the larger sex in pairs. However, in pairs where females were small and males were large, males could occasionally be the larger sex (see Figure 3 in result section). Males 10% larger than the respective female was observed, suggesting that moderate intra-pairwise male biased SSD is not constricted by mechanical barriers of narrower tunnels. Extreme cases do exist, where the mechanical barriers of small tunnel diameters excavated by very small females prevent very large males from establishing a pair (pers. obs.). However, such cases are probably rare in natural populations. Based on the observations from pairs obtained from natural populations, there does not seem to be any significant constraints on pair formation.

Assortative mating

No pattern of size-assortative mating was found for either total length, elytra length, pronotum length or pronotum width from pairs in natural populations. Lack of size-assortative mating has been reported in many bark beetle species (Reid, 1999; Pureswaran & Borden, 2003; Reid & Baruch, 2010), which has led several researchers to question whether larger size confer any advantage in scolytines (Teale *et al.*, 1994; Reid & Roitberg, 1995; Pureswaran & Borden, 2003). Considering the cryptic life of bark beetles, living within small seeds and thin inner bark or phloem, being large may simply be a disadvantage, hampering the abilities of utilizing such niches. However, more recent, size-assortative mating has been reported in the cypress bark beetle *Phloesinus armatus*, associated with mutual mate choice for larger size (Baruch *et al.*, 2017).

Assortative mating is expected to be strongest in species with an even sex ratio where there is variation in quality of potential mating partners (Harari *et al.*, 1999). *Dactylotrypes longicollis* has an even sex ratio (Bernabò, 1991; Jacobsen, 2001; Halvorsen, 2006; see also result section on narrow sense heritability below), so the observed lack of assortative mating may be an indicator of little variation in quality of potential partners. Models have been proposed where prolonged pair-formation is associated with little variance in mating success (Sutherland, 1985). This may coincide with the mating strategy in *Dactylotrypes*, as males and females engage in prolonged pair-formation.

Lack of assortative mating is not necessarily a refutation of mate choice and sexual selection. As shown in the scolytine beetle *Dendroctonus ponderosae*, mutual mate choice may mask patterns of assortative mating (Reid & Baruch, 2010). Hence, the purpose of investigating mate choice even in absence of assortative mating patterns remains important.

Mate Choice experiment 1: Three females, one male

Concordant with the observations from the pairs in natural populations, the mate choice experiment revealed that males do not pair up with the most similar sized female more frequently if given a choice. Conclusively, assortative mating does not occur in *Dactylotrypes longicollis*. Neither did males tend to choose larger females over intermediate or small ones. Hence, larger body size in females was not associated with greater mating success.

Two main conclusions can be drawn from the first mate choice experiment. 1) Females never reject males. The first male reaching a female was never kicked out of the tunnel. Although females backed out of the tunnel in several trials, the male always ended up following the female into the tunnel at the end. Female backing out is therefore more likely to be a courtship behavior, rather than a rejection behavior. 2) Males did not compare potential females. Given that the tunnel was deep enough, the male always remained with the first female, and eventually blocked the tunnel entrance. Only in one out of the forty trials, the male left the first female within 10 minutes. Dissection of the seeds revealed that the tunnels was too narrow to fit the male. Conclusively, neither females nor males were discriminating in relation to body size. These observations corresponds to a random mating strategy (Janetos, 1980; Reid & Stamps, 1997).

Parker (1983) proposed a model for random mating, and suggested that its prevalence would increase if there is little variation in mate quality in both sexes and the search costs for mates are high. Although there is individual variation in body size among females, increased body size may not necessarily impose any fecundity advantages under natural conditions (Gotthard *et al.*, 2007; Berger *et al.*, 2008). It is possible that high costs of mate search may occur under some circumstances in *Dactylotrypes*. In cases of high population density, resource depletion are more likely to occur, hampering the probability of finding a partner. In such scenarios, the cost of mate search is likely to be higher, and so, the best strategy may be to keep a partner if one first is as lucky as finding one.

While both females and males are indiscriminate towards body size, the findings does not necessarily imply a lack of eventual pair assessment and preferences for other traits. However, if other traits were important during the copulation stage, we would either expect more males to compare females, or females to avoid certain males from entering. This was not the case.

Tunnel diameter – Male constraints

Tunnel diameter may be a constraint for the largest males, and smaller males may exhibit an advantage. Liu *et al.* (2017) demonstrated that males of *Dendroctonus valens* favored larger diameters over smaller ones, and that larger females bored wider tunnels. Similarly, in *Dactylotrypes*, wider females bored wider tunnels. Contrarily, males did not show any preference toward wider tunnel entrances. They did not end up in the widest tunnels more frequently, neither did they end up in pairs with wider females more frequently.

Lack of assortative mating for pronotum width was confirmed. Males did not tend to mate more frequently with the female closest to its own width. Although smaller females bored narrower tunnels, this did not seem to confer any significant constraint on pair formation. In 25% of the trials (*n* = 10), the male had greater pronotum width than the respective female in the pair, again indicating that pair formation rarely is constrained by mechanical barriers of narrow tunnels excavated by small females.

Time until encounter – Mate location

There was no association between male size and time until first encounter. It has been suggested that smaller males have an advantage over large males being more agile and maneuverable, such as reaching the females faster (Andersson, 1994; Blanckenhorn, 2000; Moya-Laraño *et al.*, 2002; 2007). This was not the case for *Dactylotrypes*.

Independent of size, males seemed rather clumsy in locating females. First, they rarely showed patterns of unidirectional walks towards seeds. Even when they stumbled upon a seed, they often spend relatively long time crawling up on it. In addition, their location of the tunnels was poor, and they could often walk in the opposite direction from it even when walking in the groove of the seed. When males were put directly in front of the tunnel entrances (during pilot studies) they usually gained interest immediately and was quickly engaged in tactile interactions with the female. I therefore find it unlikely that poor localization by the males is due to low interest of mating. When stumbling upon frass, males started with antennal waving and digging, subsequently ending up finding the female. Based on these behaviors, I find it unlikely that precopulatory mate choice related to long-distance pheromones and eventual female stridulations are prominent in *Dactylotrypes*. Overall, the behavior coincides well with a random mating pattern. If males in general are poor at locating females, this may an indication of high search costs, which results in a strategy of first encounter pair establishment.

Mate Choice experiment 2: One female, three males

As implied by the '*time until encounter*' from the first mate choice experiment, body size did not relate to which male that first encountered the female. However, contrary to the first mate choice experiment, the number of males not entering tunnels given a first female encounter occurred relatively often. There was a significant difference between the proportion of males not entering tunnels (mate choice experiment 1) and departed males (mate choice experiment 2) between the two mate choice experiments. This suggests that male-male competition could be important in *Dactylotrypes longicollis*. Normally, males encountered the tunnel sequentially. If a male was able to copulate and later follow the female inside the tunnel without interruptions from other males, replacements were not likely to occur (Figure 6A). Tunnel diameters were too narrow for secondary males to force immersed males out from the tunnel. In cases where males approached the tunnel almost simultaneously tactile interactions and competition between males occurred. In such cases, males were often tactile, pushing and butting each other with the frons against the elytra (Figure 6B; see also videos in Appendix 3). The effectiveness of such interaction varied, and may have depended on the positioning of the first male, or on each male's motivations to copulate. Individual variation in motivation for male-male interactions seems to vary, as in many cases subsequent arriving males often left without any tactile involvement. Dislodgement of males only occurred when males were at least partly outside of the tunnel, indicating that females backing out of the tunnel for courting may be a vulnerable period during which other males can dislodge the opposite male. Male-male competition has been reported in both polygamous and monogamous taxa (Rudinsky & Michael, 1974; Oester & Rudinsky, 1975; Ryker & Rudinsky, 1976; Vernoff & Rudinsky, 1980; Smith & Cognato, 2011), and is probably common in female initiated mating systems (Kirkendall, 1983; Kirkendall *et al.*, 2015). Although male competition may be common, reported dislodgment from bark beetle taxa remain scarce. This is probably due to few studies on male-male competition in monogamous bark beetles, rather than rareness of such occurrences. However, in the only reported case I know of, dislodgment of males was rarely seen in the bark beetle *Leperisinus oregonus* (Vernoff & Rudinsky, 1980). While some enforced male-male interactions have been reported to be brutal, involving torn of bodily part within tunnels and damaged individuals (Rudinsky & Michael, 1974), such extremes were not observed in *Dactylotrypes*. Damaged individuals and torn of bodily parts were never observed, and the male-male interactions observed in *Dactylotrypes* were seemingly harmless, and resembled those tactile male-male interactions as described by Smith & Cognato (2011) for *Camptocerus* spp..

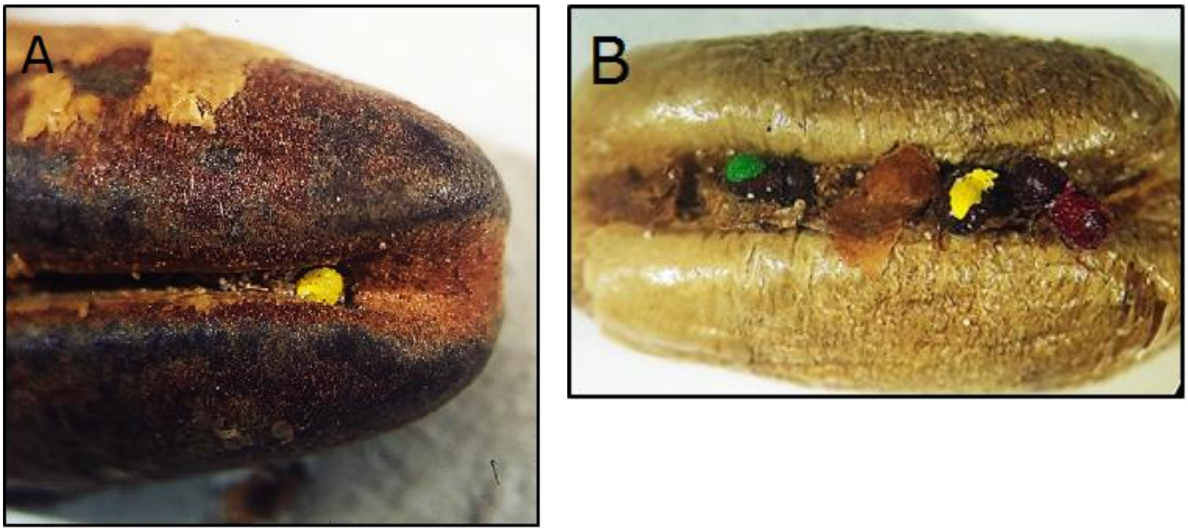

Figure 6. A) A male is totally inside the tunnel, and is unlikely to be dislodged by any subsequent males. B) Yellow and red male approached the tunnel almost simultaneously, and were pushing each other for access to the female.

Male-male competition in *Dactylotrypes* may be common in certain settings, especially under scenario of resource depletion associated with explosive population outbursts. Although male dislodgement was common, the outcome had no apparent connection with body size. As seen from the continuous observation during the 10 first minutes, dislodgements occur independent of size. Summed up, male total length did not confer any advantage for mating success. Smaller males did not confer any advantage by being more agile or sneaking into smaller crevices compared to larger males. The variation in male size is probably too little that eventual size differences will have any impact.

Notes on males not in pairs

If a female was occupied, and the male in pair was within the tunnel, the two other males often started to bore their own tunnels (Table 3). Vernoff & Rudinsky (1980) reported that dislodgement of the male already in tunnel could occur when intruding males bored intersecting tunnels whereby they ended up forced the initial male upwards. Although solitary males in *Dactylotrypes* bored tunnels, and the majority was close to the established pair, many of the tunnels were also bored far away from the residing pair, suggesting that the aim of male tunnel boring may serve other functions besides 'sneaky' mating tactics. In addition, if the female already has copulated with the residing male, I find it unlikely that she will be receptive for subsequent mating events with potential intruding males (see part 2 of this thesis). The behavior of males not in pair (e.g. boring new tunnel, walking in petri dish, etc.) could not be foreseen by the size of the male.

Mate Choice – Additional Notes

Female size may be important for initiation of tunnel excavation. During establishment of females within the seeds, I found that small females (< 1.90 mm in total length) rarely started to bore tunnels. As female excavation of tunnels is a prerequisite for pair formation, female receptivity thus may be related to female size, where smaller size causes a disadvantage. If small females fail to bore tunnels, they will not be receptive for mating and they will therefore fail to reproduce. This suggests a directional selection for larger females. Why the smallest females do not initiate tunnels remains unknown.

Narrow-sense heritability

Dactylotrypes exhibited low heritability of total length for all parent-offspring regressions. None were significant. The phenotypic variation of body size is a product of both genes and the environment, as well as the interaction between the two (Roff, 1997). The calculated heritabilities indicate that environmental effects explains a larger proportion of the observed phenotypic variance in total length in *Dactylotrypes*. Insignificant heritability estimates however, does not imply absence of genetic component for body size, but rather that the fraction of environmental effects is exceeding the impact of genes. Foelker & Hofstetter (2014) also found low, non-significant heritabilities on total length for the monogamous species

Dendroctonus frontalis and *D. brevicomis*. On the other hand, higher and significant heritabilities were found for some parent-offspring regressions in *Ips pini* and *I. lecontei*, which are polygamous.

A non-significant additive genetic component to the variation in body size in *Dactylotrypes longicollis* may explain why mate choice with respect to body size has not evolved. As the size of the partner does not have any guarantee for siring more fit offspring, there is simply no reason for discriminating among potential partners. This would rely on the assumption that larger females do not produce more eggs, which remains to be tested for *Dactylotrypes*.

In bark beetles, individual variation in body size may be under influence of both abiotic (Bentz *et al.*, 2001; Bentz *et al.*, 2011; Bracewell *et al.*, 2013) and biotic factors (Anderbrant & Schlyter, 1989; Six, 2012). Jacobsen (2001) demonstrated that abiotic factors such as temperature and humidity have an impact on the developmental time and brood size in *Dactylotrypes*. Whether such abiotic factors can influence body size evolution, and whether this operates differently on males and females remains to be tested.

Although abiotic environmental factors such as temperature and humidity may be significant for phenotypic variance of body size in natural populations, they are unlikely to explain the outcome of the heritability experiment, as all pairs were bred under same temperature and humidity regimes. Biotic effects such as intraspecific larval competition within broods and differences in seed quality are more likely determinants to the observed phenotypic variance in body size. Larval development in bark beetles may be affected by food availability, larval density, temperature and humidity (Rudinsky, 1962; Anderbrant & Schlyter, 1989; Kirkendall, 1989; Sargent & Reid, 1999; Jacobsen, 2001), with potential consequences for the final body size in mature adults. This may explain the poor resemblance between parents and offspring.

As the estimation of the intraclass correlation coefficient (ICC) showed, offspring usually did not resemble their parents. There was less variation among broods than within broods for each sex, indicating that the four different parental treatments had little effect on offspring size. Greater variation within single broods may strengthen the hypotheses of intraspecific larval competition in determination of body size. I do not necessarily think that overall larval density is the strongest factor for body size. Even in less dense broods, there was great variation in body size among siblings. I suggest that larval competition can be relatively random. As an example, a larva can be badly positioned if it is enclosed by two neighboring sibling larvae, independent of the larval density. It is not necessarily about numbers of larvae, but rather position in relation to other surrounding siblings. This suggestion, of course, remains to be tested.

By definition, narrow-sense heritability does not include the effect of dominance and epistasis, as it only refers to the proportion of phenotypic variability that is due to additive genetic variance (Falconer & Mackay, 1996; Roff, 1997). Although dominance and epistatic interactions might be prevalent, their effect are often poor predictors of resemblance between offspring and parents, and they do not necessarily contribute to a great portion of the genetic variation within a population *per se* (Lande, 1980; Falconer & Mackay, 1996).

Narrow-sense heritability is commonly used to infer the potential for a trait to be under selection (Houle, 1992). However, the validity of heritability estimates as an indicator of selective potential remains controversial, and some claim that heritability and selective potential not necessarily correlate at all (Hansen *et al.*, 2011). The criticism mainly relates to high heritability estimates, which not necessarily imply a correct level of genetic component in relation to environmental factors. Heritability estimates may be artificially high during homogeneous environmental conditions in laboratory conditions. Another fallacy may arise if the trait of interest is insensitive to the environmental factors (Hansen *et al.*, 2011).

Despite the discussed shortcomings in exact estimation of the heritability coefficients, the overall result remain unambiguous. Body size in *Dactylotrypes* is under greater influence by environmental conditions than genes. This suggest that body size in *Dactylotrypes longicollis* is not under any current selective pressure.

Sexual size dimorphism and size distribution

Concordant with the findings from the pairs from the natural populations, the mean total length differed significantly between sons and daughters disregarding broods. Females were larger than males. The estimated sexual dimorphic index based on mean values of total length was slightly larger than what was calculated from the natural population (7.7% versus 6.3%). Overall, the findings from the reared offspring suggests that the sample from the natural population was a reliable predictor for the estimation of the sexual size dimorphism, although the data were obtained only from already established pairs.

Sex ratio

The sex ratio of the progeny was not significantly different from 1:1. This corroborates previous findings for *Dactylotrypes longicollis* (Bernabò, 1991; Jacobsen, 2001; Halvorsen, 2006), and fits with the expected sex ratio for a monogamous mating system (Maynard Smith, 1978). The sex ratio in *Dactylotrypes* has been tested both of the emerging offspring (Jacobsen, 2001; Halvorsen, 2006), and from individuals found by dissection of seeds in nature (Bernabò, 1991). Brief asynchronous periods of female receptivity together with continuous sexual active males might skew the operational sex ratio (Emlen & Oring, 1977). However, as males and females forms a prolonged relationship in *Dactylotrypes* and both sexes presumably only mate once during their lifetime, such asynchronous periods are not likely to occur.

Still, the operational sex ratio in bark beetles may be altered by differences in mortality between the sexes (Lachowsky & Reid, 2014), or by symbiotic interactions with bacteria such as *Wolbachia* (Kawasaki *et al.*, 2016). Differences in larval mortality seem to be insignificant in *Dactylotrypes*, as larval mortality was low during rearing in laboratory conditions (pers. obs.). Sex ratio alteration associated with *Wolbachia* has been reported in other seed-breeding bark beetles such as *Hypothenemus hampei* (Vega *et al.*, 2002) and *Coccotrypes dactyliperda* (Zchori-Fein *et al.*, 2006). Although such interactions may be present in *Dactylotrypes*, distorted sex ratios is probably the exception rather than the rule. *Dactylotrypes longicollis* has an even sex ratio, *prima facie*.

Future works – Why are females larger than males?

Answering why sexes differ is a complex task beyond the scope of this thesis, as fecundity selection, natural selection, sexual selection and phenotypic plasticity all can operate on body size evolution. Some remarks on the sexual size dimorphism in *Dactylotrypes* can still be deduced from the experiments. There is no indication from the results pointing towards current sexual selection operating on body size in *Dactylotrypes longicollis*. Although a random mating pattern may cause variation in mating success (see Sutherland, 1985), random mating strongly indicates lack of sexual selection (Andersson, 1994). In addition, the narrow-sense heritability showed that a greater proportion of the observed variation in body size is due to environmental factors than genes. For a trait to evolve by selection it must be heritable (Hedrick & Temeles, 1989). Although genetic components related to body size may be heritable, this will have insignificant effects on phenotypic variation in offspring, and produce non-significant evolutionary response.

In order to better understand the selective forces acting upon body size in bark beetles in general, aspects related to different selection pressures needs to be scrutinized. With more data emerging in the future, evolutionary patterns of body size evolution in bark beetles can be tested with greater resolution across phylogenetic lineages.

CONCLUSIONS

Dactylotrypes longicollis display a typical slight sexual size dimorphism, where females tend to be larger than males, and exhibit greater variance in total length than males. No pattern of assortative was found, neither for pairs in natural population nor pairs from mate choice experiments. Lack of mate choice with respect to body size and lack of constrains by tunnel diameter may explain absence of assortative mating patters. *Dactylotrypes longicollis* exhibit a random mating strategy in relation to body size, mating with the first encountered mate. Body size did not confer any advantage in mating success in the experiments reported here. Male-male competition do occur, probably also in natural settings, but neither large, intermediate nor small body size was associated with a greater chance of dislodging other males or ending up in a pair at end. Males did not seem to have any preference for larger sized females. The estimated narrow-sense heritability indicated that the additive genetic variance is insignificant compared to environmental factors. With respect to body size, offspring do not necessarily resemble their parents, and there was pronounced intrasexual variation of body size within broods. There is no indication that the observed sexual size dimorphism in *Dactylotrypes longicollis* is under any current selective pressure related to sexual selection. The observed pattern from the experiments coincides with the prediction that sexual size dimorphisms will be slight in monogamous species with an even sex ratio (Emlen & Oring, 1977; Maynard Smith, 1978). There are nothing abnormal in the observations of *Dactylotrypes longicollis* in relation to mate choice and intrasexual competition, and the findings corresponds well with the conventional sexual selection theory as predicted by Darwin (1871).

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APPENDICES

Appendix 1: Sampled sites

Table A-1: All sites located in La Gomera, except from Los Cristianos (Tenerife) and Montpellier (France).

Date-ID	Site	Geographic position	Species
180721-1	Hermigua, close to Finca Piñero	28.153 N, -17.198 W	*Dactylotrypes longicollis*
180722-1	Visitor centre to Garajonay NP	28.178 N, -17.213 W	*Dactylotrypes longicollis*
180724-1	Las Hayas	28.130 N, -17.289 W	*Dactylotrypes longicollis*
180724-2	Mirador de Abrante	28.125 N, -17.312 W	*Dactylotrypes longicollis*
180724-3	Los Granados	28.112 N, -17.315 W	*Dactylotrypes longicollis*
180724-4	Vueltas	28.098 N, -17.331 W	*Dactylotrypes longicollis*
180725-1	Hermigua (site 2)	28.153 N, -17.198 W	*Dactylotrypes longicollis*
180725-2	Hermigua, close to Finca Piñero	28.153 N, -17.198 W	*Dactylotrypes longicollis*
180726-1	Vallehermoso	28.187 N, -17.264 W	*Dactylotrypes longicollis*
180726-2	Vallehermoso, Botanical garden	28.187 N, -17.263 W	*Dactylotrypes longicollis*
180728-1	Hermigua (site 2)	28.153 N, -17.198 W	*Dactylotrypes longicollis*
180728-2	Lepe	28.185 N, -17.186 W	*Dactylotrypes longicollis*
180729-1	San Sebastian[1]	*NA*	-
180731-1	Los Cristianos, Tenerife[2]	*NA*	*Coccotrypes dactyliperda*
180818-1	Montpellier, France[3]	*NA*	*Dactylotrypes longicollis*

1. All fruits of *Phoenix canariensis* were immature in San Sebastian, La Gomera at the sampling date. Several sites were searched, hence, no coordinates. All fruits were without hardened seed suited for infestation. No beetles were found.

2. Sampling in Los Cristianos, Tenerife was conducted at several sites, hence no data on coordinates. Only *Coccotrypes dactyliperda* was identified within the collected seeds.

3. L. Kirkendall collected seeds from an unidentified palm outside Le Corum convention center in Montpellier. Only *Dactylotrypes longicollis* was present within the seeds. No coordinates were taken from the sampling site.

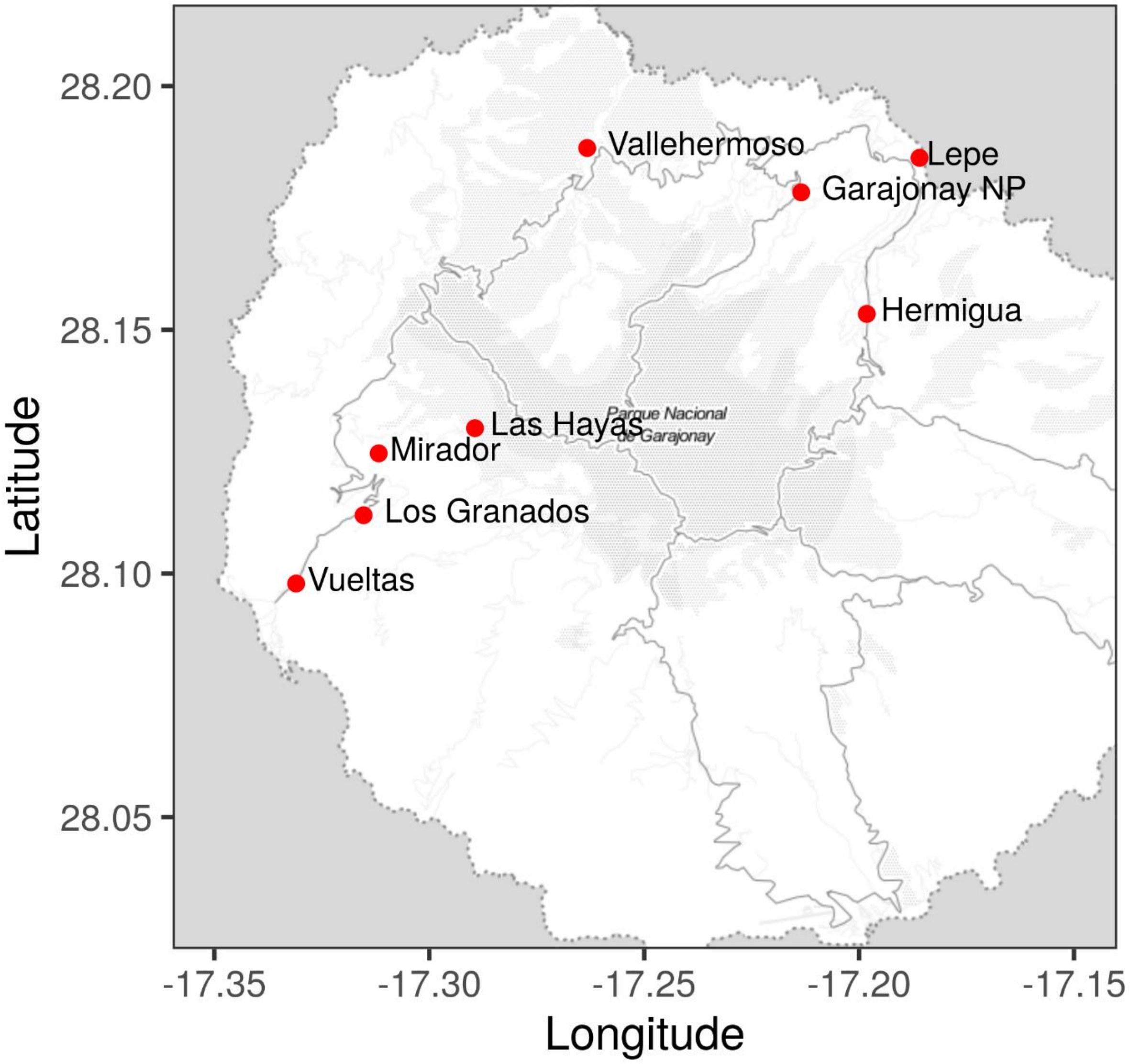

Figure A-1.1: Sampled sites on La Gomera. Several samples took place in Hermigua and Vallehermoso, however they had the same coordinates and are therefore not differentiated on the map. San Sebastian were also sampled, but due to no recorded coordinates, and absence of *Dactylotrypes longicollis*, it was excluded from the map.

Appendix 2: External suppliers of seeds

Seeds from *Phoenix canariensis*, *P. sylvestris* and *P. roebelenii* were bought from Especiesbelize (www.especiesbelizetropicalseeds.com [website down]). I suspect that the advertised *P. canariensis* seeds must have been either a hybrid or another species than *P. canariensis*. Compared to our handpicked seeds from La Gomera, the seeds were significantly smaller and much darker in coloration. These seeds were not preferred by the beetles. None of the seeds bought from Especiesbelize were used for the experiments, but exclusively for rearing.

Additional seeds of *Phoenix canariensis* were bought from Sheffield's Seed Co., Inc. New York (www.sheffields.com). These provided to be successful both for rearing procedures and experiments. *P. canariensis* seeds from this supplier were used for all experiments.

Appendix 3: Videos

Links to videos of general observations and mate choice experiments can be streamed or download from the following web pages:

1. Video of two males at the tunnel entrance recorded during pilot studies. The male to the right has a greater proportion of his body inside the tunnel. The other male is rubbing his frons towards the other male's elytra. https://vimeo.com/361413286

2. Cross-section of a seed (*Phoenix canariensis*), opened in the field in Hermigua, La Gomera. A residing pair of *Dactylotrypes longicollis* was inside. The female is furthest inside, while the male is residing at the tunnel entrance. https://vimeo.com/361413099

3. One of the trials in the male mate choice experiment. The yellow male blocks the tunnel. Red male is pushing yellow male, frons against elytra. Green male seems to have started to bore an own tunnel. https://vimeo.com/362168798

Password for all videos: Dactylotrypes

All videos recorded by the author.

Appendix 4: Supplementary figures for statistics

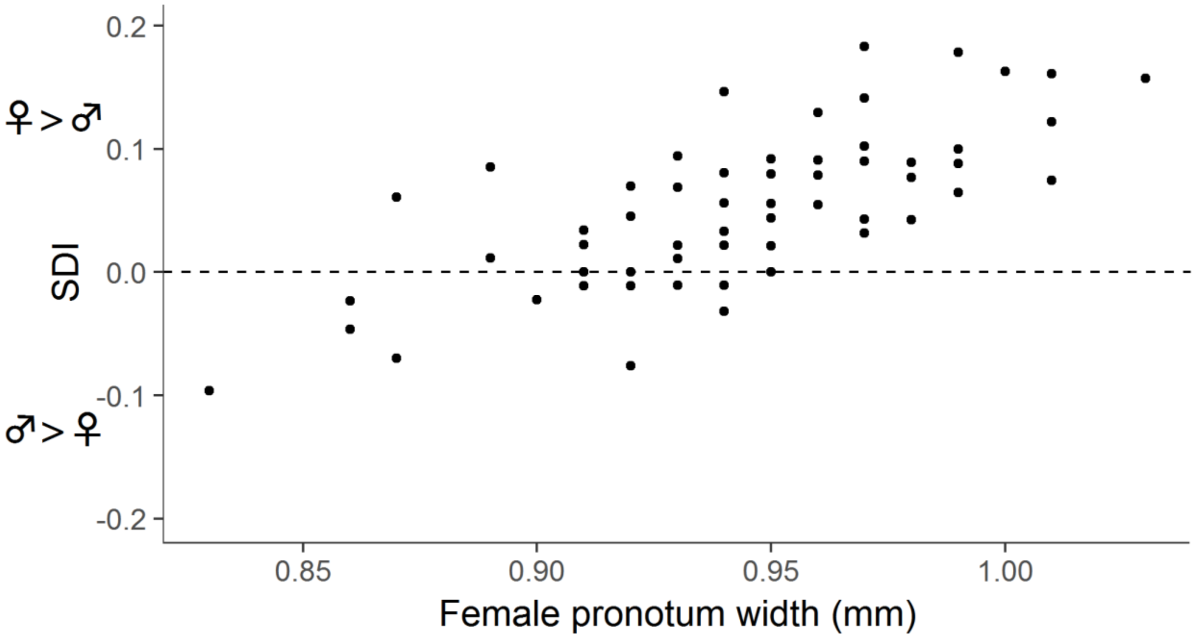

Figure A-4.1. Relationship between calculated SDI values for each pair and the pronotum width of females. SDI = (female size/male size-1) when females are larger. SDI = - (male size/female size-1) when males are larger. Every point refer to the size differences between the sexes of individual pairs. The horizontal shattered line (SDI = 0), refers to equal size of male and female. Dots under the line constitute negative SDI-values, which means that males were larger than their respective female. Contradictory, dots over the line constitute positive SDI-values, and refers to pairs where a female were larger than their respective male.

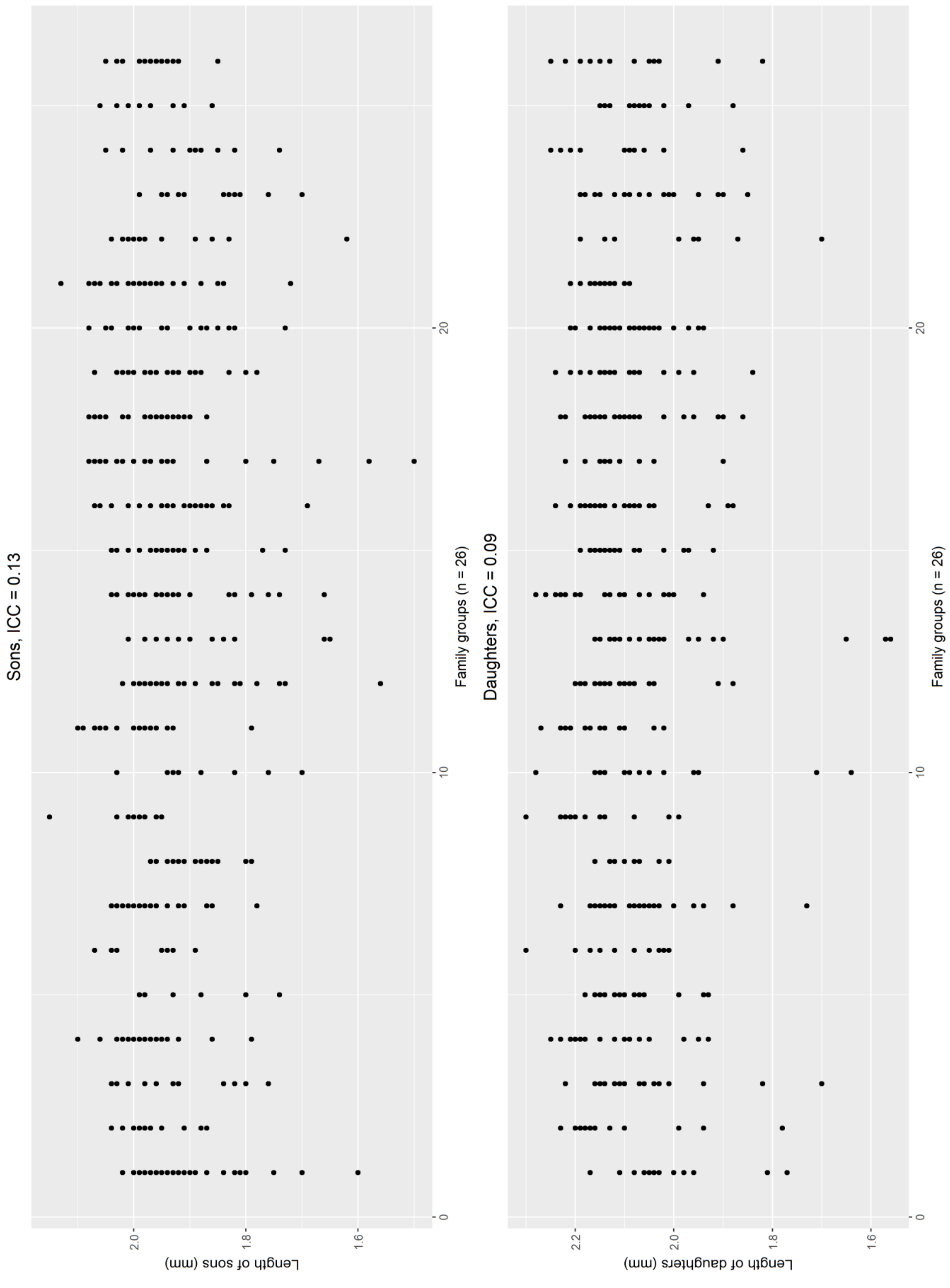

Figure A-4.2. Dot-plots for sons (*n* = 497) and daughters (*n* = 461) from different broods/family groups (*n* = 26). The estimated intraclass correlation coefficient was lower for daughters than for males.

Appendix 5: R-scripts

Sexual size dimorphism and assortative mating in natural populations

Welch’s two-sample t-test to test for differences in mean sized between sexes

```
#TOTAL LENGTH

t.test(assortative.df$Male.length, assortative.df$Female.length, data=assortative.df)

#ELYTRA LENGTH

t.test(assortative.df$Male.elytra.length..mm., assortative.df$Female.elytra.length..mm.,
data=assortative.df)

#PRONOTUM LENGTH

t.test(assortative.df$Male.pronotum.length..mm., assortative.df$Female.pronotum.length..mm.,
data=assortative.df)

#PRONOTUM WIDTH

t.test(assortative.df$Male.pronotum.width..mm., assortative.df$Female.pronotum.width..mm.,
data=assortative.df)
```

F test to compare variances between sexes

```
#Total Length

var.test(assortative.df$Male.length, assortative.df$Female.length)

#Pronotum width

var.test(assortative.df$Male.pronotum.width..mm., assortative.df$Female.pronotum.width..mm.)


# ANOVA to test if differences of mean sizes differed between the three sample sites for each sex

#Males

# Total length:

fit.lm <- lm(Male.length~Site, data=assortative.df)

anova(fit.lm)


# Pronotum width

fit2.lm <- lm(Male.pronotum.width..mm.~Site, data=assortative.df)

anova(fit2.lm)


#Females

# Total length:
```

```
fit3.lm <- lm(Female.length~Site, data=assortative.df)
anova(fit3.lm)

# Pronotum width:
fit4.lm <- lm(Female.pronotum.width..mm.~Site, data=assortative.df)
anova(fit4.lm)

#Correlation between pronotum width and total length for each sex separately
# MALES
cor.test(assortative.df$Male.length, assortative.df$Male.pronotum.width..mm., method = "pearson")
# FEMALES
cor.test(assortative.df$Female.length, assortative.df$Female.pronotum.width..mm., method = "pearson")

# Test whether the two correlations differ
cocor.indep.groups(0.83, 0.73, 67, 65, alternative = "two.sided", test = "all", alpha = 0.05, conf.level = 0.95, null.value = 0, data.name = NULL, var.labels = NULL, return.htest = FALSE)

# Pearson Correlation for assortative mating between the sexes #
#TOTAL LENGTH:
cor.test(assortative.df$Male.length, assortative.df$Female.length, method = "pearson")
#ELYTRA LENGTH:
cor.test(assortative.df$Male.elytra.length..mm., assortative.df$Female.elytra.length..mm, method="pearson")
#PRONOTUM LENGTH:
cor.test(assortative.df$Male.pronotum.length..mm., assortative.df$Female.pronotum.length..mm., method="pearson")
#PRONOTUM WIDTH:
cor.test(assortative.df$Male.pronotum.width..mm., assortative.df$Female.pronotum.width..mm., method="pearson")

# Wilcoxon signed ranks to examine whether females within each par was larger than males.
# Total length
wilcox.test(assortative.df$Male.length, assortative.df$Female.length, paired=T)
```

```
test<-wilcox.test(assortative.df$Male.length, assortative.df$Female.length, paired=T)
zstat<-qnorm(test$p.value/2)
abs(zstat)/sqrt(65)
# Z = Effect size * Sqrt(N)
# In this case, Z = 0.6195032 * sqrt(66) = 5.03
0.6242504 * sqrt(65)


# Pronotum width #
wilcox.test(assortative.df$Male.pronotum.width..mm., assortative.df$Female.pronotum.width..mm.,
paired=T)
test2<-wilcox.test(assortative.df$Male.pronotum.width..mm.,
assortative.df$Female.pronotum.width..mm., paired=T)
zstat2<-qnorm(test2$p.value/2)
abs(zstat2)/sqrt(67)
# Z = Effect size * Sqrt(N)
# In this case, Z = 0.6195032 * sqrt(66) = 5.03
0.6527492 * sqrt(67)


# Correlation for SDI-values #
cor.test(sdi.df$SDI.TL, sdi.df$FTL, method = "pearson")
```

Mate Choice Experiment 1

```
# Random or comparative?
# Males enter tunnel, n = 36
# Males do not enter tunnel, n = 4
# Random or comparative mating?
binom.test(36, 40, 0.5, alternative="two.sided")


# Assortative mating
# Closest size, n = 23
# Not closest size, n = 17
binom.test(23, 40, 1/2, alternative="two.sided")
```

```
# Do males pair up with the largest female/widest tunnel?
# Total length
# observed <- c(large, intermediate, small)
observed <- c(12, 12, 16)
expected <- c(1/3, 1/3, 1/3)
chisq.test(observed,p=expected)

# Widest female
# observed <- c(large, intermediate, small)
observed <- c(9, 13, 18)
expected <- c(1/3, 1/3, 1/3)
chisq.test(observed,p=expected)

# Widest tunnel
# observed <- c(large, intermediate, small)
observed <- c(10, 11, 19)
expected <- c(1/3, 1/3, 1/3)
chisq.test(observed,p=expected)

# Linear regression for female pronotum width and tunnel diameter
lm(TD~Female.pronotum.width, data=diameter.df)
fit.lm<-lm(TD~Female.pronotum.width, data=diameter.df)
summary(fit.lm)

# TIME OF ENCOUNTER
# Linear regression
lm(encountertime.s.~Total.Length.Male, data=time.df)
fit.lm<-lm(encountertime.s.~Total.Length.Male, data=time.df)
summary(fit.lm)
```

Mate Choice Experiment 2

```
# FIRST ENCOUNTER:
# observed <- c(large, intermediate, small)
observed <- c(33, 32, 28)
expected <- c(1/3, 1/3, 1/3)
chisq.test(observed,p=expected)

# AT END:
observed <- c(30, 31, 32)
expected <- c(1/3, 1/3, 1/3)
chisq.test(observed,p=expected)

# COMPARISON OF PROPORTIONS OF MALES NOT IN TUNNEL AFTER 10 MINS
proportion <- prop.test(x = c(41, 4), n = c(93, 40), alternative='two.sided')
proportion
```

Narrow-sense heritability

```
# FINDING SLOPES BY LINEAR PARENT-OFFSPRING REGRESSION:
# Father-son:
regression1<-lm(meansons~fatherlength, data=mean.df)
summary(regression1)
# Father-daughter:
regression2<-lm(meandaughters~fatherlength, data=mean.df)
summary(regression2)
# Mother-son:
regression3<-lm(meansons~motherlength, data=mean.df)
summary(regression3)
# Mother-daughter:
regression4<-lm(meandaughters~motherlength, data=mean.df)
summary(regression4)
```

```
# Two-sample t-test: Used for comparing the mean of two groups
#TOTAL LENGTH
t.test(Total.length~Sex, data=testsize.df)

# F test to compare variances between sexes
#Total Length
var.test(Total.length~Sex, data=testsize.df)

# Intraclass correlation coefficient #
#SONS
ICCest(Group, Total.length, data = iccsons.df, alpha = 0.05, CI.type = c("THD"))
#DAUGHTERS
ICCest(Group, Total.length, data = iccdaughters.df, alpha = 0.05, CI.type = c("THD"))

# Sex Ratio #
#born males
males <- 497
#born females
females <- 461
binom.test(c(males, females), p=0.5, alternaive="two.sided")
```

Part 2:

An overview of *Dactylotrypes longicollis* based on literature review and own laboratory observations

Cross-section of a seed (*Phoenix canariensis*) with a residing pair of *Dactylotrypes longicollis*. The female is first, with a following male. Photo: Anders Isaksen.

Abstract

Despite discovered over 150 years ago, little is known about the biology of the bark beetle *Dactylotrypes longicollis*. Here, I present an overview of *Dactylotrypes longicollis* based on a handful of existing papers integrated with own observations and measurements in the laboratory. The first paragraphs deals with the discovery, taxonomical history and morphology. The latter part focus on life-history traits and reproductive ecology, which is given greater emphasis, as there is a current gap of such information in existing papers.

Discovery and Taxonomy

Dactylotrypes longicollis (Coleoptera: Curculionidae: Scolytinae) was first scientifically described in 1864 by the British entomologist Thomas Vernon Wollaston as *Xyloterus longicollis* (Wollaston, 1864). In 1927, Eggers – unaware of Wollaston's descriptions – erected the genus *Dactylotrypes*, and included two species, *D. draconis* and *D. uyttenboogaarti*, based on associations with two different host species, the Canary date palm (*Phoenix canariensis*) and the dragon tree (*Dracaena draco*) (Eggers, 1927; Wood & Bright, 1992). Uyttenboogaart (1937) pointed out similarities between *D. uyttenboogaarti* and *D. draconis*, but it was not until Schedl *et al.*, (1959) revised the genus *Dactylotrypes*, that the aforementioned species were regarded as synonymous. Today, *Dactylotrypes longicollis* is regarded as a monotypic genus in the subtribe Dryocoetina (LaBonte & Takahashi, 2012).

Distribution & risk as pest

Dactylotrypes longicollis is native to the Canary Islands (Wollaston, 1864; Eggers, 1927; Uyttenboogaart, 1927; Enderlein, 1929). Today, it has been reported from all of the Canary Islands, except from El Hierro (see Schedl *et al.*, 1959; Israelson *et al.*, 1982; Machado & Oromi, 2000). Palm groves of *Phoenix canariensis* are absent on El Hierro (Obón *et al.*, 2018), which might be an explanation for poor establishments of *Dactylotrypes* on this particular island. Later, *Dactylotrypes* was also reported from Madeira (Liebmann, 1939; Jansson, 1940; Lundblad, 1958). During the past fifty years, it has spread throughout the entire Mediterranean region. It is established in France (Balachowsky, 1949; Perrot, 1955; Noblecourt, 2004), Spain (Palacios, 1973; Whitehead, 1993; Lombardero & Novoa, 1994; Lombardero, 1995; Riba, 1996), Italy (Sampò & Olmi, 1975a, 1975b; Bernabò, 1991; Longo *et al.*, 1991), Croatia (Whitehead *et al.*, 2000), Malta (Mifsud & Colonnelli, 2010), and Greece (L. Kirkendall pers. obs.). More recently, *Dactylotrypes* has even been found in North America (LaBonte & Takahashi, 2012) and in Chile in South America (Kirkendall, 2018). In addition, it has also been reported from gardens in Switzerland (Bovey, 1987) and in an attic in Haag, Netherlands (van Rossem *et al.*, 1974). However, *Dactylotrypes* has probably not been established in the two latter countries. Excluding the Macaronesian islands, *Dactylotrypes longicollis* is considered an invasive species in Europe (Kirkendall & Faccoli, 2010). Both *Phoenix canariensis* and *P. dactylifera* are widely used as ornamental plants across the Mediterranean (Bramwell & Bramwell, 1995; Morici, 1998; Pérez, 2000), and is probably one of the main reasons for the current distribution of *Dactylotrypes longicollis*. Some regard it as a serious pest (Sampò & Olmi, 1975a, 1975b; Longo *et al.*, 1991), as boring into seeds hamper the propagation, not only of ornamental palms, but potentially also of native flora (Kirkendall, 2018).

Morphology

General taxonomic treatments can be found in several sources (see Eggers, 1927; Enderlein, 1929; Lepesme, 1947; Balachowsky, 1949; Wood, 1986; Longo *et al.*, 1991; Pfeffer, 1995; LaBonte & Takahashi, 2012). ♀ Long: 1.5 – 2.3 mm. ♂ Long: 1.5 – 2.1 mm. Females on average slightly larger than males. Cylindrical body, dark brown in coloration and densely coated with short hairs. Head is relatively spherical, usually pulled under pronotum. Frons dimorphic; impressed in males, flattened or convex in females. Eyes are emarginated and kidney-shaped. The base of the antennal scape springs out anterior to the ventroproximal part of the eye. The antenna is club-shaped and consists of four segments between the club and the scape. Suture on the club is skewed towards the apical region. Pronotum is rounded and has approximately the same length as width, is slightly curved and densely punctuated. Elytra is approximately 1.4 times the length of the pronotum, and is parallel along the lateral margins for 2/3 of its length. Scutellum almost absent. In profile, the posterior part of the elytra is strongly declivous, and is not impressed adjacent to suture. The elytral declivity do not have any protruding spines. Tibiae are characteristic, and is an important structure for separation between similar bark beetles, such as *Coccotrypes*. The rounded distal margin of each tibia has 8-9 socketed teeth (Figure 1).

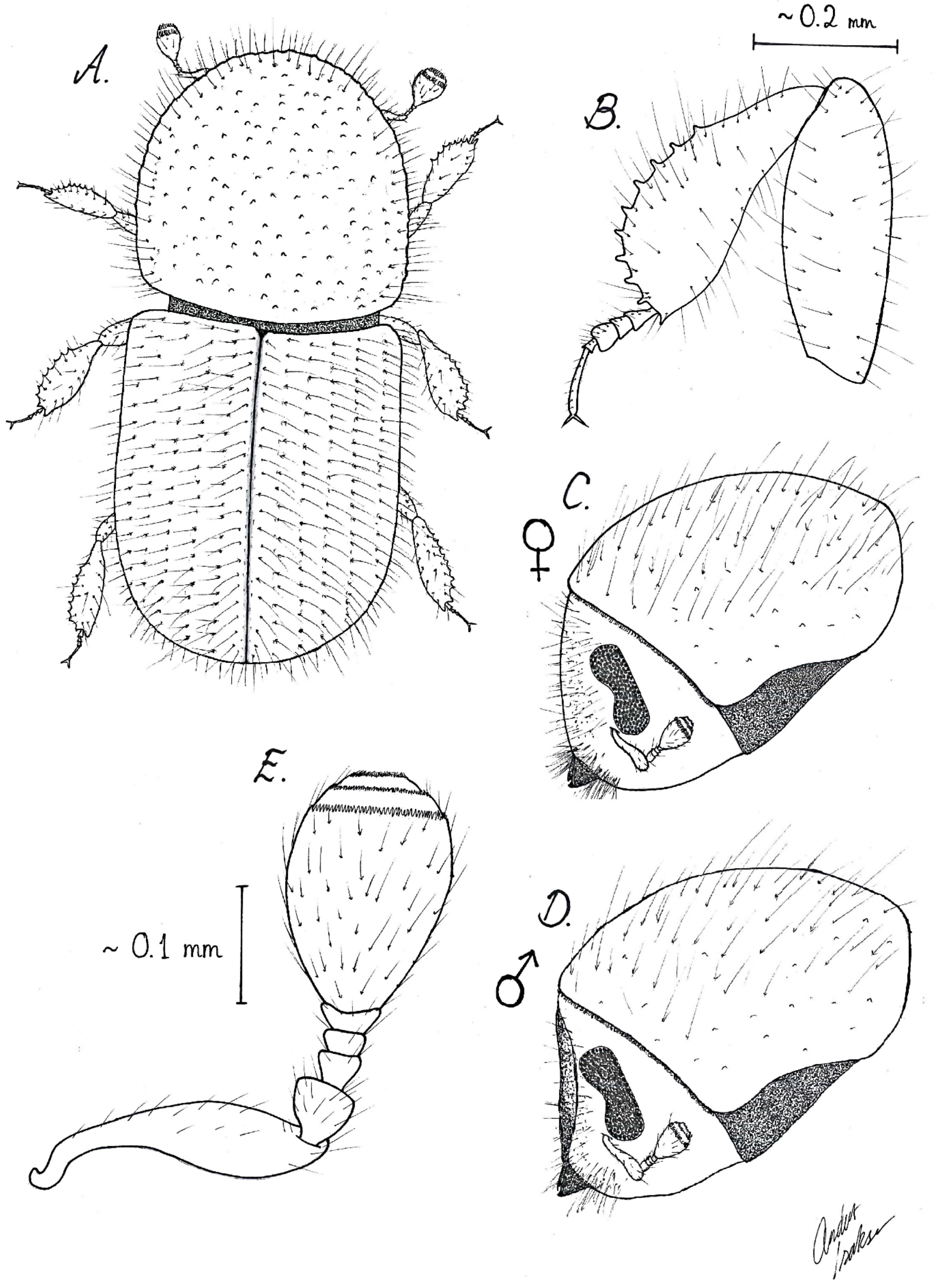

Figure 1. A) Dorsal habitus of *Dactylotrypes longicollis*. Total length ♀: 1.5 – 2.3 mm, ♂: 1.5 – 2.1 mm. B) Close-up of tibia. Notice the socketed teeth along the rounded margin. C-D) Lateral view of the pronotum and head of C) female and D) male. Notice the difference in frons, which in males are impressed, while in females it is rounded. E) Close-up of antenna. Notice the four segments between the club and the scape, which is only visible in microscope. Drawings by Anders Isaksen.

Plant hosts

Dactylotrypes longicollis is a spermatophagous species, breeding and feeding in seeds of a wide variety of plant hosts. Originally, *D. longicollis* feed and breed in the endocarp of the seeds of the date palm *Phoenix canariensis* (Figure 2), and the dragon tree, *Dracaena draco*, both native to the Canary Islands (Pérez, 2000). However, *Dactylotrypes longicollis* can be regarded as a seed generalist, inhabiting seeds from several other hosts, such as *Phoenix dactylifera* (Uyttenboogaart, 1927; Kleine, 1935; Jacobsen, 2001), *Trithrinax brasiliensis*, *Rhapis excelsa* (LaBonte & Takahashi, 2012), *Butia eriospatha*, *Chamaerops humilis*, *Phoenix pumila* and *Trachycarpus excelsus* (Wood & Bright, 1992; LaBonte & Takahashi, 2012). Some of these host relationships may be uncertain, as *D. longicollis* occasionally may have been misidentified as *Coccotrypes dactyliperda* (Balachowsky, 1949; Whitehead *et al.*, 2000). In laboratory, it has also been breeding and feeding in seeds of *Phoenix sylvestris* and *P. roebelenii* (pers. obs.).

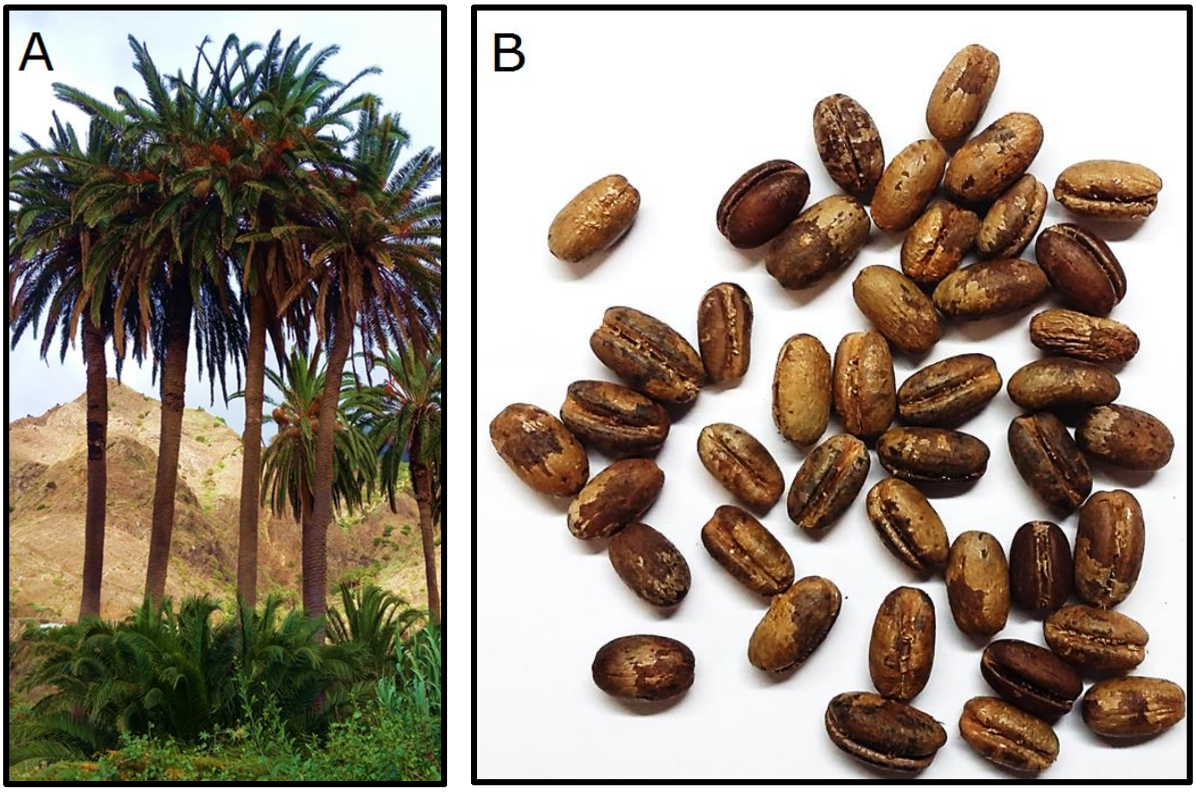

Figure 2. A) A cluster of *Phoenix canariensis* in Hermigua, La Gomera. B) Seeds of *Phoenix canariensis*. Size of single seeds approximately 15 x 10 mm. Photos: Anders Isaksen.

Interspecific interactions

The bark beetle *Coccotrypes dactyliperda* is widespread throughout the Mediterranean region, and is also abundant on the Canary Islands, where it breeds and feeds in the same seeds as

Dactylotrypes longicollis (Schedl *et al.*, 1959). *Coccotrypes dactyliperda* is relatively similar to *Dactylotrypes longicollis* in terms of ecology and life history traits (see Kleine, 1935; Balachowsky, 1949; Longo *et al.*, 1991). Resource depletion of seeds are more likely to occur if both species are present simultaneously within the same seed, as larvae of both species will be in stronger competition for food. Uyttenboogaart (1937) suggested that interspecific competition between *Dactylotrypes* and *Coccotrypes* might have been a driving factor in making the former species bore tunnels into the seeds of *Dracaeno draco*. During fieldwork, I never found *Dactylotrypes* and *Coccotrypes* residing within the same seed, *Dactylotrypes* was exclusively found in La Gomera while *Coccotrypes* exclusively was found in Tenerife. Jacobsen (2001) examined the possibilities of temporal segregation between the two species in Tenerife, and found evidence for coexistence by utilization of same hosts at different times of the year.

Other arthropods have been observed in close proximity to or within same seeds as *Dactylotrypes*, but their interspecific interactions with *Dactylotrypes* have never been investigated. Uyttenboogaart (1927) reported findings of *Corticarina delicatula* (= *Corticaria tenella*) (Coleoptera: Latridiidae) from Gran Canaria, and Longo *et al.* (1991) reported findings of the flat grain beetle *Cryptolestes (=Laemophloeus) juniper* (Coleoptera: Laemophloeidae) together with *Dactylotrypes* in Italy. Similarly, I found a species of *Cryptolestes* during the rearing procedure (Figure 3), which I identified to be *C. ferrugineus*, by the key in Biege & Partida (1976). In addition, Longo *et al.* (1991) found the ant beetle *Thanasimus formicarius* (Coleoptera: Cleridae) together with *Dactylotrypes*. *Thanasimus formicarius* is a known predator of several other bark beetle species. They prey on both larvae and mature adults (Herard & Mercadier, 1996; Wegensteiner *et al.*, 2015), and may in some cases drastically reduce brood sizes (Schroeder, 1997). The presence of *Thanasimus formicarius* together with *Dactylotrypes*, strongly suggest that *Dactylotrypes* also may be a common prey species. A hymenopteran species within the genus *Laelius* (Bethylidae) has also been reported in seeds of *Phoenix canariensis* (Longo *et al.*, 1991), although not together with *Dactylotrypes*. Several parasitoid wasps from the family Bethylidae (e.g. *Laelius elisae*) attack bark beetles (Wegensteiner *et al.*, 2015). Although no direct predation on *Dactylotrypes* have been observed, I find it likely that predation from both *Thanasimus formicarius* and *Laelius* sp. may be evident.

Mites and fungi were common within the boxes during the rearing procedure in the laboratory, especially in populations with old seeds. In addition, nematodes, bacteria and viruses are probably abundant within seeds. More research are needed to infer the effect of these interactions (but see Hofstetter *et al.*, 2015).

Figure 3: *Cryptolestes* sp. found together with *Dactylotrypes longicollis* during the rearing procedure. Photo: Anders Isaksen.

Life-history

Seed infestation

Normally, *Dactylotrypes longicollis* bore tunnels in seeds after they have fallen to the ground. The entrance holes are usually bored in the longitudinal hollow line of the seeds. However, females can also bore through fresh and sundried fruit flesh in order to reach the seed. During fieldwork, I never observed beetles boring tunnels in fruits still attached to the trees, although such has been reported previously (Longo *et al.*, 1991; LaBonte & Takahashi, 2012). I suppose that infestation on attached fruits is a strategy when the population density is high and the majority of the seeds on the ground are infested. Both females and males are able to fly (Eggers, 1927; pers. obs.), which enable both sexes to disperse in order to seek for uncolonized seeds or receptive females in less dense areas.

Mating system and gender roles

Except from copulation, which occur at the tunnel entrance, the entire reproductive life cycle of *Dactylotrypes longicollis* takes place within seeds. By the criteria in Kirkendall (1983), *Dactylotrypes longicollis* is monogamous. The female initiates a single longitudinal tunnel (Figure 4), whereby a male follows. When ready to court, the female position herself at the tunnel entrance, whereby the male bumps his frons against the female's elytra. If the female is receptive, she backs out of the excavated tunnel, exposing her posterior part of the abdomen. The male climbs up and inseminates the female. When copulation is finished, the females re-enters the tunnel. The male spend most of his time blocking the tunnel entrance, but as the female extends the tunnel and start deposit eggs, the male occasionally follows inside for brief periods. The males usually remain at the tunnel entrance during the oviposition period, and are thought to play a significant role for the numbers of produced offspring (Halvorsen, 2006), which may be enhanced by removal of frass and general maintenance of the tunnel.

Figure 4. Cross section of a seed where a female is boring a longitudinal tunnel. No male has arrived yet. Photo: Anders Isaksen.

Eggs

The eggs of *Dactylotrypes longicollis*, as in other scolytines, are oval in shape with a smooth surface and translucent white color (Figure 5A). They are deposited in rows of slight impressions alongside the tunnel walls, with the longitudinal axis of the egg parallel to the tunnel length (Figure 5B). The eggs are covered partially in white boring dust, which suggestively is held together by an oral secretion from the maxillary glands (Wood, 1982; Kirkendall, 1983; Figure 5B). In temperatures at 25°C, single females reportedly lays around

40-60 eggs during the oviposition period (Longo *et al.*, 1991), but according to Jacobsen (2001), single pairs may be able to breed 80-100 individuals under ideal conditions in large seeds of *Phoenix canariensis*. This is considerably higher numbers than observed by myself and by Longo et al. (1991), and I find these numbers to be artificially high. From breeding pairs (*n* = 26) in room temperatures, I obtained an average of 37 individuals per brood, whereby no dead individual larvae, pupa or unhatched eggs were observed. The number of oviposited eggs probably depends on abiotic factors such as temperature and humidity, whereby humidity seems to be more important than temperature in *Dactylotrypes longicollis* (Jacobsen, 2001).

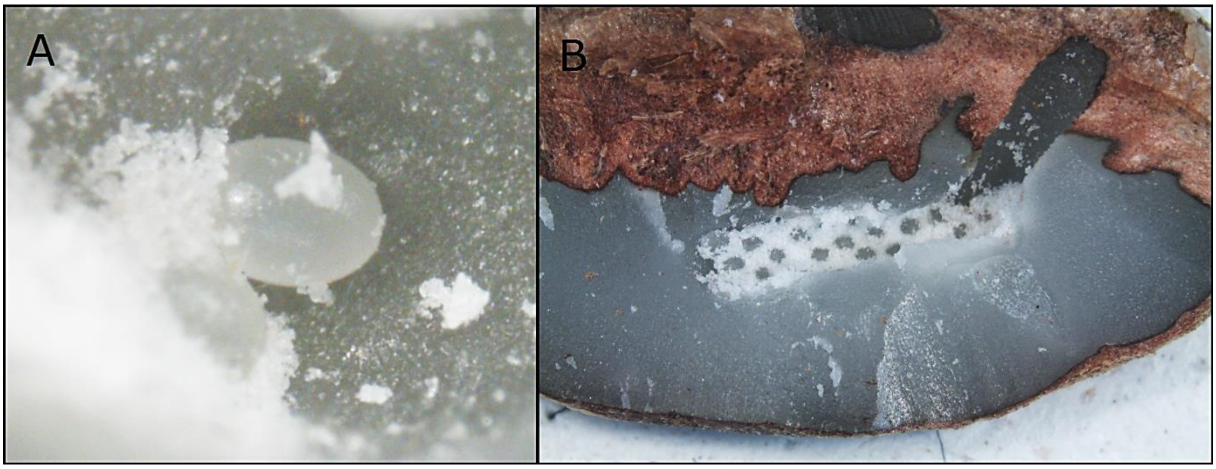

Figure 5. A) Close-up of eggs in the tunnel. B) Cross-section of a seed with deposited eggs in tunnel, covered in white boring dust. Photos: Anders Isaksen

A subsample of eggs (*n* = 20) were measured during the rearing period. The mean length with standard errors was 0.48 ± 0.008 mm and the mean width was 0.39 ± 0.006 mm. There is seemingly little variation in egg size. The eggs are relatively large compared to the female, and constitute approximately ¼ of the female's total body length. Hence, the oviposition period must be an extreme energy load for females. The eggs are fragile, and almost impossible to remove from the tunnels without breaking. Still, the majority of the eggs hatch, and no eggs or remains are usually found within seeds when dissected after the first individuals have started to emerge. The eggs seems to be relatively cold-resistant as they did not freeze after 15 hours exposure in - 8^{o}C. However, it is not known whether exposure to such cold regimes will result in hatching of the eggs or not. Concordant with most other bark beetle species (Wood, 1982; 2007), the eggs usually hatches within 10 days under normal conditions (in room temperature 22 ± 2^{0}C and relative humidity (RH) of 40 ± 20%).

Larvae

The larval period commences after hatching of the eggs. Similar to other weevils, the larvae are *C*-shaped and legless (Figure 6A). The head is sclerotized, and mandibles are present (Figure 6B). I measured a single outstretched larva (~2.4 mm) to be longer than the total length of adult female individuals. I assume that the variation in larval length may vary considerably within broods as intraspecific larval competition probably are significant within seeds. Larval development probably ranges from 30-40 days under ideal conditions, but last longer in conditions with lower temperatures and decreased humidity (Jacobsen, 2001; pers obs). The larvae bores tunnels perpendicular to the initial tunnel, and radiates towards the outer margin of the seeds. They feed on the endocarp within the seed, which over time gets more depleted (Figure 6C). The number of larval instars remains unknown.

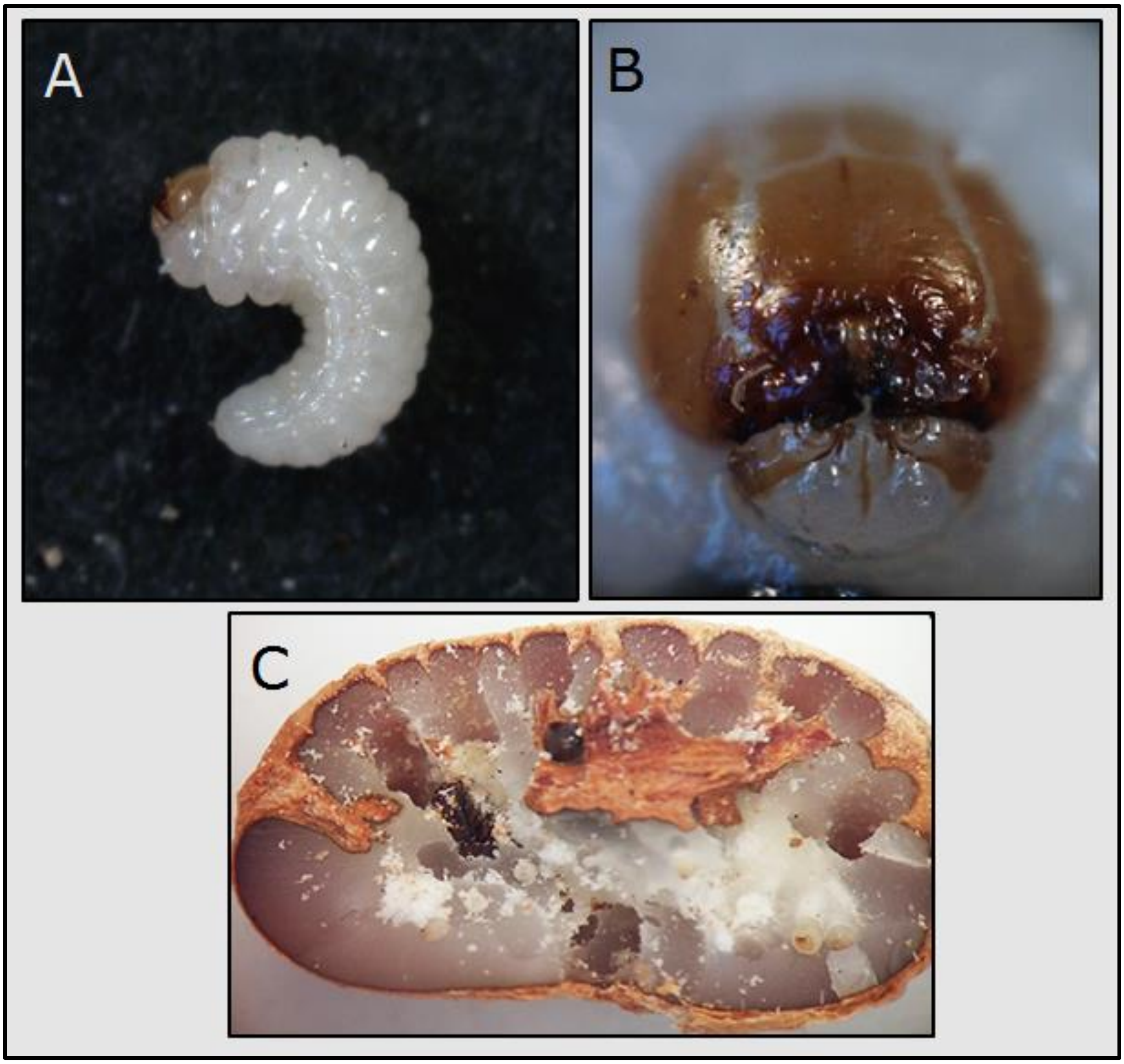

Figure 6. A) Lateral habitus of larvae. B) Apical view of head, which is clearly sclerotized. Mandibles are present. C) A cross section of a seed of *Phoenix canariensis*. Two larvae can be seen in the bottom right corner of the picture. Photos: Anders Isaksen.

Pupae & Immature adults

Pupae can be found in separate chambers towards the margins of the seed (Figure 7A). The chambers are cleared of frass. In the beginning, the pupae are eyeless, the shape of the pronotum is apparent, but the elytra remains absent (Figure 7B). Right before last transformation into adult stage, the pupae have developed eyes, mandibles have been formed, and legs are present (Figure 7C-D). The pupal stage lasts for approximately a week (Halvorsen, 2006; pers. obs.), as in similarly sized bark beetles (Wood, 1982). After final molt into the adult stage, there is probably still a short period where immature adults remain within seeds until reaching maturity. Immature individuals are characterized by being pale and yellow (in contrast to dark brown), which is due to incomplete sclerotization of the exoskeleton (Raffa *et al.*, 2015). Under ideal conditions, adults probably emerge relatively quick when mature. Most beetles emerge through either the initial tunnel or through an additional excavated exit tunnel. Emerging beetles will seek fresh seeds, and a start a new reproductive cycle.

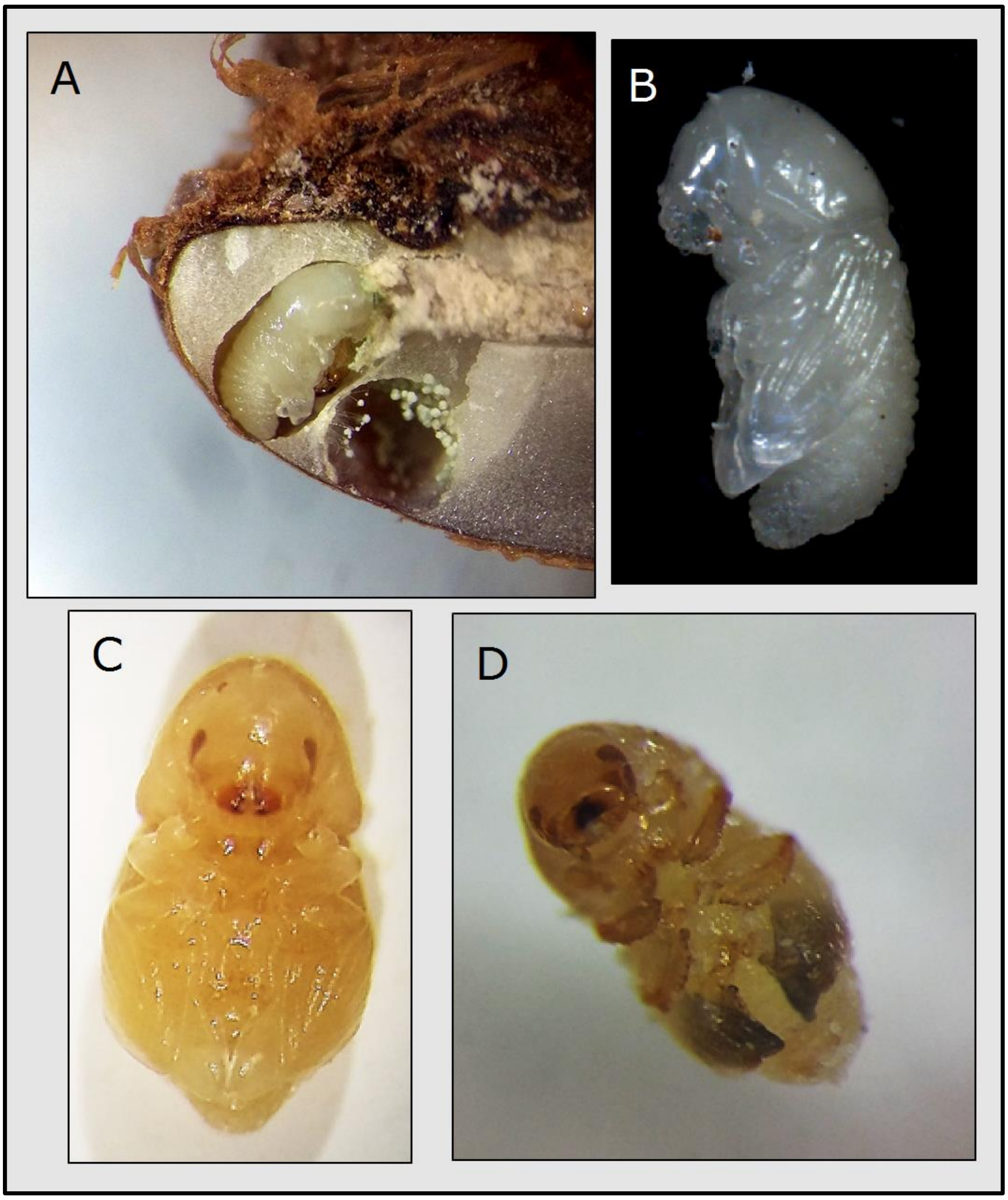

Figure 7. A) Pupa within a pupal chamber. B) Lateral view of pupa. C-D) Metamorphosis from the pupal stage into mature adult. C) Ventral view of pupa at a late stage. Legs are not fully developed, but eyes and mandibles are present. D) Last transformation from pupa into immature adult. Legs have developed, but the elytra is still not shaped. The pupa starts to resemble a mature adult. Photos: Anders Isaksen

Longevity and duration of the reproductive cycle

Adult longevity is hard to designate, especially for oviposited females as they usually do not emerge from seeds, but remain within seeds for the rest of their life. I observed a male living for at least 79 days after been put together with a female to mate. Males tend to come out of the tunnel after the oviposition period, but were often found deceased after a couple of days after re-emergence. Occasionally, both females and males from a pair can be found deceased within the seed, which makes an accurate determination of their longevity difficult.

Under ideal conditions, the duration of developing individuals from oviposition of the eggs until mature adults takes approximately 2 months (Table 1). However, Uyttenboogaart (1927) reported 5 months for completion of one generation, while Longo *et al.* (1991) experienced a period of 3 months. The duration of the reproductive cycle in *Dactylotrypes longicollis* depends largely on environmental factors, such as temperature and humidity (Jacobsen, 2001), and probably also biotic factors such as intraspecific larval competition (e.g. Anderbrant *et al.*, 1985) and interspecific interactions (see paragraph above).

Table 1. Duration of a complete reproductive cycle in *Dactylotrypes longicollis*, including the longevity of mature adults (only observed for males). Data based on rearing under ideal conditions (T=25°C, RH=75%)

Stage	Duration (days)
Egg:	7-10
Larvae:	30-40
Pupae:	7-10
Immature adults:	~3-5?
Mature adults (males):	~70-80?
Total:	~120-145

Further notes on mating system

A monogamous mating system usually does not exclude the potential for additional mating besides the initial pair (see for example Wittenberger & Tilson, 1980; Wickler & Seibt, 1983). However, I find it unlikely that subsequent copulations are very common in *Dactylotrypes*. Females do not actively seek additional partners as they are confined to their excavated tunnel for the rest of their life. Males usually stays with the female during the whole oviposition period, sometimes even for the rest of his life, and thereby prevents other males from entering. If disturbed (by for example shaking a seed), males may leave the tunnel and discard the female (pers. obs. in field and laboratory). Whether such ‘unreliable’ males remate with other females remain uncertain, but in the laboratory, it was possible to pair virgin females with previous mated males. I suppose that the longevity of adult beetles is so short, that this will result in less paternal care during the oviposition period. Whether fleeing males are occurring in natural settings remains uncertain.

In laboratory, virgin males did follow into tunnels of already mated females in cases where the initial male was removed artificially. Non-virgin females did not seem to be interested in mating with a subsequent male, as she never was observed backing out of the tunnel. I assume that she invests her time and energy in oviposition rather than prioritizing additional copulations. Subsequent encountered males usually left the tunnel within a day, assumingly without copulating. Based on these observations, I find it likely that both females and males tend to mate only once during their lifetime.

In lab, I never observed females mating more than once. I suppose that this is normal, as the duration of the oviposition period are long-lasting. Although males probably are able to transform ejaculates several times, they also tend to mate only once, as their prolonged period of parental care prevent covert mating. The first ejaculate probably contains enough sperm for the female to fertilize all her eggs, over a prolonged oviposition period. I find it unlikely that covert female mating can lead to sperm displacement and sperm competition even if the first mate leaves the tunnel. Hence, it is unlikely that post-copulatory mate choice by for example sperm competition and sperm displacement by the female occurs in *Dactylotrypes*. As mentioned, the effect of paternal care in *Dactylotrypes* has been demonstrated to be important for the number of offspring within a single brood (Halvorsen, 2006). I therefore suggest that the prolonged relationship between a female and a male mainly stems from enhanced reproductive output, and not male guarding of females to ensure paternity.

Dactylotrypes longicollis is considered an outbreeding species. However, inbreeding seems to occur occasionally in laboratory (Hestvik, 2002; pers.obs), especially in cases where additional fresh seeds are not available and the original seed is not depleted for resources (pers.obs.). If the endocarp in a seed remain partly intact, emergence is probably not a requirement for initiation of a reproductive life cycle. Under such circumstances, beetles may remain and assumingly form pairs between full-siblings. This suggestion indicates that resource availability may have important evolutionary consequences on mating strategies. However, the magnitude and scope of inbreeding in *Dactylotrypes* are probably low or may even be absent under natural conditions. The proposed occurrences of inbreeding are still speculative, and should ideally be confirmed by genetic analyses.

Sometimes resources will be scarce. In scenarios with high population density, suited seeds for infestation may be over-exploited by high numbers of beetles. In field, I observed several individuals of both females and males that were occupying the entire groove of seeds (Figure 8). In such scenarios, some females within the population might fail to initiate tunnels. Following, the operational sex ratio may be distorted, setting the stage for male-male competition for limited females, or females may compete for access to limited resources. As inferred by the results from the first part of this thesis, body size does not confer any advantage under such circumstances.

Figure 8. Competition over limited resources. Several females and males in the groove of a seed of *Phoenix canariensis*. This picture was taken during rearing in laboratory, but similar scenarios was observed in the field in Hermigua, La Gomera in July 2018. Photo: Anders Isaksen

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AFTERWORDS

It is approximately one year since I first put my foot on the scenic island of La Gomera starting my scientific foray into exploring the world of *Dactylotrypes longicollis*. The year has been overwhelmingly educational. The micro-cinematographer Dietmar Fill once said: "When I look into a microscope, I might as well look through a telescope, into the universe. It's the same thing. The spaces are the same." I find these words to neatly sum up my feelings for this thesis. Occasionally, the thesis feels somewhat insignificant. On the other hand, it feels bigger than what I can express with words. When I observe the beetles in the microscope, watching their behaviors, in their surroundings, I feel lucky to get a glimpse of their *umwelt* – their universe!