

Reproductive traits and number of matings in males and females of *Cerambyx welensii* (Coleoptera: Cerambycidae) an emergent pest of oaks

L.M. Torres-Vila*, F.J. Mendiola-Díaz, Y. Conejo-Rodríguez and Á. Sánchez-González

Servicio de Sanidad Vegetal, Consejería de Agricultura DRMAyE, Gobierno de Extremadura, Avda, Luis Ramallo s/n, 06800 Mérida, Badajoz, Spain

Abstract

The longhorn beetle *Cerambyx welensii* is an emerging pest involved in oak decline episodes, whose damage is increasingly reported in dehesa open woodlands. Knowledge of the reproductive biology of *C. welensii* is a crucial goal due to its new pest status. In this study, we assess the reproductive traits of both sexes in the laboratory (25°C and 60% relative humidity). In females, body length was 44.9 ± 0.9 mm (mean \pm SE), fecundity 132 ± 12 eggs, fertility 70 ± 1 %, longevity 70 ± 3 days, pre-oviposition period 2 ± 0.2 days, oviposition period 44 ± 3 days and postoviposition period 19 ± 3 days. Fecundity was positively correlated with female size, longevity and oviposition period. Daily fecundity was 3.0 ± 0.2 eggs/day and showed a fluctuating synovigenic pattern with a slight decreasing trend over time. Egg length was 4.24 ± 0.01 mm and egg volume 8.14 ± 0.04 mm³. Egg size was correlated with female size but the relative size of eggs was larger in smaller females. Incubation time was 13.9 ± 0.1 days and hatching did not depend on egg size. Neonate size was positively correlated with egg length. Females were polyandrous (more than 20 lifetime matings) but multiple mating did not increase fecundity, fertility or longevity. In males, body length was 43.7 ± 0.6 mm and longevity 52 ± 3 days. Unlike with females, longevity was positively correlated with male size. Males were polygynous (up to 30 lifetime matings) but mating history did not affect male longevity. Rather to the contrary, long-lived males mated more times because they had more mating chances. Lastly, *C. welensii* reproductive traits were compared with those other Cerambycidae species and discussed from an adaptive perspective. Our data will be useful to improve management of *C. welensii* in order to prevent or mitigate its impact in dehesa woodlands and other oak forests.

Keywords: oak decline, body size, reproductive output, longevity, daily fecundity, egg size, oviposition period, mating number, polyandry, polygyny

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Introduction

The beetle *Cerambyx welensii* Küster (= *C. velutinus* Brullé) (Coleoptera: Cerambycidae: Cerambycinae) is among the largest longhorn species in the Palaearctic fauna (Bense, 1995). Its host plants include species in the genus *Quercus*, although this wood-boring beetle has been also occasionally reported from other broadleaf trees (Bense, 1995; Vives, 2000). Populations of *C. welensii* are most abundant in the southern part of its

*Author for correspondence
 Phone: +34 924 00 25 30
 Fax: +34 924 00 22 80
 E-mail: luismiguel.torres@gobex.es, luismiguel.torresvila@gmail.com

European distribution, particularly in dehesa ecosystems, a singular type of Mediterranean open woodland similar to savannah landscape, typically populated by holm oak (*Quercus ilex* L.) and cork oak (*Quercus suber* L.) (Montero *et al.*, 1998). Like other wood-boring longhorns developing on oaks, *C. welensii* was thought to primarily utilize old, decayed and diseased trees, forming part of the highly diverse assemblage of saproxylic insects. The activity of this functional group of species – often in combination with wood-degrading saprophyte microorganisms – is considered essential in ecological and biodiversity terms, particularly for being primary producers of arboreal cavities and shelters exploited as niches for an array of animals, including invertebrate, reptile, birds and mammals (Grove, 2002; Buse *et al.*, 2008).

The impact of *C. welensii* has changed drastically in last decades. This insect is now found causing injuries to healthy and young trees, threatening the high resilience and stability of the dehesa ecosystem (Martín *et al.*, 2005; López-Pantoja *et al.*, 2008; Carrasco, 2009; Torres-Vila *et al.*, 2012a, 2013). A large study on 6000 trees across Extremadura (Southwestern Spain) showed that nearly 40% of oak trees had longhorn galleries and 10% had larval activity (Naveiro *et al.*, 1999). Tunnelling activity of larvae into cambium and xylem can alter sap flow, trigger wilting, die-back, leaf fall, vigour loss and tree decay. Larvae bore increasingly wider and longer galleries into sapwood and heartwood along the tree trunk and main branches causing huge physiological, mechanical and structural damage, and even tree death (Torres-Vila *et al.*, 2012a, Sallé *et al.*, 2014). In addition, larvae favour the spread of oak pathogens such as the charcoal disease by creating entryways to the inner tissues (Martín *et al.*, 2005). Therefore, *C. welensii* is considered a major inciting factor involved in oak decline in the Iberian Peninsula (López-Pantoja *et al.*, 2008; Carrasco, 2009; Torres-Vila *et al.*, 2012a; Morales-Rodríguez *et al.*, 2015), and acts similarly to that reported for other wood-boring insects (Führer, 1998; Thomas *et al.*, 2002; Evans *et al.*, 2004; Sallé *et al.*, 2014).

The increase in oak decay in Spain is often attributed to the mismanagement and overuse of dehesa woodlands in recent decades. Abusive pruning, improper decorking, poor forestry practices (lack of reforestation and protection of seedlings/saplings), livestock overgrazing and trampling, reduced use of soil-conditioning crops, low natural regeneration and ultimately progressive ageing are major factors predisposing to oak decay (Carrasco, 2009; Torres-Vila *et al.*, 2012a). Among them, inappropriate pruning and decorking practices, providing suitable egg-laying sites and promoting early larval survival, have likely contributed to a population increase of *C. welensii* making it an oak pest. Additionally, oak decay due to wood-borers and other insects is likely to be intensified under the current climate change scenario. There is increasing evidence that the impact of secondary oak pests may increase with warming and drought, especially in Southern Europe (Allen *et al.*, 2010; Sallé *et al.*, 2014) and *C. welensii* could be an example of this. In any case, *C. welensii* is currently considered an emerging pest and a serious threat to dehesa woodlands, and there is a need to control it, at least in the short term (Carrasco, 2009; Torres-Vila *et al.*, 2012a, 2013).

Adults of *C. welensii* are large and striking beetles whose occurrence in the wild does not go unnoticed to amateur and professional entomologists. Hence, *C. welensii* records are frequent in national and local faunas and its geographic distribution is well known (Bense, 1995; Vives, 2000; González-Peña *et al.*, 2007) even if occasional

misidentifications or misuses by confusion with *Cerambyx cerdo* L. have occurred (Del Moral *et al.*, 1989; Sallé *et al.*, 2014). The large size, robustness and taxonomic value of the chitinous structures of *Cerambyx* have even enabled the study of adult remains in aspects such as the fossil record (Harding & Plant, 1978) and the raptor diets (Fattorini *et al.*, 1999).

The knowledge of the distribution of *C. welensii* contrasts sharply with the poor understanding of its reproduction and mating system. These aspects of its biology have received little attention in this longhorn as in most cerambycids (Hanks, 1999). Several factors could have limited field and laboratory research, including adult crepuscular habits, absence of long-distance sex pheromones, long adult diapausing stage, extended life cycle and particularly the difficulty of laboratory rearing (Hanks, 1999). Moreover, due to the seemingly reduced impact of *C. welensii* in the past, this species has been generally regarded as a secondary pest (or not a pest at all), so that the scientific literature dealing with its biology is very scarce (Sallé *et al.*, 2014). As a result of the new status quo of *C. welensii*, recent research has shed some light on important aspects of its ecology, including flight behaviour, dispersal potential, adult lifespan, population density, semiochemical attraction, mass trapping and natural enemies (López-Pantoja *et al.*, 2008; Torres-Vila *et al.*, 2012a, 2013; Sánchez-Osorio *et al.*, 2015; Morales-Rodríguez *et al.*, 2015).

The prevalence and impact of *C. welensii* in dehesa open woodlands is largely dependent on its reproductive output and fitness, aspects that remain to be investigated. The knowledge of *C. welensii* fecundity is a critical goal, not only to understand population dynamics and potential damage to host trees, but also to improve pest control strategies. In fact, means of managing bark and borer insects associated with oaks are almost non-existent (Evans *et al.*, 2004). Predicting the pattern of abundance of bark and boring beetles would be highly desirable to prevent oak decline in Europe (Führer, 1998). Consequently, this study deals with assessing potential reproductive output, daily reproductive patterns, number of matings and related biological traits in both sexes of *C. welensii* under optimal laboratory conditions.

Materials and methods

Study species

C. welensii is univoltine flying from late May to early August. Adults are large (25–60 mm long) with a blackish-brown body and show sexual dimorphism. Females are slightly larger than males, but antennae are longer in males (twice as long as the body) than in females (just extend to the elytral apex). Adults feed mainly on sap and tree exudates while larvae are xylophagous. Daily activity of adults (feeding, flight, male fights for mates, mating and egg-laying) takes place mainly at dusk and early evening. Mated females wander over the host tree probing the bark with the ovipositor and lay eggs into suitable bark crevices and pruning wounds. After hatching, neonate larvae bore directly into the inner bark and initiate feeding. Larval development usually lasts 2–3 years. Pupation occurs in late summer within a pupal cell in the sapwood. Adults emerge from pupae in the autumn and overwinter protected within the pupal cell in a prereproductive status until the following year (late spring to early summer) (Vives, 2000; Torres-Vila *et al.*, 2012a).

Insect origin and adult preparation for tests

Insects used in tests were collected in dehesa open woodlands at about 50 locations across Extremadura (southwestern Spain) during three consecutive years (2012–2014). Collections were made taking advantage of a parallel study dealing with the specific distribution and assemblage of large apoxylic cerambycids in *Quercus* species (mostly holm oak, *Q. ilex* L. and cork oak, *Q. suber* L.). Virgin adults were obtained in three ways: (1) collecting overwintering adults during October–April by cutting host trees with a chainsaw (mainly recently fallen branches) and carefully opening the logs using metal wedges; (2) rearing the mature larvae collected to adulthood on an agar-based artificial diet (Morales-Rodríguez *et al.*, 2015); and (3) rearing larvae to adulthood from eggs obtained from field-collected ovipositing females on the same diet in the laboratory, although this method was not very profitable as artificial rearing is difficult in this species. The proportion of adults obtained by each of the three methods was about 80, 18 and 2%, respectively. Larvae were individually reared at room temperature (22–28°C and 50–70 relative humidity (r.h.)) in aerated 140 ml plastic containers. Pupae were held in rolls of laboratory blotting paper from Albel[®] (15–20 mm in diameter, 21 cm long), which were sealed on both ends with staples and arranged horizontally in plastic trays. Paper roll diameter was adjusted simulating a pupal cell, so that pupae were neither too loose nor too tight. In this way, the adults properly extended the wings and elytra. After emergence (September–October) diapausing adults were kept in the dark in a refrigerator (6–10°C) to overwinter.

In early June all adults were slowly warmed to 25°C to avoid thermal shock and allowed to complete sexual maturation before starting experiments (about 1–2 weeks later). Adults were considered to be sexually mature when they expelled the meconium and began feeding (see below). That day was assumed to be the day of emergence from the host tree and was scored as ‘day one’ to determine adult longevity. Adults were then sexed, measured (body length) and individually marked with identifying numbers by sex. A fine layer of waterproof white correction fluid (Tipp-ex[®], BIC, France) was applied on each elytron (a rectangle of about 6 × 12 mm²) and after drying the reference number was written twice with a black permanent fine marker (Lumocolor[®], Staedtler, Germany). In some cases, numbers were written directly on the elytra using a fine white permanent-ink marker (Paint Marker[®], Pentel, Japan). In males, a small white spot was also painted on the pronotum to readily distinguish both sexes under the reduced lighting of the artificial dusk used in the experiments (see below).

Laboratory tests: general procedures

Laboratory tests were conducted in the summer coinciding with the presence of active adults in the field. We used 161 cardboard cages with a transparent cover as mating and oviposition chambers. A single cage was assigned to each female throughout her life to avoid handling errors. Males shared cages with females during tests and were kept in well-aerated 240 ml clear plastic containers during inactivity periods. Caged males and females were regularly sprayed with water and fed ad libitum on a saturated sugar–water paste simulating host tree exudates. Wood disks were prepared as an egg-laying substrate for females. Freshly cut cork oak branches

70–80 mm in diameter were sliced with a circular saw to produce disks 20 mm thick that were frozen until use. Cork layer was detached in one piece from cambium with a penknife and was put back and held in place with a rubber band. Decorking of disks greatly facilitated daily inspections and egg removal (see below). A wood disk bearing the female reference number was used per cage (being replaced by a new as necessary).

All tests were performed in a controlled environmental room at 25 ± 1°C, 60 ± 10% r.h. and a L(14 + 2):D8 photoperiod, simulating typical summer conditions in the study area (July). The first 14 photophase hours were at a 1000 lux luminosity and the last 2 h at 25 lux simulating dusk. The sexual activity of adults was continuously observed at dusk, and observations were continued during the dark phase when necessary using a small red light LED lantern to avoid disturbing adults. Under the conditions described, adults fed, mated and oviposited normally.

Female tests

An array of variables were recorded to characterize the reproductive output of *C. welensii* females ($n = 45$), including fecundity (total eggs), fertility (per cent of eggs that hatched), preoviposition period (the elapsed time between mating and first oviposition), oviposition period (the time between first and last oviposition), postoviposition period (the time between last oviposition and female death), longevity, daily fecundity and egg size. Some variables were just studied in a random subset of females (see below).

The effect of number of matings (polyandry) on fecundity was assessed by allowing virgin females to mate singly or multiply, from one to more than 20 times. Females were scored in seven classes according to their lifetime mating number (1, 2, 3, 4–5, 6–10, 11–20 and 20 < matings/female). Two marked males were caged with each 3–4-day-old virgin female, 15–30 min before the onset of artificial dusk. If mating occurred, the unmated male was removed quickly to avoid male fights leaving the pair-bonded adults in the cage. The mated male was removed the next morning when the mating was completed. If mating did not occur within the dusk period, the two males were removed leaving the female isolated in her cage to prevent unobserved matings overnight. New males were routinely added/removed every 2–5 days following the same protocol until the predetermined number of matings was achieved for each female. Matings (both pair-bonding and male intromissions) were always verified at dusk (fig. 1A, B).

Wood disks were daily inspected after removing the cork layer and eggs counted to assess daily fecundity (fig. 1C). The small room between the cambium and cork layer showed to be extremely attractive for ovipositing females as most eggs were found in this location (>95%). Eggs were carefully detached, stored by date in well-aerated 30 ml plastic vials and incubated at 25°C in the environmental chamber. Per cent fertility was assessed after hatching. Eggs damaged when they were removed from wood disks were excluded from fertility estimates. Some unhatched eggs in which a dead larvae was clearly visible were considered fertile (<5%). Mated but unfertilized females (100% unhatched eggs) were excluded from the data analysis. Incubation time (the elapsed time between egg laying an eclosion) was also determined in a random sample of 523 eggs obtained from 33 females over the first 3 weeks of the oviposition period.

Mean egg size was estimated for each tested female over 3–4 weeks from five eggs randomly chosen per oviposition

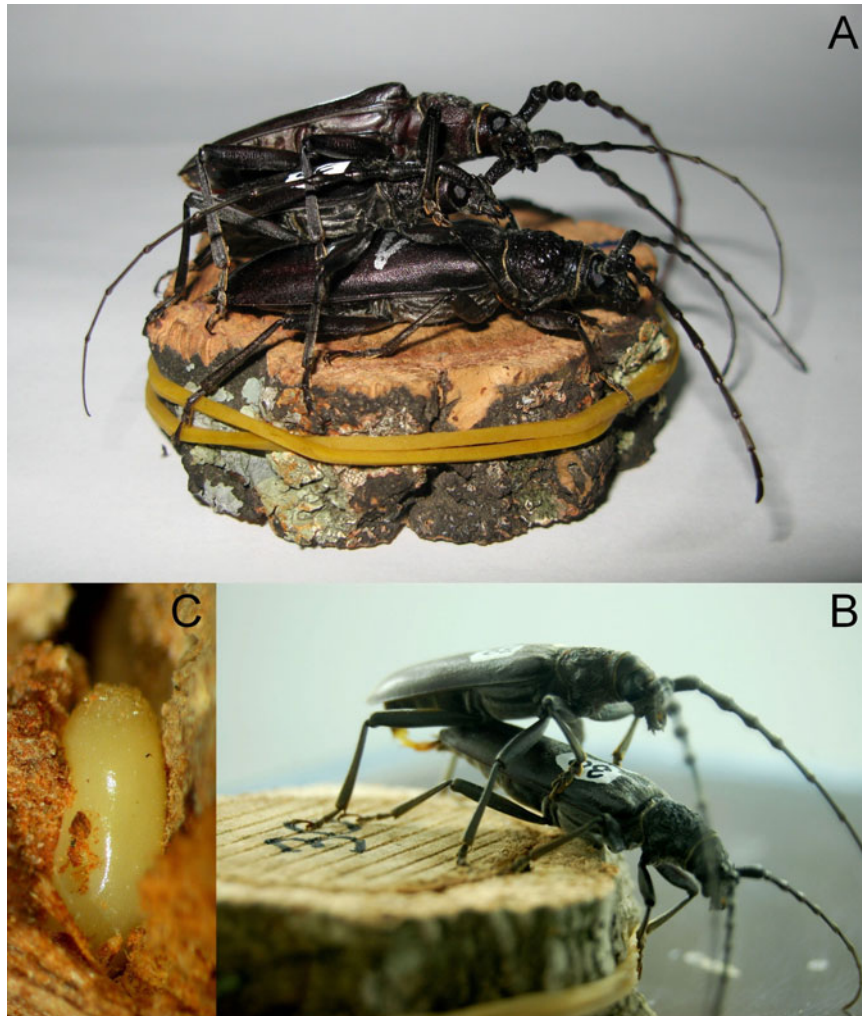


Fig. 1. (A) A conflict between two *C. welensii* males for a female on the cork oak disk used as egg-laying substrate, (B) mating at dusk of *C. welensii* during male intromission and (C) a recently laid egg of *C. welensii* with the micropylar region visible on the top.

day, or from all eggs if fewer eggs were available in a given day (total sample $n = 1600$ eggs). Eggs were measured using a Nikon DS-U1 digital camera connected to a Leica S6D stereomicroscope. Resulting images were analysed with Eclipse Net 1.20 software to determine egg dimensions: length l , width w and thickness t . Egg volume (V , mm^3) was calculated as an ellipsoid according to the formula $V = \pi/6 (l \cdot w \cdot t)$. The correlations between egg size (estimated as either length or volume) and neonate size (head width), and between head width and mandible size (length from condyle to apex) was determined in a sample of 50 eggs from 29 females. We used as estimator of mandible size the average length of both mandibles as the left one tended to be longer (see results).

Male tests

The reproductive output of *C. welensii* males ($n = 78$) was assessed in terms of number of matings (polygyny) and longevity. Both variables were obtained from the set of males that were allowed to mate with the tested females. In addition, the effect of number of matings on male longevity was also

investigated following an experimental approach. We compared two randomly chosen male groups (10–12 males per group) that were allowed to mate at either a high (one mating every 2–3 days) or low (one mating per week) mating rate. Each male was caged with two females 15–30 min before dusk, the unmated female was removed after pair-bonding and the mated female was removed the next morning when mating was completed. If mating did not occur at dusk, the two females were removed to prevent unobserved matings overnight. New females were routinely added/removed according to the required mating rate and this protocol was repeated throughout the male's lifetime.

Data analysis

All analysed variables were tested for a departure from normality prior to statistical test computation using probability plots. Only per cent fertility was arcsine transformed. Linear regression analysis was used to test the correlation between some reproductive variables, both in females (lifetime fecundity, daily fecundity, fertility, longevity, oviposition

period, number of matings, egg size, neonate size and female length) and males (longevity, number of matings and male length). Nested and Model I analysis of variance (ANOVAs) (either one- or multiple-way) were computed for comparison of means and to explore the interaction between some of the studied traits. A nested ANOVA was computed to assess the effect of egg size (nested to female as random factor) on egg hatching. We exploited a subset of 22 females in which a sufficient sample of hatched (chorions) and unhatched eggs was available. We measured five eggs per egg-laying day or all eggs if fewer eggs were available as usual ($n = 1117$ eggs). The effects of female and oviposition week on incubation time were explored through a two-way ANOVA, in which both oviposition week (two classes: eggs laid either on the first week or on the second-to-third week) and female itself were considered fixed factors. Analysis of covariance (ANCOVA) was used to examine the effect of female mating number (fixed factor) on fecundity using female size as covariate (Sokal & Rohlf, 1995). ANCOVA assumptions were verified prior to analysis (see results). All analyses were performed with Systat (2000) software.

Results

Females

Female length (mean \pm SE) was 44.9 ± 0.9 mm (range 34–58) in the studied sample ($n = 45$). Female fecundity was 132 ± 12 eggs (range 20–286) and per cent fertility was 70 ± 1 % (range 60–78). Female longevity was 70 ± 3 days (range 22–117). The preoviposition period was 2 ± 0.2 days (range 1–7), the oviposition period 44 ± 3 days (range 10–84) and the post-oviposition period averaged 19 ± 3 days (range 1–71). Fecundity was positively correlated with female length (fig. 2A) and with female longevity ($R^2 = 0.13$, $F_{1,43} = 6.47$, $P = 0.015$). However, the linear relationship was stronger between fecundity and oviposition period ($R^2 = 0.34$, $F_{1,43} = 22.12$, $P < 0.001$). Larger females did not live longer ($R^2 < 0.001$, $F_{1,43} = 0.0008$, $P = 0.98$) but showed longer oviposition periods ($R^2 = 0.12$, $F_{1,43} = 5.82$, $P < 0.05$). Per cent fertility was not related to either fecundity ($R^2 = 0.05$, $F_{1,43} = 2.14$, $P = 0.15$) or female length ($R^2 = 0.01$, $F_{1,43} = 0.33$, $P = 0.57$).

Mean daily fecundity (\pm SE) averaged 3.0 ± 0.2 eggs/day and ranged from about 0.8–5.8 eggs/day, so that there was considerable fluctuation even between consecutive days (fig. 3A). There was a slight, but significant, decrease in daily oviposition over the female's oviposition period (fig. 3A). Maximum daily fecundity ranged widely depending on female size and egg laying day, with some large females reaching values of up to 30–40 eggs/day (fig. 3B). Mean daily fecundity of each female positively correlated with her body size, results being similar when days with no eggs laid were excluded (fig. 2B). Consequently, large females had higher reproductive output than small females over the whole oviposition period (fig. 3A, B).

Mean (\pm SE) egg dimensions, length, width and thickness were $l = 4.24 \pm 0.01$ mm (range 2.7–5.6), $w = 2.25 \pm 0.01$ mm (range 1.5–3.1) and $t = 1.64 \pm 0.01$ mm (range 0.8–2.3) ($n = 1600$ eggs). Therefore, egg volume averaged 8.14 ± 0.04 mm³ (range 3.8–16.1). Egg size increased very slightly during the first egg-laying week, remained relatively constant for much of the oviposition period and showed a substantial drop in the fourth week (fig. 3C). Egg size was positively correlated with female length when the first-day eggs were considered,

and a similar relationship was obtained when considering the first-week eggs (fig. 4A). However, the relative size of eggs (the ratio egg length/female length) was negatively correlated with female length (fig. 4B), so that smaller females produced proportionally larger eggs. A nested ANOVA showed differences in egg size among females ($F_{21,22} = 12.93$, $P < 0.001$) but the size of hatched and unhatched eggs was not significantly different ($F_{22,1073} = 1.11$, $P = 0.33$) showing that hatchability did not depend on egg size.

Incubation time was 13.9 ± 0.1 days (range 7–22, $n = 523$ eggs) and was unrelated to mother size ($F_{1,31} = 0.15$, $P = 0.70$, $n = 33$ females). Eggs laid by a female within a single day did not hatch synchronously, variation of up to one week was observed. A two-way ANOVA showed that incubation time was significantly affected by individual female ($F_{32,457} = 5.01$, $P < 0.001$), oviposition week ($F_{1,457} = 9.05$, $P < 0.01$) and their interaction ($F_{32,457} = 3.27$, $P < 0.001$). The interaction arose because differences among females in incubation time depended on the oviposition week (i.e. female age) which was evidenced by successive one-way ANOVAs for each female: in 24 females (73%) incubation time did not change with age, but in eight females (24%) incubation time decreased and even in one female (3%) increased. Neonate size (head width) was positively correlated with egg length ($R^2 = 0.17$, $F_{1,48} = 9.89$, $P < 0.01$) but unexpectedly was unrelated to egg volume ($R^2 = 0.01$, $F_{1,48} = 0.41$, $P = 0.53$). Lastly, the left mandible tended to be longer than the right one ($F_{1,49} = 5.26$, $P < 0.05$, single larvae computed as blocks in the ANOVA) but mean mandible size was correlated with head width ($R^2 = 0.17$, $F_{1,48} = 9.99$, $P < 0.01$).

C. welensii females were highly polyandrous and some of them mated more than 20 times during their lifetime. ANCOVA results showed that the number of matings did not significantly affect fecundity ($F_{6,37} = 1.35$, $P = 0.26$) when controlling for female size ($F_{1,37} = 53.15$, $P < 0.001$). ANCOVA assumptions were verified prior to analysis: there was linear independence between mating number and female length ($F_{6,38} = 0.82$, $P = 0.56$), linear dependence between fecundity and female length ($F_{1,43} = 66.66$, $P < 0.001$) and homogeneity of regression slopes ($F_{6,31} = 0.61$, $P = 0.72$). Linear regression also showed that the number of matings did not affect fertility ($R^2 = 0.06$, $F_{1,43} = 2.71$, $P = 0.11$) nor longevity ($R^2 = 0.002$, $F_{1,43} = 0.08$, $P = 0.78$).

Males

Male length (mean \pm SE) was 43.7 ± 0.6 mm (range 31–54) in the studied sample ($n = 78$) and longevity was 52 ± 3 days (range 11–144). Thus, females were larger and lived longer than males ($F_{1,121} = 14.13$, $P < 0.001$). Unlike what was observed with females, male longevity was positively correlated with body size, so that large males lived longer than small males ($R^2 = 0.13$, $F_{1,76} = 11.58$, $P < 0.01$; fig. 5A). Results showed that *C. welensii* males were extremely polygynous, some of them being able to mate up to 30 times during their lifetime. Number of matings had no detrimental effect on male longevity ($R^2 = 0.01$, $F_{1,76} = 0.65$, $P = 0.42$). Rather to the contrary, long-lived males mated more times throughout their lifetime because they had more mating opportunities (fig. 5B). Consistently, the longevity of males that mated once a week did not differ significantly from those that mated once every 2–3 days ($F_{1,20} = 2.21$, $P = 0.15$). It follows that male mating history did not affect longevity, even if lifetime sexual activity was threefold higher in males that mated

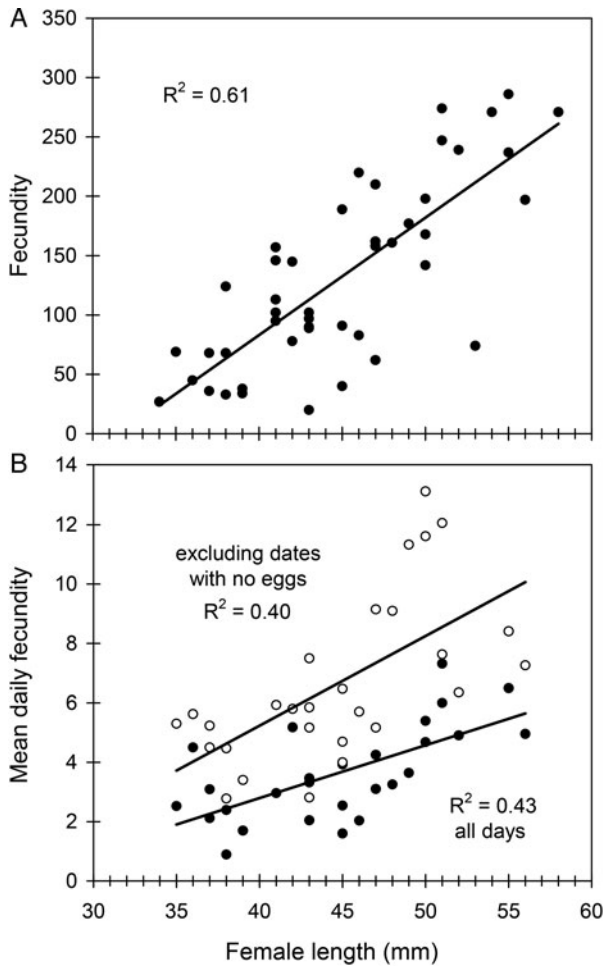


Fig. 2. The effect of female size (female length) on lifetime fecundity (A) and mean daily fecundity (B) in *C. welensii*. In the lower graph two regression lines are plotted, either including all egg-laying days (full circles) or excluding those days in which no eggs were laid (open circles). Regression equations and statistics were: (A) $y = 9.88x - 312.22$, $F_{1,43} = 66.67$, $P < 0.001$; (B) $y = 0.30x - 6.85$, $F_{1,26} = 17.24$, $P < 0.001$ (excluding dates with no eggs), and $y = 0.18x - 4.33$, $F_{1,26} = 19.81$, $P < 0.001$ (all days).

multiple times a week (20.2 ± 1.7 matings/male) than in those that just mated once a week (6.4 ± 0.6 matings/male) (fig. 5B).

Discussion

Mean fecundity of *C. welensii* was quite close to the average of the subfamily Cerambycinae (table 1), but individual values covered a quite broad range between 20 and 286 eggs/female, depending largely on female size. A positive correlation between fecundity and female size is widespread in insects (Honěk, 1993) irrespective of nutritional background (Torres-Vila *et al.*, 1999) and specifically in longhorns beetles, both Cerambycinae (Iwabuchi, 1988; Matsumoto & Irianto, 1998; Wang *et al.*, 1998, 2002; Kato *et al.*, 2000) and Lamiinae (Lawrence, 1990; Keena, 2002; Togashi, 1997, 2007; Togashi *et al.*, 2009). Fitness benefits for females of being large are likely to be especially important in these and other wood-boring

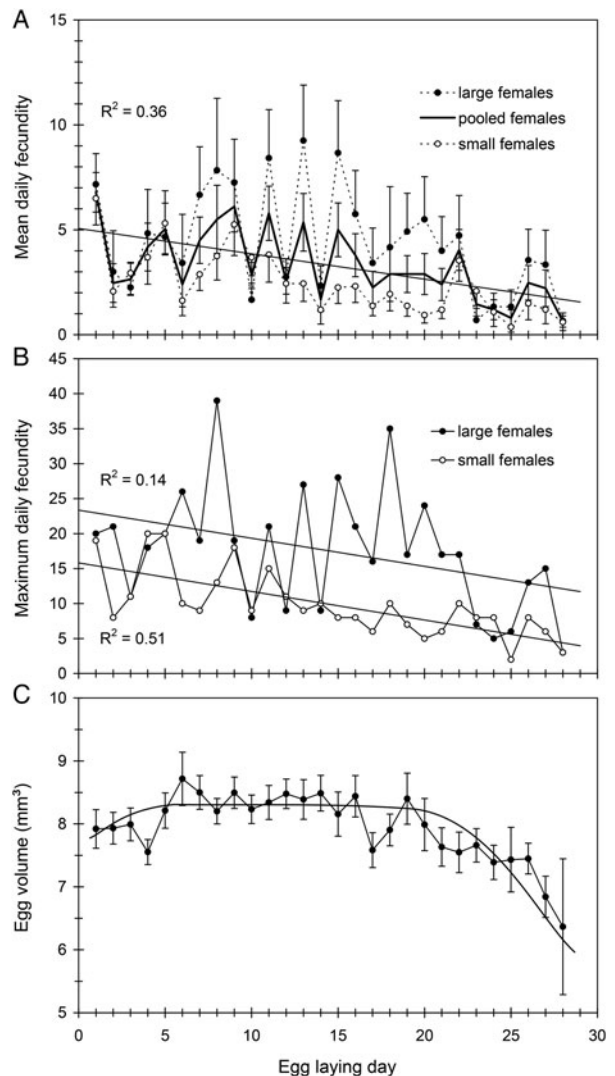


Fig. 3. (A) Mean daily fecundity (by female size and pooled), (B) maximum daily fecundity (by female size) and (C) egg size (egg volume) variation over the oviposition period in *C. welensii*. In the upper graphs, females were scored in two body size classes (large and small, above and below mean female length: 44.9 mm, see text). Vertical lines represent the SE of the mean.

species. A wide variation in adult size frequently occurs within these species because larvae (usually legless) cannot move between hosts and have restricted mobility within host, so that they are incapable of improving their nutritional status if host quality is poor. When adverse conditions arise, the larvae of these species produce smaller adults rather than fail to complete their development (Andersen & Nilssen, 1983). In this scenario, females able to choose the best egg-laying sites would have a significant fitness advantage.

Adults of *C. welensii* were long-lived (around 2 months), longevity records being in most cases higher than in other Cerambycinae and more similar to Lamiinae species (table 1). However, *C. welensii* longevity showed to be extremely variable in both sexes, from 2 to 3 weeks to almost 5 months. Mean longevity recorded in this study was much higher than

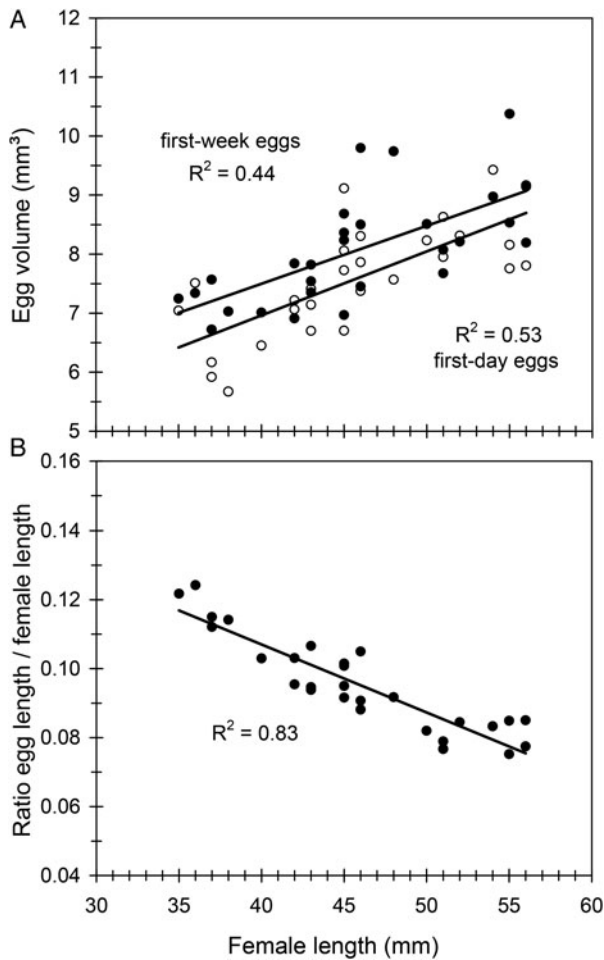


Fig. 4. The effect of female size (female length) on egg size (egg volume) (A) and the effect of female length on the ratio egg length/female length (B) in *C. welensii*. In the upper graph two regression lines are plotted, either considering the first-day eggs (open circles) or the first-week eggs (full circles) (see text). Regression equations and statistics were: (A) $y = 0.10x + 3.56$, $F_{1,26} = 20.43$, $P < 0.001$ (first-week eggs), and $y = 0.11x + 2.62$, $F_{1,26} = 29.29$, $P < 0.001$ (first-day eggs); (B) $y = -0.002x + 0.186$, $F_{1,26} = 124.02$, $P < 0.001$.

the 2 weeks reported in a previous work conducted in the same experimental conditions, but in which adults remained grouped throughout lifetime (Torres-Vila *et al.*, 2012a). This suggests that *C. welensii* longevity was reduced by the stress derived from the continuous interaction between adults, competition between females for egg-laying sites, male fights to gain mates and intense male harassment of females. Longevity records in the present study were also much higher than the 2–3 weeks of lifespan estimated under field conditions with mark–recapture methods (Torres-Vila *et al.*, 2013), which was attributed to a better adult feeding in the laboratory and the predation risk in the field. Female longevity was positively correlated with fecundity as in other longhorn species (Iwabuchi, 1988; Wang *et al.*, 1998; Togashi *et al.*, 2009) although there are also exceptions (Jikumaru *et al.*, 1994; Keena, 2002).

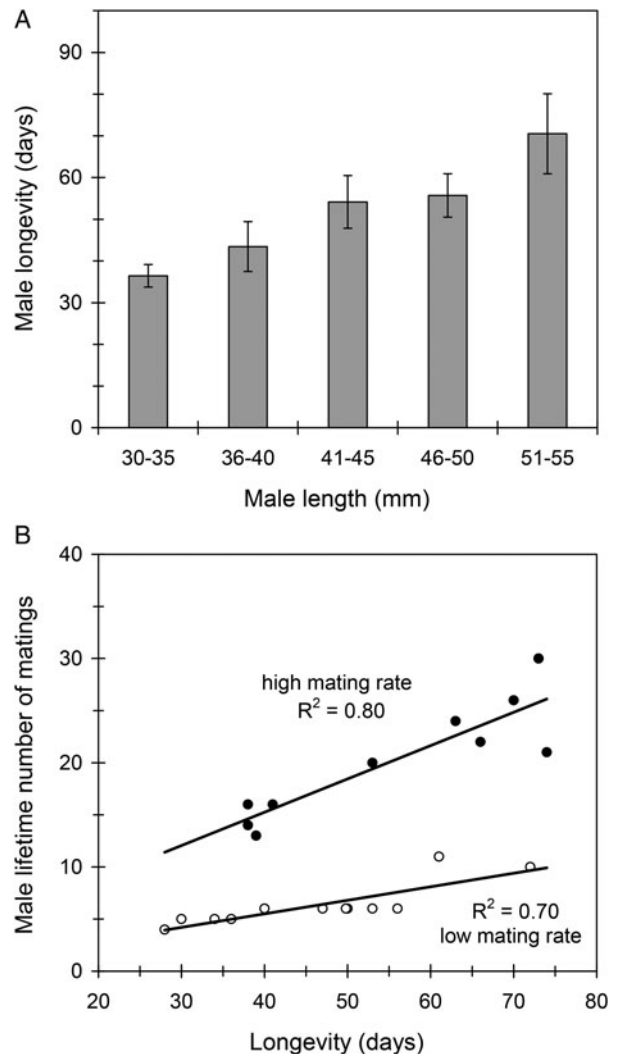


Fig. 5. The effect of male size (male length) on longevity (A) and the effect of male longevity on lifetime number of matings (B) in *C. welensii*. Vertical lines represent the SE of the mean. The lower graph includes data from two experimental groups of males that were allowed to mate at either one mating every 2–3 days (full circles) or one mating per week (open circles). Regression equations and statistics were: (B) $y = 0.32x + 2.45$, $F_{1,8} = 30.98$, $P < 0.001$ (high mating rate), and $y = 0.13x + 0.32$, $F_{1,10} = 23.05$, $P < 0.001$ (low mating rate).

The oviposition period was highly variable among females but the mean value (one month and a half) was similar to the average of the subfamily Cerambycinae (table 1). The post-oviposition period was very irregular and unexpectedly long in some individuals, suggesting a non-adaptive situation derived from an imbalance between the reproductive and somatic reserves. Many females did not oviposit for several weeks at the end of their life (up to 10 weeks) but their fecundity was not significantly reduced. Superior adult nutrition in our experimental conditions could partly explain these unexpected results. The irregularity among females in the postoviposition period determined both (1) the lack of correlation between longevity and female size, and (2) the better

Table 1. Reproductive and biological traits of Cerambycidae species belonging to the Cerambycinae and Lamiinae subfamilies.

Species subfamily	<i>n</i>	Lifetime fecundity (eggs)	Daily fecundity (eggs)	Fertility (%)	Oviposition period (days)	Female longevity (days)	Male longevity (days)	Reference
<i>Cerambyx welensii</i> Küster	28–78	132 ± 12	3.0 ± 0.2	70 ± 1	44 ± 3	70 ± 3	52 ± 3	This study
<i>Oemona hirta</i> F.	23	51 ± 9	2.8	60 ± 1	18 ± 2	26 ± 3	–	Wang <i>et al.</i> (1998)
<i>O. hirta</i> F.	23	52 ± 9	–	–	–	33 ± 3	52 ± 4	Wang <i>et al.</i> (2002)
<i>Phoracantha recurva</i> Newman	100<	284 ± 27	1.7–2.3	91 ± 2	120–168	68 ± 2	42 ± 2	Millar <i>et al.</i> (2003)
<i>Phoracantha semipunctata</i> F.	40<	131 ± 39	–	98	–	49 ± 11 ¹	–	Hanks <i>et al.</i> (1993)
<i>P. semipunctata</i> F.	100<	162 ± 23	1.6–2.9	90 ± 3	56–104	33 ± 1	31 ± 2	Millar <i>et al.</i> (2003)
<i>Xylotrechus pyrrhoderus</i> Bates	89–94	53 ± 11	3.8–7.6	95<	7–14	31 ± 1	30 ± 1	Iwabuchi (1988)
<i>Xystrocera festiva</i> Thomson ²	19	169 ± 10	126 ± 11	–	1–2 [<7]	5 ± 1	10 ± 1	Matsumoto & Irianto (1998)
<i>Xystrocera globosa</i> Olivier ²	12	162 ± 25	>20.3	–	<8	8 ± 1	7 ± 1	Matsumoto <i>et al.</i> (1996)
<i>Semanotus japonicus</i> Lacordaire	20	91	5.0	–	18 ± 1	24 ± 1	16 ± 1	Shibata (1987)
<i>S. japonicus</i> Lacordaire	19	80 ± 11	–	–	–	10–58	–	Kato <i>et al.</i> (2000)
Subfamily Cerambycinae	–	124 ± 21	21.0 ± 15.1	84 ± 6	41 ± 17	35 ± 6	30 ± 6	
<i>Acalolepta luxuriosa</i> Bates	16	287 ± 21	3.8	–	75 ± 5	85 ± 5	–	Akutsu (1985) ³
<i>Anoplophora glabripennis</i> Motschulsky	100<	67 ± 6	0.8 ± 0.1	83 ± 2	85–105	80 ± 3	103 ± 6	Keena (2002)
<i>A. glabripennis</i> Motschulsky	45	113 ± 12	1.1 ± 0.1	48–66	80–120	95 ± 6	–	Smith <i>et al.</i> (2002)
<i>Anoplophora malasiaca</i> Thomson	17	194 ± 16	2.9	78–100	ca. 67	78 ± 5	–	Adachi (1988)
<i>Apriona germari</i> Hope	15	117 ± 7	2.8 ± 0.1	–	43 ± 2	51 ± 4	63 ± 4	Hussain & Buhroo (2012)
<i>Monochamus alternatus</i> Hope	10	33	0.9 ± 0.2	–	56 ± 11	42 ± 8	38 ± 7	Shibata (1987)
<i>M. alternatus</i> Hope	11	124 ± 10	1.7 ± 0.1	–	69 ± 4	96 ± 3	–	Togashi (1997)
<i>Monochamus carolinensis</i> Olivier	20	250	4.7 ± 0.7	–	<80	56 ± 7	51 ± 8	Togashi <i>et al.</i> (2009)
<i>Monochamus galloprovincialis</i> Olivier	37	67 ± 6	1.2	93 ± 1	54 ± 4	64 ± 6	61 ± 7	Naves <i>et al.</i> (2006)
<i>M. galloprovincialis</i> Olivier	16	93 ± 15	4.3	–	32 ± 6	75–113	–	Koutroumpa <i>et al.</i> (2008)
<i>Monochamus saltuarius</i> Gebler	19	92 ± 2	2.2	–	42 ± 1	57 ± 1	–	Jikumaru <i>et al.</i> (1994)
<i>M. saltuarius</i> Gebler	22–28	40 ± 8	2.0 ± 0.4	73 ± 7	21 ± 4	33 ± 5	35 ± 6	Nakayama <i>et al.</i> (1998)
<i>Paraglenea fortunei</i> Saunders	26	275 ± 39	8.0 ± 0.4	–	32 ± 4	39 ± 4	43 ± 4	Togashi (2007)
<i>Psacotha hilaris</i> Pascoe	15	418 ± 37	5.5 ± 1.1	–	79 ± 6	90	–	Iba (1963, 1982) ³
Subfamily Lamiinae	–	155 ± 30	3.0 ± 0.6	79 ± 6	60 ± 7	69 ± 6	56 ± 9	
Family Cerambycidae	–	142 ± 19	9.5 ± 5.6	82 ± 4	53 ± 8	54 ± 6	42 ± 6	

Values are the mean, mean ± SE or variation range. Averaged values are given in those studies in which several experimental treatments were tested (e.g. larval feeding, adult feeding, female mating number and temperature). In those studies in which the numerical data were not provided, relevant values were recovered by measuring the figures. Note that the reproductive and biological traits compiled in this table are merely illustrative, as experimental conditions were variable among species/studies.

¹Sexes pooled.

²*X. festiva* and *X. globosa* have very short-lived adults and females often laid eggs in a single cluster.

³In Togashi (2007).

correlation of fecundity with oviposition period than with longevity, as also reported in *Monochamus galloprovincialis* Olivier (Koutroumpa *et al.*, 2008).

Daily fecundity in *C. welensii* showed a typical synovigenic pattern, characterized by females that produce, mature and lay eggs throughout an extended oviposition period. Synovigeny is characteristic of species that rely on feeding in adult stage (the so-called income breeders) to gather vital energetic resources for both reproduction and somatic maintenance (Stearns, 1977). There was a slight decreasing trend over time in daily fecundity (both mean and maximum values) with oviposition rate being always higher in large than small females. Thus, daily fecundity was positively correlated with female size, but negatively correlated with female age, as observed in other cerambycids (Lawrence, 1990; Keena, 2002; Smith *et al.*, 2002). Mean daily fecundity (3 eggs/day) was similar to the values reported in most longhorn beetles when *Xystrocera* species were not taken into account given their quite dissimilar reproductive biology (table 1). Large fluctuations in oviposition rate occurred because many females did not laid eggs every day, and moreover, after a day without oviposit, egg-laying was often accentuated the next day. A high fluctuation in oviposition rate is often reported in cerambycids (Shibata, 1987; Jikumaru *et al.*, 1994; Keena, 2002; Koutroumpa *et al.*, 2008) and attributed to intraspecific differences in female size, oogenesis rate and even ovariole number (Togashi *et al.*, 2009). In the case of *C. welensii*, also keep in mind that mating duration is extremely long (8 h in average), so that females are constrained by males on mating days, even if pair-bonded females are able to feed and oviposit between intromissions (Luis M. Torres-Vila, personal observation).

Eggs of *C. welensii* were large (length \times width: 4.2×2.3 mm²), values being similar to those reported in previous studies: 4.5×2.3 mm² (Hernández, 1991) and 4.7×2.3 mm² (Vitali, 2001). A large size of *C. welensii* eggs was explained by the allometric scaling between female size and egg size, the so-called 'Bauplan' relationship (Wiklund & Karlsson, 1984). However, in relative terms (in relation to female size) the eggs of *C. welensii* – as those other Cerambycinae – were proportionally smaller than in Lepturinae and Lamiinae species (Hernández, 1991), which evidences a phylogenetic background regulating the trait. In any case, larger *C. welensii* females produced larger eggs as in most insects (Fox & Czesak, 2000) and particularly in cerambycids (Togashi *et al.*, 1997; Kato *et al.*, 2000). Egg size remained large and relatively constant throughout most of the oviposition period and just decreased on the fourth week, probably by a depletion of energetic resources not compensated for by adult feeding (Torres-Vila & Rodríguez-Molina, 2002).

There was a positive correlation between neonate size (head width) and egg size (length) as reported in others cerambycids (Kato *et al.*, 2000; Walczyńska, 2008). However, head width was unrelated to egg volume, signifying that yolk surplus in large eggs was incorporated to larval body rather than to increase head size. The adaptive significance of egg size in insects is controversial as there is not a generalizable relationship between neonate size and larval performance. In some species, however, large neonates have an adaptive advantage over small ones especially in adverse environments (Torres-Vila & Rodríguez-Molina, 2002; Torres-Vila *et al.*, 2012b). The adaptive advantage of larger progeny size could be especially important in *C. welensii* and other xylophagous cerambycids that develop on hardwood trees. A better performance of large neonates could result from the larger

head/mandible size (Murphy *et al.*, 1983; Nakasuji, 1987) conferring advantage in the host perforation during the first tunnelling stages. Larger neonates could also be more resistant when facing the numerous physical and biochemical countermeasures that a host tree displays in response to borer injury, either to directly kill or compartmentalise the intruder. Tree resistance mechanisms include lignification of cell walls, formation of impervious tissue and necrophylactic periderm, callus formation in the cambial zone, cytosolic changes with accumulation of secondary chemicals (phenolic and isoprenoid compounds), high tissue moisture content, bark turgor pressure and increased tree sap flow (Hanks, 1999; Kato *et al.*, 2000; see Sallé *et al.*, 2014 for a review). There is little evidence available in cerambycids to support this idea. In *Monochamus alternatus* Hope the larvae kill each other under the bark at high densities and the winners are larger than the losers, suggesting that the initial size of progeny affects early survival (Togashi *et al.*, 1997). In *Semanotus japonicus* Lacordaire larval survivorship to adulthood is greater in large than small progeny (Kato *et al.*, 2000). The adaptive advantage of larger neonates could also explain why small females laid larger eggs relative to their body size compared with larger females in *M. alternatus* (Togashi *et al.*, 1997) and *C. welensii* (this study). The effect of neonate size on larval performance and early survival merits further research in *C. welensii* given its potential adaptive significance.

There were huge differences in incubation time (from 7 to 22 days) among females under a constant temperature (25°C) suggesting heritable variation in the trait. Variation in incubation time was unrelated to female size but could be linked to the geographical origin of individuals tested. We observed that some *C. welensii* larvae, perfectly formed inside the chorion, delayed some days their emergence contributing to lengthen incubation time, but this variable was not measured. Similar variation in incubation time has been reported in the longhorn *Psacotha hilaris* Pascoe, in which there is also a negative trade-off between incubation time and postembryonic development time (Yumino & Togashi, 2015). What is more, in cerambycids there is evidence that incubation time is heritable, as this trait depends on phylogeny and varies among subfamilies (Lamiinae < Cerambycinae < Prioninae) (Hanks, 1999). Interestingly, in *C. welensii* there were also large differences in the incubation time within the egg complement of a single female. This could derive from a risk-spreading (bet-hedging) strategy, because a female may increase her fitness if a part of her progeny manages to avoid unfavourable and unpredictable environmental conditions (Hopper, 1999; Yumino & Togashi, 2015).

Both females and males of *C. welensii* were extremely promiscuous, accomplishing up to 20 and 30 lifetime matings, respectively. Note that our experiments were not designed to define the maximum mating potential, so they could underestimate what occurs in the wild. Male fights for mates were a commonly observed behaviour. Male coercion was not a rare event including male harassment of females and punishing resisting females, especially when males were allowed to mate with previously multiple mated females in which sexual receptivity was lower. Pair-bonded males forcefully guarded their mates, while unreceptive females walked quickly and climbed the box walls, trying to get rid of the males, hiding their genitalia under the elytra or even extruding the ovipositor and laying eggs, trying in every way possible to prevent male intromission attempts. Male coercion was early described (e.g. Rothschild, 1978) and male cerambycids often

harass and mount females to induce mating, although it has been questioned whether such behaviour implies forced copulation (Eberhard, 2002).

Male mating history did not affect longevity. Rather to the contrary, long-lived males mated more times throughout their lifetime because they had more mating chances. In females in turn, multiple mating did not affect longevity, fecundity or fertility. Our results suggest that the adaptive significance of extreme polygyny and polyandry in *C. welensii* would be to promote sperm competition and cryptic female choice rather than the acquisition of energy as nuptial gifts to increase fecundity (Arnqvist & Nilsson, 2000; Torres-Vila *et al.*, 2004; Torres-Vila, 2013). However, this notion requires further experimental support since the good adult nutrition in our tests could have biased the effects. The energetic resources gathered via nuptial gifts could become significant when adult feeding is limited or non-existent, a common situation in summer in Mediterranean dehesa woodlands. The effect of multiple mating on fecundity in cerambycids is unclear because few and contradictory studies are available. For instance, in *Tetraopes tetraophthalmus* Forster (Lawrence, 1990) and *Oemona hirta* F. (Wang *et al.*, 1998) there is a positive relationship between polyandry and fecundity, but it is lacking in *Xylotrechus pyrrhoderus* Bates (Iwabuchi, 1988), *Phoracantha semipunctata* F. (Bybee *et al.*, 2005) and *C. welensii* (this study).

Species-specific values of fecundity, daily fecundity, oviposition period and longevity in cerambycids are quite variable both between and within subfamilies (table 1). Data showed the effect of the phylogenetic background, supporting that Lamiinae tend to live longer than Cerambycinae (Hanks, 1999). Cerambycids are a very diverse group, so that an array of biological, behavioural and environmental variables may account for differences in reproductive traits. These include the main energetic source – income or capital breeders –, mate location pattern, adult dispersal/sedentary behaviour, female gnawing or not into bark for oviposition, single (iteroparity) versus clustered (semelparity) egg laying, feeding versus non-feeding adults or the occurrence/absence of adult maturation feeding (Linsley, 1959; Hanks, 1999; Allison *et al.*, 2004). Environmental conditions are also extremely important, including weather conditions: temperature, drought (Bybee *et al.*, 2004; Keena, 2006), host-plant quality: tree species, age, phenology, physiological and health status (Hanks, 1999; Keena, 2002; Smith *et al.*, 2002; Wang *et al.*, 2002), feeding resources for adults (Hanks *et al.*, 1993; Millar *et al.*, 2003) and even sublethal entomopathogenic infections (Hajek *et al.*, 2008). Differences between studies on the same target species (seven cases in table 1) support both the effects of genetic background and environmental conditions. It follows that detailed comparisons of reproductive variables between species (or populations) should be made with caution.

Conclusions

Our study provides insight on the reproductive output of *C. welensii*. Larger males lived longer and mated more times than small males while larger females had greater overall reproductive fitness than did smaller females in terms of lifetime fecundity, daily fecundity, oviposition period and offspring size. These biological data will be useful to improve pest management methods and to establish new action guidelines to prevent or mitigate the increase of *C. welensii* populations in dehesa open woodlands and other oak forests.

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