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Male-induced costs of mating for females compensated by offspring viability benefits in an insect

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Abstract

Sexual conflict facilitates the evolution of traits that increase the reproductive success of males at the expense of components of female fitness. Theory suggests that indirect benefits are unlikely to offset the direct costs to females from antagonistic male adaptations, but empirical studies examining the net fitness pay-offs of the interaction between the sexes are scarce. Here, we investigate whether matings with males that invest intrinsically more into accessory gland tissue undermine female lifetime reproductive success (LRS) in the cricket *Teleogryllus oceanicus*. We found that females incur a longevity cost of mating that is proportional to the partner's absolute investment into the production of accessory gland products. However, male accessory gland weight positively influences embryo survival, and harmful ejaculate-induced effects are cancelled out when these are put in the context of female LRS. The direct costs of mating with males that sire offspring with higher viability are thus compensated by direct and possibly indirect genetic benefits in this species.

Introduction

In lifelong monogamous species, the reproductive interests of males and females are convergent. However, strict genetic monogamy is taxonomically rare. Rather, individuals in most species typically engage in matings with multiple partners (see Smith, 1984; Birkhead & Møller, 1998; Simmons, 2001b), and under these circumstances, the reproductive interests of the sexes generally differ (Arnqvist & Rowe, 2005). Conflicting evolutionary interests between the sexes, defined as sexual conflict by Parker (1979), pervades the biology and evolution of reproduction and can lead to sexually antagonistic coevolution by creating cycles of adaptive and counter-adaptive responses between males and females (Holland & Rice, 1999; Chapman *et al.*, 2003; Arnqvist & Rowe, 2005; Chapman, 2006).

Asymmetries in parental investment between the sexes commonly generate conflict over mating rates in promiscuous species, where the net benefits of increasing the number of mating partners are higher in males than in females (Bateman, 1948; Trivers, 1972; Parker, 1979). Higher potential reproductive rates for males generate male–male competition for matings and fertilizations, and the intense sexual selection that competition generates can facilitate the evolution of male traits that are harmful to females. *Drosophila melanogaster* can be considered as the archetypical example, where sexual conflict leads to sexually antagonistic coevolution (Holland & Rice, 1999). In this species, seminal fluids transferred by the male during copulation induce a series of dramatic physiological changes in females, including a reduction in sexual receptivity, increase in the rate of oviposition, and ultimately a decrease in female lifespan (Fowler & Partridge, 1989; Chapman *et al.*, 1995; Wolfner, 2002; Kubli, 2003; Wolfner, 2009). Evidence is accumulating that in many other species males harm females during copulation, either physically or by means of the action of seminal fluid products (Chapman *et al.*, 1995; Crudgington & Siva-Jothy, 2000; Stutt & Siva-Jothy, 2001; Arnqvist & Rowe, 2005; Hotzy & Arnqvist, 2009).

Sexual conflict over mating decisions and mating rates typically entails costs to females. However, it has been suggested that indirect benefits arising from mating with harmful male partners can outweigh the direct costs

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inflicted by these males (Parker, 1979; Cordero & Eberhard, 2003). Whether direct costs to females of antagonistic male adaptations are countered by genetic benefits is central to our understanding of sexual selection and sexual conflict. Theory suggests that indirect benefits are unlikely to compensate for direct costs (Cameron *et al.*, 2003), and evidence gathered in the most studied model species so far, *D. melanogaster*, seems to support this notion (Brown *et al.*, 2004; Byrne & Rice, 2005; Orteiza *et al.*, 2005; Stewart *et al.*, 2005; Pischedda & Chippindale, 2006; Stewart *et al.*, 2008; but see Rundle *et al.*, 2007). Little is known in other species because only a few empirical studies have examined the direct and indirect pay-offs of the interactions between the sexes in an integrative way (Boake, 1985; Kokko *et al.*, 2003; Fedorka & Mousseau, 2004; Head *et al.*, 2005; Le Galliard *et al.*, 2008).

In this study, we look at the consequences of seminal fluid transfer upon female longevity and female lifetime reproductive success (LRS) in the Australian field cricket *Teleogryllus oceanicus*. This polygamous species is an ideal model to examine the implications of mating for female fitness. Male *Teleogryllus* transfer a spermatophore during mating that contains sperm and seminal fluid products secreted from the accessory glands (Loher & Rence, 1978). Therefore, some of the main costs and benefits of mating are expected to be mediated by the contents of the spermatophore. In some orthopterans, spermatophores are delivered with large edible nutrient donations, but this is not the case in field crickets (Simmons, 1988; Vahed, 1998; Bussiere *et al.*, 2006). Females that mate with multiple partners gain genetic benefits in the form of increased hatching success resulting from enhanced embryo survival (Simmons, 2001a; García-González & Simmons, 2005), and previous studies have shown significant sire effects on embryo viability as well as on male absolute investment into the accessory gland (Simmons, 2003; García-González & Simmons, 2005; Simmons & García-González, 2007). Importantly, both accessory gland weight and a male's ability to induce embryo viability exhibit additive genetic variation and are genetically correlated (CV_A = 11.08 and 21.10 for accessory gland weight and embryo viability, respectively; r_g = +0.662) (García-González & Simmons, 2005), implicating a role of seminal products on embryo survival and therefore on the genetic benefits of polyandry. Further support for the role of accessory gland products in determining embryo viability comes from a study which demonstrated that a male's seminal fluids can influence the viability of his own offspring, as well as those of his sperm competitors, indicating that seminal fluid products can exert both direct and indirect genetic effects on offspring fitness (García-González & Simmons, 2007). However, seminal products could also be detrimental to female fitness, as female fecundity has been found to trade off with the viability of her embryos (Simmons & García-González, 2007). Males could influence this evolutionary trade-off if the effects of accessory gland products on embryo viability drag the females from their naturally selected offspring number-offspring quality optimum (Smith & Fretwell, 1974).

Here, we determine whether seminal fluid effects undermine female fitness by examining the effects of mating on hatching success (embryo viability), fecundity, and female LRS. We used a full-sib breeding design to obtain groups of sisters that were mated to males that differed in their absolute investment into accessory gland tissue. We document a negative effect of male accessory gland weight on female longevity, but a positive effect on embryo viability and no net effect on female fitness as revealed from LRS data. Thus, the direct costs of mating are compensated by offspring viability benefits in this system.

Methods

Experimental crickets were sampled from 32 full-sib families, generated from the F1 offspring of field-inseminated females collected from a plantation in Carnarvon (Western Australia). These families were reared in 5-L plastic containers kept in a constant temperature room at 25 °C with a 12 : 12 h light/dark cycle, fed with cat chow *ad libitum*, and supplied with a Petri dish containing a pad of moist cotton. The sexes were separated before the penultimate instar to ensure they remained unmated.

To characterize families according to the investment that males made into their accessory gland, two 7- to 9-day-old males were randomly selected from each family and dissected. Prior to the dissections, individuals were extracted from the single sex containers and each male isolated for 3 days in a small plastic box (7 cm × 7 cm × 5 cm) supplied with food and water. The pronotum widths of these males were measured to the nearest 0.01 mm using digital callipers, and their body, accessory gland, seminal vesicle, and testes were weighed to the nearest 0.01 mg. Consistent with previous studies that have found significant additive genetic variance for accessory gland weight, testes weight, and morphological traits in *T. oceanicus* (Simmons, 2003; García-González & Simmons, 2005; Simmons & García-González, 2007), we found significant repeatabilities for these traits (see Table 1). The absence of a relationship between accessory gland weight and body weight (r = 0.22, P = 0.22, n = 32 families), pronotum width (r = 0.07, P = 0.69), or soma weight (r = 0.01, P = 0.94) further supports previous findings indicating the independence of male investment into accessory gland weight from body size (Simmons, 2003). For this reason and because it is accessory gland weight in absolute terms what has been found to be genetically associated with embryo viability (García-González & Simmons, 2005), we focus here on the effects of this absolute measure on female reproduction.

Table 1 Family means (including SE and range) of reproductive and morphological traits (n = 32 families, two males per family), and repeatabilities, R (= intraclass correlation coefficient), including SE and P values following one-way ANOVAS on un-transformed variables (after Becker, 1984).

	Mean	SE	Min.	Max.	R	R SE	P
Pronotum (mm)	6.38	0.04	5.88	6.76	0.34	0.18	0.025
Body size (mg)	667.66	11.65	513.81	766.98	0.62	0.13	< 0.001
Testes (mg)	33.38	0.67	23.83	39.36	0.39	0.18	0.011
Accessory gland (mg)	19.23	0.70	11.96	28.87	0.29	0.19	0.048
Seminal vesicle (mg)	1.41	0.09	0.66	2.91	0.36	0.18	0.018

Experimental design

This study investigates the fitness consequences for females of mating with males that differ intrinsically in their accessory gland weight. However, female reproductive success is influenced to a great extent by female identity (e.g. fecundity is strongly influenced by ovary weight, which exhibits substantial additive genetic variance; Simmons, 2003; Simmons & García-González, 2007). Thus, to account for female effects, we designed a mating scheme that controlled for female family identity. Our experimental females consisted of six sisters from each of 15 families. The six sisters from each family were mated to two males belonging each to a different family (unrelated to each other and unrelated to the female) such that three sisters were mated singly to one male and the remaining three sisters were mated singly to the second male (Fig. 1). In this way, by analysing the difference between the accessory gland weight of the two males and the difference in the mean LRS, fecundity, or longevity between the two sets of sisters mated to them, we were able to examine the influence of accessory gland weight on female reproduction while controlling for intrinsic female effects.

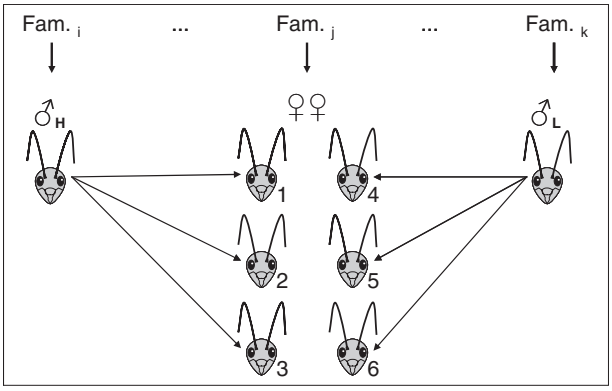

Fig. 1 Outline of one block in the experimental mating design. Six sisters were mated to two unrelated males belonging each to a different family such that three sisters were mated singly to one male and the remaining three sisters were mated singly to the second male. The two males were from families with different levels of investment into accessory gland weight (H, High, or L, Low). Individuals were extracted from 32 families and 15 blocks were performed. See text for details.

The 15 pairs of males mated to each set of sisters were established so that variation in the differences in investment in accessory glands between the two males within each pair was maximized: the 32 families of crickets were ranked according to mean accessory gland weight and matched first with last, second with second last, and so forth. Each male within each pair was defined High (H) or Low (L) depending on the mean accessory gland weight of the family to which he belonged. Each of the 15 families (referred to as blocks) of sisters mated to a pair of males defines the unit for the analysis. Some females escaped after the matings were carried out or failed to be fertilized as assessed from inspection of egg development, and thus the number of females analysed is < 90. In total, 82 females belonging to 15 families were mated to 30 different males belonging to 30 different families (mean number of sisters mated to the same male: 2.73 ± 0.1). A nested ANOVA with family (random factor) and treatment (females mated to H or L males) nested within family showed that female body size did not vary between treatments ($F_{15,52}$ = 1.57, P = 0.11).

Matings

To avoid unknown but potential influences of age upon the dynamics of investment into reproductive tissue, matings were initiated soon after establishing the levels of investment into accessory glands across families (mean number of days between dissection of brothers and commencement of matings for males = 8.9 ± 0.54). In addition, to further minimize the influence of age effects on reproduction, we carried out the matings as quickly as possible and used individuals matched for age. Males were approximately 2 weeks old when used [mean age (days) 15.9 ± 0.54, n = 30]. Females were mated when they were about 3 weeks old [mean age (days) 22.9 ± 0.54, n = 82]. Matings were carried out in small plastic boxes (7 cm × 7 cm × 5 cm). Within blocks, the first matings for both the L male and the H male were initiated at the same time. Each female was mated twice to the same male. Each male thus performed a total of six matings across three sisters. Pairs were observed closely to ensure matings occurred. After mating, the male was left to guard the female to prevent her from removing the spermatophore, and we ensured that the spermatophore remained attached to the female for 40 min to allow complete sperm and seminal fluid transfer (Simmons *et al.*, 2003). After this period, the male and the female were separated and the empty spermatophore removed from the female with the aid of forceps. Once the male produced a new spermatophore

and was ready to mate again (minimum 60 min after first mating), the pair was again housed together and the mating procedure repeated. The mean interval between matings for the same female was 0.4 ± 0.06 days (n = 82), the mean interval between matings of the same male across females was 2.0 ± 0.21 days (n = 30), and the mean interval between matings for all the sisters within a family was 4.0 ± 1.13 days (n = 15 female families).

Female fitness assays

After completing their two matings with the same male, females were housed for 7 days in small boxes with water, food, and oviposition pads made of wet sand in a 5.5-cm Ø Petri dish. At the end of this period (week 1), eggs were rinsed from the sand and counted. From the batch of eggs laid from each female during week 1, 50 eggs were haphazardly collected and placed in rows on moist filter paper in a 9- cm Ø Petri dish. This sample of eggs was used to obtain a measure of hatching success (hatching success is a proxy of embryo viability; Simmons, 2001a), unaffected by any potential confounding effects of egg density on hatching success. The remaining eggs were placed in a box (16.5 cm × 11 cm × 5.5 cm) on a piece of damp cotton for an additional measure of hatching success. Eggs were again spread on the cotton to avoid any density-dependent problems that might affect hatching success. Eggs were incubated in a controlled temperature room at 25 °C with a 12 : 12 h light/dark cycle. Females were changed to new boxes on a weekly basis until death, with the exception that from week 2 onwards the oviposition pads consisted of cotton wool instead of sand. From this week onwards, the eggs were placed, uncounted, in the damp cotton in a box. These eggs were checked exclusively for the measures of LRS. Hatching was checked regularly in the plates and boxes until no eggs had hatched for a period of 2 weeks (eggs take 14 days to hatch). Nymphs were collected and counted as they emerged.

Measures of fecundity and hatching success were taken from eggs laid during the first week, whereas total number of offspring across lifetime provided a measure of female LRS. Across the 82 females, over 70% (71 ± 2%) of offspring were produced during the first 2 weeks after mating. Both hatching success calculated in the Petri dishes (50 eggs per female) and hatching success calculated in the boxes (mean number of eggs 418 ± 21, n = 82) were highly correlated across females (Arcsin transformed proportion of hatched eggs, r = 0.854, P < 0.0001, n = 82). Thus, we use the average between these two measures of hatching success in further analyses. Eggs were collected weekly for as long as females were alive, and females were checked for deaths on a daily basis. Female survival was calculated as the number of days since mating to death.

Data analysis

We analysed the relationship between the difference in accessory gland weight between the two males mated to sisters (as assessed from family dissections) and the difference in the mean hatching success, fecundity, and LRS between the two sets of sisters mated to these males. Our experimental design and subsequent paired analyses sacrifice sample size but present two important advantages: it controls for female effects on female life-history traits and allows for inspection of the continuous relationship between accessory gland weight and female fitness. All variables (accessory gland weight, hatching success, survival, and LRS) were normally distributed (following K–S tests) and were therefore used untransformed when calculating Pearson correlations. Data analyses were carried out with Statistica 6.0 (StatSoft, 2001), and confidence limits for the correlation coefficients were calculated by bootstrapping using PopTools 3.0.6 (Hood, 2008). All means are presented with ±1 SE. All analyses involving investment into the accessory glands exclude the weight of the seminal vesicle. Notwithstanding, results are unaffected if the analyses are performed on the accessory gland and seminal vesicle weights combined.

Results

We found significant family effects for accessory gland, seminal vesicle, testes, and body weight, as well as for pronotum width (Table 1), indicative of the additive genetic variance for these traits reported in previous studies (Simmons, 2003; García-González & Simmons, 2005). All together, we collected data for life-history traits from 82 females (mean hatching success, 27 ± 2%; fecundity during first week after mating, 469 ± 21 eggs; female survival, 38 ± 1 days since mating; LRS, 340 ± 20 total number of hatched offspring).

We looked at the influence of male accessory gland weight on female reproduction and LRS by analysing the difference between accessory gland weight between the two males within a block and the reproductive fate of the females mated to these males. There was a significant negative relationship between the weight of the accessory gland in males and the lifespan of the females mated to them (Fig. 2 and Table 2), revealing a longevity cost of mating for females. However, accessory gland weight in males had no effect on female LRS (lifetime number of hatched offspring) (Table 2), and the compensation of the longevity cost likely occurs through the beneficial effects of accessory gland products on embryo viability, as evidenced by a significant positive relationship between the weight of the accessory gland in males and the hatching success of the embryos they sire (Fig. 3, Table 2, and see Introduction and Discussion).

It is unlikely that fecundity costs (i.e. costs arising from egg production and oviposition) mediate the relationship

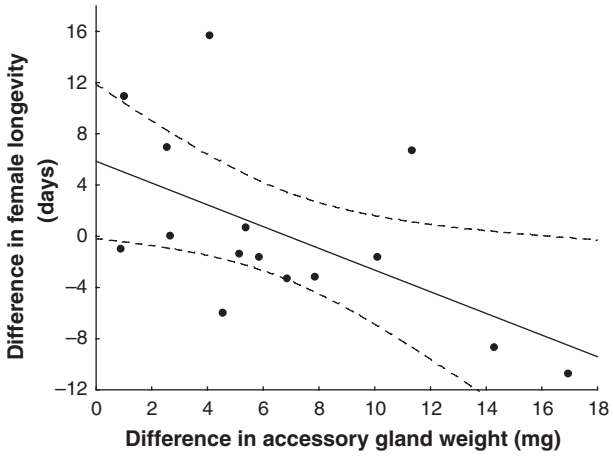

Fig. 2 Relationship between investment into accessory gland in males and female longevity. The plot shows the negative and significant relationship between the difference in accessory gland weight between the two males mated to a group of sisters and the difference in the mean lifespan between the two sets of sisters mated to these males. Each of the 15 data points belongs to one block, i.e. to one statistically independent group of full-sibling sisters. Discontinuous lines indicate the 95% confidence intervals for the fitted line. See Table 2 and text for details.

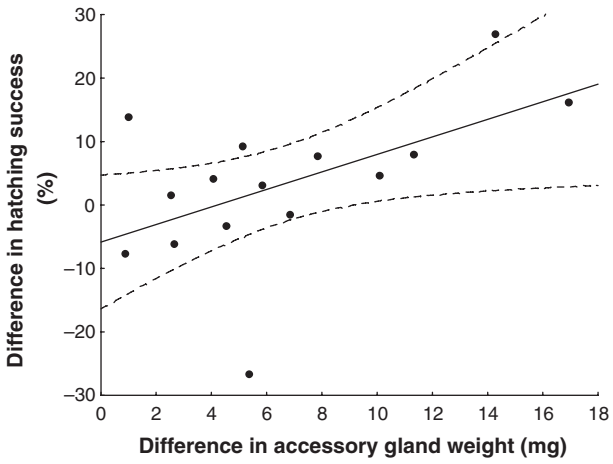

Fig. 3 Relationship between investment into accessory gland in males and hatching success in females. The plot shows the association between the difference in accessory gland weight between the two males mated to a group of sisters and the difference in the mean hatching success between the two sets of sisters mated to these males. Each of the 15 data points belongs to one block, i.e. to one statistically independent group of full-sibling sisters. Discontinuous lines indicate the 95% confidence intervals for the fitted line. See Table 2 and text for details.

Table 2 Effects of male accessory gland weight on hatching success, female fecundity, female longevity, and female lifetime reproductive success (LRS). Pearson correlation coefficients and *P* values, plus mean correlation coefficients and confidence limits from bootstrapping (10 000 iterations) are shown.

Trait	*r*	*P*	Mean *r* and CL
Hatching success	0.53	0.040	0.53 (0.01, 0.88)
Fecundity	0.33	0.237	0.32 (−0.12, 0.69)
Female longevity	−0.56	0.030	−0.54 (−0.86, −0.03)
LRS	0.13	0.651	0.12 (−0.39, 0.63)

between accessory gland weight and female lifespan, given that the weight of the accessory gland had no significant influence on fecundity (Table 2). Furthermore, the negative and significant relationship between accessory gland weight and female survival remains when fecundity and hatching success are controlled for (partial correlation $r = -0.62$, $P = 0.024$, $n = 15$).

Discussion

Male influences on female reproduction are a common currency for exploring interactions between the sexes, because a male's reproductive success is contingent on the reproductive output of his mating partner/partners. Male influences on female reproduction are increasingly thought to generate sexual conflict, because they are often found to reduce components of female fitness. However, to conclude that antagonistic sexual interests drive the evolution of sexual interactions can be misleading unless net female fitness is examined (Parker, 1979; Cordero & Eberhard, 2003). In this study, we looked at the effects of male investment into accessory glands on female LRS in the cricket *T. oceanicus*. We found that females had shorter lifespan when mated with males with an intrinsically greater investment into their accessory glands. However, the offspring of these females enjoyed improved survival during the embryonic stage of development. Importantly, we found that, overall, female LRS (total number of hatched offspring across lifespan) was not affected by the detrimental effects on survival of male investment into accessory gland products. These findings reveal that in *T. oceanicus*, the direct costs of mating are compensated by direct and, by virtue of the heritability of paternal effects, indirect genetic benefits of improved offspring viability (García-González & Simmons, 2005, 2007). The results also highlight the fact that investigations into the net fitness pay-offs of sexual interactions are critical for understanding the role played by sexual conflict in a natural and sexual selection framework.

Male field crickets cannot physically coerce females into mating, implying that the act of mating results to a great extent from female choice. In at least one species (*Gryllus lineaticeps*), it has been shown that female mating preferences based on male song translate into fecundity and fertility benefits that have been interpreted as resulting from the transfer of seminal fluid products during mating (Wagner *et al.*, 2001; Wagner & Harper, 2003). To date, we do not know whether there is a

similar association between male attractiveness and the production of seminal products in *T. oceanicus*. Nonetheless, the investment into accessory gland weight is heritable and influences positively embryo survival (García-González & Simmons, 2005), and therefore, our present findings are informative of the female fitness consequences of mating with high-quality (and potentially preferred or more competitive) males (see Pitnick & García-González, 2002; Friberg & Arnqvist, 2003; Head *et al.*, 2005; Stewart *et al.*, 2008; Taylor *et al.*, 2008). We do know that female *T. oceanicus* are choosy of their mates and that both courtship song and cuticular hydrocarbon blends contribute to male attractiveness (Rebar *et al.*, 2009; Thomas & Simmons, 2009). In one of the few studies that have looked at the net fitness consequences of mate choice to date, Head *et al.* (2005) showed that females of the field cricket *Acheta domesticus* mating with attractive males experience survival costs that are ameliorated by, and that can even be outweighed by, indirect benefits. Whether seminal fluids play a role in driving costs or benefits in *A. domesticus* is not known, although ejaculate compounds have also been implicated as oviposition stimulants in this species (Destephano & Brady, 1977).

The positive effects of accessory gland products on hatching success found in this study are consistent with previous findings in this species (García-González & Simmons, 2005, 2007), but whether these effects compensated for potential direct costs of mating was not known. In the light of our results, we conclude that the evolution of sexual interactions in *T. oceanicus* is most likely explained by traditional sexual selection rather than by a history of sexual conflict. Nonetheless, in this study, we only allowed a female to mate twice with the same male. Whether increased mating frequencies elevate the costs incurred by females (e.g. Kuijper *et al.*, 2006) remains to be tested in this species, although multiple mating might also enhance the potential for genetic benefits. In fact, previous studies have demonstrated clear benefits of polyandry in *T. oceanicus* (Simmons, 2001a). In addition, we think that our interpretation that sexual conflict is not prevalent in this system is conservative given that LRS measures do not take into account the potential benefits for females of producing sons that accrue high fitness because of their higher investment into accessory gland products.

There is increasing evidence that in some orthopterans, the transfer of seminal fluids entails longevity costs for females (Wedell *et al.*, 2008), although positive influences of seminal fluid products on female lifespan are common in others (Arnqvist & Nilsson, 2000; Wagner *et al.*, 2001; Wagner & Harper, 2003). We have found that the difference in the mean longevity of sets of sisters mated to males that vary in their investment into accessory gland weight ranges from 0 to 16 days (Fig. 2), with a mean longevity across all females of 38 ± 1 days after mating (approx 58 ± 1 days after adult emergence). Our results on the effect of accessory gland weight on female survival raise the question of whether this effect really represents a cost of mating. This question arises because accessory gland weight has no apparent effects on fecundity (measured during the first week after mating), and our interpretation of a cost rests on the assumption that females can expect to survive long enough in natural populations for the reduction in lifespan to manifest itself. Several lines of evidence suggest that the effects on lifespan represent a biologically relevant longevity cost. First, female *T. oceanicus* lay eggs continually throughout their lives. Thus, the reduced longevity impacts reproductively active females. Second, in our study, females mated only twice (with the same male), but natural female mating rates in this species are high. A recent study, in which the offspring produced by field-collected females were genotyped, revealed that the minimum number of mates ranged from 2 to 6 (Leigh W. Simmons, unpublished). This is a minimum estimate because females may mate repeatedly with the same male and may also have mated with different males of the same genotype. This means that the detrimental (as well as the beneficial) effects of accessory gland products are likely underestimated in our study. Third, the lifespan of females in our study is comparable to those documented for this and related species in natural or semi-natural conditions. From a sample of 63 *T. oceanicus* females collected from an Australian population, Simmons & Zuk (1994) reported a mean age of 11 days (maximum 20 days), whereas Zajitschek *et al.* (2009) found that the mean lifespan since adult emergence for females of the sister species *Teleogryllus commodus* maintained under semi-natural conditions was 42 days (max. 70 days). Likewise, the lifespan of females in a natural population of European field crickets, *Gryllus campestris*, ranged from 1 to 68 days (Rodríguez-Muñoz *et al.*, 2010). Of course, the magnitude of any lifespan cost is expected to be environment dependent and could be exaggerated under benign laboratory conditions, where sources of background mortality are very much reduced compared to natural conditions. Nevertheless and even if the longevity of females in our study was artificially high, the longevity costs of mating for females are also expected to be underestimated because of the fact that females are polyandrous. For all these reasons, we interpret the reduction in lifespan observed in this study as a biologically realistic longevity cost, which nonetheless carries no net fitness consequences because of the compensation resulting from the effects of accessory gland products on embryo viability.

The important role that accessory gland products play in reproduction, beyond the obvious functions of sperm nourishing and maintenance, is becoming increasingly clear. Accessory gland products can stimulate ovulation, egg maturation and egg laying, or influence female remating rates, or even the onset of female reproductive

senescence (Leopold, 1976; Eberhard, 1996; Simmons, 2001b; Gillott, 2003; Poiani, 2006; Reinhardt *et al.*, 2009). The transfer of seminal fluids can be either beneficial (e.g. cases of nuptial gifts in insects; Vahed, 1998; Arnqvist & Nilsson, 2000; but see Sakaluk *et al.*, 2006; Wedell *et al.*, 2008) or detrimental to females. In the later case, male harm could be adaptive, but the hypothesis that harmful traits evolve as a side effect of selection has received greater empirical support (Parker, 1979; Johnstone & Keller, 2000; Morrow *et al.*, 2003; Lessells, 2005; Hotzy & Arnqvist, 2009). In particular, the toxicity of ejaculates has been suggested to arise as a pleiotropic effect of the role of accessory gland products on sperm competition (Civetta & Clark, 2000; Pitnick & García-González, 2002; Fiumera *et al.*, 2005). However, the reality is that with the exception of a few cases (most notably the fruit fly; Kubli, 2003; Wolfner, 2009), the function of accessory gland products remains incompletely known in the majority of species.

In crickets, as many as 30 seminal proteins, suspected to be under positive selection, have been identified (Andrés *et al.*, 2006). In the genus *Teleogryllus*, accessory gland proteins are transferred to the female together with prostaglandin synthetase from the testes and converted in the female to prostaglandin, which stimulates vitellogenesis and oviposition (Loher & Edson, 1973; Stanley-Samuelson & Loher, 1983, 1986; Stanley-Samuelson *et al.*, 1986, 1987). Interestingly, it has been suggested that in *T. commodus*, females try to maintain the upper hand, using excretory pathways to eliminate prostaglandin from their circulatory system (p. 147 in Arnqvist & Rowe, 2005 and references therein). Our results show that in the closely related *T. oceanicus*, males can harm females through components of the ejaculate, but the fact that some of these components influence positively embryo survival begs the question as to whether female resistance would be favoured in this species.

Unlike *D. melanogaster*, where males with larger accessory glands have higher reproductive success in competitive contexts (Wigby *et al.*, 2009), in *T. oceanicus* accessory gland weight does not seem to influence paternity (Simmons, 2003; Simmons *et al.*, 2003). Little is known about the mechanisms involved in the accessory gland effects on female longevity and embryo viability. The effects of accessory gland products on embryo viability might be mediated by females allocating more resources to embryos when mating with males that invest heavily in their accessory gland products. If so, there could be a trade-off between resources allocated to offspring and female longevity (e.g. a kind of terminal investment; Johnstone & Keller, 2000; Lessells, 2005). Our data do not support this view, as increased accessory gland investment impacts negatively on female lifespan independently of its effects on hatching success. However, more data are needed to fully explore the relationship between increased hatching success and female lifespan. The possibility that females play an active role in the modulation of the effects of accessory gland products upon their reproduction needs full attention (see García-González & Simmons, 2007). A recent study using radiolabelling of ejaculate compounds has found that while the direct action of ejaculate compounds on eggs cannot be ruled out, these compounds may have a major effect on maternal allocation to offspring given that they are incorporated predominantly into the female's somatic tissue (Leigh W. Simmons, unpublished). These results support the notion that female allocation may mediate many of the effects attributed to the direct action of accessory gland products (Eberhard, 1996).

Rates of embryo survival in the highly fecund *T. oceanicus* are typically low but highly variable (around 30% in this study and around 50%, 20%, or 80% in other studies: Simmons, 2001a; García-González & Simmons, 2005, 2007; Simmons & García-González, 2007), in line with the values found in other cricket species (Simmons, 2005). Why these animals exhibit such high rates of embryo mortality is puzzling. Previously, we found a negative genetic correlation between fecundity and embryo viability in *T. oceanicus* (Simmons & García-González, 2007). Given the known effects of accessory gland products on female reproduction and embryo survival in this species, we suggested that males might shift females from the natural selected fecundity–embryo viability balance that maximizes maternal fitness and thus generate sexual conflict. However, as we show here, after direct and indirect fitness pay-offs are integrated, it seems unlikely that sexual conflict over embryo viability would occur.

Our results illustrate the importance of measures of net fitness to examine the consequences of sexual selection (e.g. Qvarnstrom *et al.*, 2006). Promising areas of research to integrate direct costs and indirect benefits of mating or female choice include the analyses of female LRS (e.g. this study, and Fedorka & Mousseau, 2002; Dunn *et al.*, 2005; Le Galliard *et al.*, 2008; Bilde *et al.*, 2009), an adequate estimator of fitness (Brommer *et al.*, 2004). In addition, classical approaches to the study of genetic benefits arising from sexy sons, good genes, sexy sperm, and good sperm (Orteiza *et al.*, 2005; Fisher *et al.*, 2006; Stewart *et al.*, 2008), and in particular those accounting for the multigenerational nature of indirect benefits which influence the production of grandchildren (Head *et al.*, 2005; Rundle *et al.*, 2007; Bilde *et al.*, 2008; Le Galliard *et al.*, 2008) are highly valuable. Research into the consequences of indirect genetic effects of paternal origin (i.e. paternal effects, Mousseau & Fox, 1998) on offspring performance and female fitness can also be revealing. These effects have only recently been implicated in the benefits of multiple mating and female choice. For example, in *T. oceanicus*, a female mating with a male that invests heavily in accessory gland products does not only have the viability of the embryos sired by that male enhanced, but the benefits of seminal fluids on embryo survival spill over to embryos sired by other

partners (García-González & Simmons, 2007). Similarly, in *D. melanogaster*, ejaculates containing exclusively seminal fluids have been found to be responsible for increases in daughters' fitness (Priest *et al*., 2008; but see Long *et al*., 2009).

In conclusion, we have shown that the benefits derived from mating with males that invest intrinsically more into the production of accessory gland products compensate the longevity costs associated with mating with these males. As such, the evolution of female reproductive traits in this species is most likely explained by the positive fitness returns of mating with males expressing traits that entail higher costs. Our data highlights the value of empirical studies that measure net female fitness to discern whether indirect selection can maintain costly sexual interactions.

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