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Increased copulation duration before ejaculate transfer is associated with larger spermatophores, and male genital titillators, across bushcricket taxa

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prolonged copulation;
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Abstract

Copulation duration varies considerably across species, but few comparative studies have examined factors that might underlie such variation. We examined the relationship between copulation duration (prior to spermatophore transfer), the complexity of titillators (sclerotized male genital contact structures), spermatophore mass and male body mass across 54 species of bushcricket. Using phylogenetic comparative analyses, we found that copulation duration was much longer in species with titillators than those without, but it was not longer in species with complex compared with simple titillators. A positive relationship was found between spermatophore size and copulation duration prior to ejaculate transfer, which supports the hypothesis that this represents a period of mate assessment. The slope of this relationship was steeper in species with simple rather than complex titillators. Although the data suggest that the presence of titillators is necessary to maintain long copulation prior to ejaculate transfer, mechanisms underlying this association remain unclear.

Introduction

Copulation duration varies greatly across species (Simmons, 2001). Extended copulation may be costly for both sexes, in terms of energy expended and increased predation risk, although the balance between costs and benefits may differ between the sexes (Arnqvist & Rowe, 2005). Various functions have been proposed for extended copulation, partly depending upon which phase of copulation is extended in relation to ejaculate transfer (Alexander *et al.*, 1997): when extended copulation occurs following ejaculate transfer, it may function as a form of post-copulatory mate guarding (Alcock, 1994; Simmons, 2001); where ejaculate transfer occurs during prolonged copulation, males can transfer larger ejaculates and thereby displace rival sperm or transfer substances that affect female reproductive behaviour (Simmons, 2001). In many taxa, such as bushcrickets (Orthoptera: Tettigoniidae), the copulation period is independent of the insemination period, with males transferring a spermatophore at the end of copulation (Alexander *et al.*, 1997). The insemination process begins following spermatophore transfer and normally continues for several hours after copulation (Gwynne, 2001). Thus, males delay ejaculate transfer until long after correct genital contact has been achieved, requiring continued genital contact before sperm transfer (Eberhard, 1985, 1996). The adaptive significance of prolonged copulation prior to ejaculate transfer is unclear. In certain taxa, this period may function as a form of precopulatory mate guarding or may be associated with sperm removal in some cases (Simmons, 2001). It has been proposed that extended copulation prior to spermatophore transfer in bushcrickets and other insects may function as a period of mate assessment for males (cryptic male choice: Simmons, 2001; Bonduriansky, 2001). In bushcricket species that produce large spermatophores, males are predicted to be particularly selective when it comes to

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mating: larger spermatophores are more costly to produce, limiting the potential mating rate of the male (Gwynne, 2001; Vahed, 2007a).

The spermatophore in bushcrickets consists of two parts, the sperm containing ampulla and an attached spermatophylax. This spermatophylax is eaten by the female after copulation, and functions mainly to prevent the ampulla from being removed before sperm have entered successfully into the female (Vahed, 1998, 2007b). The bushcricket spermatophylax can be an important nutrient source for females (Gwynne, 2001; Voigt *et al.*, 2005, 2006, 2008), with females being choosy about male gift giving ability (Gwynne, 1982; Lehmann & Lehmann, 2008). As in other courtship-feeding animals, the bushcricket spermatophore appears to have evolved via sexual selection and sexual conflict (Gwynne, 2001, 2008; Vahed, 2007b).

In bushcrickets, males typically possess two main types of sclerotized copulatory structures: cerci and titillators. The cerci act as nongenitalic contact devices (sensu Eberhard, 2010), allowing the male to maintain hold of the female (Rentz, 1972; Lehmann & Heller, 1998) and, in some cases, take control of copulation duration (Vahed & Carron, 2008). The titillators (Fig. 1) are paired structures that are normally concealed and are absent in some subfamilies (Beier, 1955, 1972). The titillators are inserted into the female's genital chamber prior to spermatophore transfer and move rhythmically with contractions of the male's abdomen and phallic complex (Gerhardt, 1913, 1914; Boldyrev, 1928; Duijm *et al.*, 1983; Hartely & Warne, 1984; Vahed, 1997). Little is known about the function of titillators or the adaptive significance of titillator complexity. Like other genitalic structures, bushcricket titillators are widely used as important taxonomic characters and show considerable variation across species in structure, shape and the extent to which they are spined (Harz, 1969; Rentz, 1985, 1993, 2001). Exaggerated male genitalia are a widely observed evolutionary phenomenon, with sexual selection being regarded as providing the most likely driving force behind genital diversification (Eberhard, 1985, 1996; Arnqvist, 1998). Modern comparative techniques have confirmed predicted associations between genital complexity and mating patterns across species (Hosken & Stockley, 2004; Eberhard, 2010). However, there have been few previous comparative studies of the relationship between the complexity of genital contact structures in males and copulation duration (for examples, see Dixson, 1995; Larivière & Ferguson, 2002; Takami & Sota, 2007). Here, we examine the relationships between copulation duration, titillator complexity and spermatophore size across bushcrickets, using phylogenetic comparative analyses to test the following hypotheses:

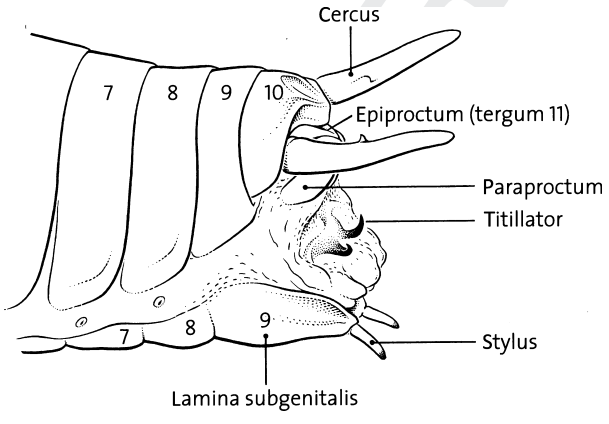

Fig. 1 Abdomen of a male bushcricket showing the position of the cerci and titillators (A. Coray).

1 The hypothesis that copulation duration (prior to ejaculate transfer) represents a mate assessment period predicts that copulation duration will increase with relative spermatophore size. Such cryptic choice could represent either cryptic female choice (Lehmann, 2007) or cryptic male choice (Wedell, 1992; Bateman & Ferguson, 2004).

2 The hypothesis that sclerotized male genitalia act as anchors, allowing males to prolong copulation (in cases where the optimal copulation duration for the male exceeds that of the female, see Arnqvist & Rowe, 2005; Edvardsson & Canal, 2006), predicts that copulation prior to ejaculate transfer will be longer in species with more complex titillators and longer in species with titillators than without. It should be noted, however, that if the female's genital chamber has coevolved to resist the complexity of the male's titillators, there might be no influence of titillator complexity on copulation duration. The genital chamber in bushcrickets, however, is unsclerotized (see Gwynne, 2001), and there do not appear to be any obvious anatomical characters that might receive the titillators. It could be argued that the predicted association between titillator complexity and copulation duration is also consistent with the hypothesis that male genitalia may act as copulatory courtship devices to ensure female acceptance of the ejaculate (Eberhard, 1985, 1996): the selection pressures favouring increased stimulation of the female could select for greater titillator complexity and longer copulation duration in concert. Note that our study is not designed to distinguish between these hypotheses.

3 Alternatively, the mating efficiency hypothesis predicts that copulation duration prior to ejaculate transfer will be shorter in species with more complex titillators and shorter in species with titillators than without. This hypothesis proposes that more complex male genitalia help males to lock more effectively into females, facilitating rapid spermatophore transfer and thereby enabling a decreased time investment in a single copulation (Takami & Sota, 2007; Holwell *et al.*, 2010).

Our results indicated that copulation duration prior to ejaculate transfer across the bushcrickets was longer in species with larger spermatophores. Copulation duration was also much longer in species with titillators than those without, but it was not longer in species with complex compared with simple titillators.

Materials and methods

Copulation duration

Data on the mean copulation duration were obtained for 54 species of tettigoniid (Table S1). Novel data for this variable were obtained for 30 of the species, whereas data for the remaining species were obtained from the literature (Table S1). Because in many species, the male engages and disengages his cerci and/or the female pulls the tip of her abdomen away from the male several times before the start of copulation *per se*, we define the copulation duration as the time from which the male establishes a firm grip with his cerci into the gonangulum until the moment when the ampulla of the spermatophore becomes visible. This definition is identical with the ones used in our previous studies (Vahed, 1996, 1997; Lehmann & Lehmann, 2000a, 2008; Lehmann, 2007; Vahed & Carron, 2008). We were unable to use some copulation duration data from the literature because it was not clear whether the duration of copulation given occurred prior to or following spermatophore transfer. Additional data on copulation duration were obtained from tettigoniids collected as adults or late stage nymphs and where sexes were maintained separately before the observation of mating. Species were sampled in a variety of European locations and held either at the University of Derby, UK, following methods described in Hartley & Dean (1974) (Vahed & Gilbert, 1996; Vahed, 2006, 2007a), or studied during field work in Greece (Lehmann, 1998; Lehmann & Lehmann, 2000a,b, 2008, 2009; McCartney *et al.*, 2008, 2010). Individual pairs were assigned to observation cages at a time of day corresponding to the peak activity period for that species. Data from two Australian species were from mating observations in field cages (Lehmann, 2007; Lehmann & Lehmann, 2007). Pairs were observed, and if copulation occurred, the duration of copulation prior to spermatophore transfer was timed to the nearest second with a digital stopwatch.

Spermatophore mass and male body mass

Data on male body mass and spermatophore mass (combining ampulla mass and spermatophylax mass) for most of the species were obtained from the literature, whereas novel data for these variables and/or extra replicates were obtained for nine of the species (Table S1). Males were weighed on an electrobalance to an accuracy of 0.01 mg immediately prior to being introduced to the observation cages. Following the end of copulation, the spermatophore was removed from the female using watchmaker's forceps and weighed.

Titillator structure

Data on titillator morphology were obtained from taxonomic sources (Table S1), chiefly Harz (1969), for the European species. We developed a ranked classification system to reflect titillator complexity (Table 1), with emphasis on the complexity (e.g. presence/absence of visible spines; clumping of spines at the tip; single or double pair of projections; see Table 1) of the apical part of the titillators (median projection) which makes contact with the female during copulation. The titillators were scored blind (by G & A Lehmann), i.e. without the knowledge of copulation duration. For the purposes of analysis, titillators were grouped into three categories according to complexity: 'None' (class 0), 'Simple' (classes 1–3) or 'Complex' (classes 4–6).

Analysis

Data were ln-transformed to meet the assumptions of parametric linear regression. Data were then analysed by fitting phylogenetic generalized least squares models (PGLS; Grafen, 1989; Martins & Hansen, 1997) using the CAICR package (http://r-forge.r-project.org/R/?group_id=140; see Freckleton, 2009) in R 2.10.0 (R Core 5

Table 1 The titillator classification scale used in this study.

Numerical classification	Explanation	Examples
0	Titillators absent	*Poecilimon*
1	No sclerotized titillators, but a densely covered field of small tubercles	*Kawanaphila*
2	One pair of sclerotized titillators, apical part (median projection) conical and not strongly projecting, may have minute teeth	*Ruspolia*, *Yersinella*
3	One pair of sclerotized titillators: apical part strongly projecting, with no teeth (the tip, however, can be hooked)	*Metrioptera roeselii*
4	One pair of sclerotized titillators: apical part strongly projecting with clearly visible teeth	*Anonconotus*, *Decticus*
5	One pair of sclerotized titillators: apical part strongly projecting with teeth concentrated on the club-shaped tip	*Metrioptera saussuriana*
6	Two pairs of sclerotized titillators: apical part strongly projecting with teeth on one or both pairs	*Gampsocleis*, *Antaxius*

Development Team, 2008). PGLS fits a linear model, but rather than assuming that all errors are drawn from a univariate normal distribution, as in ordinary least squares, PGLS instead assumes that errors will covary with species relatedness. The degree of covariance will depend upon (i) how far apart species are on the phylogeny and (ii) the assumed model of evolution along branches. The error term in the model is therefore multivariate normal and is represented by a variance–covariance matrix. This matrix describes the expected covariance among pairs of species in the variance of the distributions from which their respective errors are assumed to be drawn (Martins & Hansen, 1997). We used the function pglmEstLambda in CAICR, which assumes a Brownian motion model of trait evolution while simultaneously estimating Pagel'sλ, a measure of how closely covariance in the model residuals matches the structure of the phylogeny (Pagel, 1999; Freckleton *et al.*, 2002). Model fit was assessed visually using standard residual plots. The phylogeny used in the analysis was based primarily on the morphological phylogeny developed by Naskrecki (2000) (this phylogeny did not use titillators as a character). For the subfamily Tettigoniinae, we used the morphological phylogeny provided by Rentz & Colless (1990) (majority consensus tree of 50 equally short cladograms) because many of the genera were not included in the study conducted by Naskrecki (2000). For phylogenetic relationships within the genus *Anonconotus* (Tettigoniinae), we used an unpublished molecular phylogeny based on mtDNA (R. Szabo, G. Carron, K. Vahed & M. Ritchie). For the genus *Poecilimon* (Phaneropterinae), we used the phylogeny given by Ullrich *et al.* (2010). Branch lengths were not available and so were arbitrarily set to 1. As candidate predictor variables to explain copulation duration, we included titillator complexity, male body mass and spermatophore mass, plus all possible interactions between these variables. We fitted multiple models under an Information Theoretic framework, using Akaike's Information Criterion, corrected for sample size (AICc) as a criterion for model selection. Models were ranked according to their AICc, and models within 2 points of the lowest-ranked model were treated as equally likely (Burnham & Anderson, 2002).

The prediction that copulation duration will be longer in taxa with titillators and that the presence of titillators will affect the relationship between copulation duration and spermatophore size required us to compare taxa with titillators against those without titillators. The prediction that copulation duration will correlate positively with titillator complexity required us to compare species with simple titillators against those with complex titillators. Accordingly, to test these predictions, once the best models had been established, we conducted two appropriate *a priori* orthogonal contrasts among the three levels of titillator complexity: 1, 'None vs. (Simple or Complex)'; 2, Simple vs. Complex.

We first conducted our analyses on the full data set for which all relevant data were available ($n = 54$) and then again on each bushcricket subfamily. For the Tettigoniinae ($n = 22$), we were able to fit the full set of candidate models. For the other subfamily with > 10 data points (Phaneropterinae, $n = 17$), data were too few to fit multiple models, so we fitted a simple main effects PGLS model incorporating titillator complexity, spermatophore size and male size as predictor variables and tested the significance of each using likelihood-ratio tests.

Results

Bushcricket copulation duration varied from 8 s to 105 min (Table S1). Among all species in our data set ($n = 54$), there was one single best model of copulation duration (delta AICc < 2; Table 2a). This model contained titillator complexity and spermatophore mass, plus their interaction, indicating that the relationship between copulation duration and spermatophore mass was different depending on titillator complexity. Deviance increased significantly upon dropping each predictor variable from this model (likelihood-ratio tests, $P < 0.01$). Copulation duration was much longer in species with titillators as opposed to without (Contrast 1, $t = 2.96$, df = 1, $P < 0.005$) but actually was no longer in species with complex compared with simple titillators (Contrast 2, $t = -1.48$, df = 1, $P = 0.12$; Fig. 2). The interaction term indicated that, in addition to its main effect upon copulation duration, titillator complexity also affected the spermatophore size/copulation duration relationship. Copulations were always longer in species with larger spermatophores, and the slope of this relationship was no different in species with no titillators as opposed to species possessing any kind of titillators (Contrast 1, $t = 0.92$, df = 1, $P = 0.36$; but see also the separate analysis of the Phaneropterinae, described later). Among species with titillators, though, this relationship was actually steeper in species with simple rather than complex titillators (Contrast 2, $t = 2.94$, df = 1, $P < 0.005$, Fig. 3 and Table 3a). Phylogenetic structure in copulation duration was weak after accounting for predictor variables ($\lambda \sim 0$, Table 2a).

Within the Tettigoniinae ($n = 22$), which all possess titillators, the best four models again contained titillator complexity plus either spermatophore size or male size, with one model containing an additional titillator complexity by spermatophore size interaction (delta AICc < 1, Table 2b). The relationship was similar in form to that obtained in the wider analysis; copulation duration was generally shorter in species with more complex titillators but increased with spermatophore size (or male size; Table 3b). One of these four best models also contained the interaction between titillator complexity and spermatophore size; in this model, the copulation duration/spermatophore size relationship was steeper in species with simple titillators than with

Table 2 AICc table for analysis of the (a) full data set, (b) Tettigoniinae. Top model is shown in bold type.

Model	*k*	AICc	ΔAICc†	Relative likelihood‡	Akaike weight§	*λ*
(a)						
TTL + MALE + SPM + TTL:SPM	**7**	**128.79**	**0.00**	**1.00**	**0.53**	**< 0.001**
TTL + MALE + SPM + TTL:SPM + MALE:SPM	8	130.98	2.20	0.33	0.18	< 0.001
TTL + MALE + SPM + TTL:MALE	7	132.25	3.46	0.18	0.09	< 0.001
TTL + MALE + SPM	5	132.29	3.50	0.17	0.09	< 0.001
TTL + MALE + SPM + TTL:MALE + MALE:SPM	8	133.93	5.15	0.08	0.04	< 0.001
TTL + MALE + SPM + SPM:MALE	4	134.36	5.57	0.06	0.03	< 0.001
TTL + MALE + SPM + TTL:MALE + TTL:SPM	9	135.57	6.78	0.03	0.02	< 0.001
TTL + MALE + SPM + TTL:MALE + TTL:SPM + MALE:SPM	10	137.33	8.55	0.01	0.01	< 0.001
TTL + SPM	4	138.33	9.54	0.01	0.00	< 0.001
TTL + SPM + TTL:SPM	6	139.60	10.81	0.00	0.00	< 0.001

Model	*k*	AICc	ΔAICc†	Relative likelihood‡	Akaike weight§	*λ*
(b)						
TTL + SPM	**4**	**43.13**	**0.00**	**1.00**	**0.25**	**< 0.001**
TTL + SPM + TTL:SPM	**6**	**43.50**	**0.37**	**0.83**	**0.20**	**< 0.001**
SPM	**2**	**43.62**	**0.48**	**0.79**	**0.19**	**< 0.001**
TTL + MALE	**4**	**43.87**	**0.74**	**0.69**	**0.17**	**< 0.001**
MALE + SPM	3	46.12	2.99	0.22	0.06	< 0.001
TTL + MALE + TTL:MALE	6	46.63	3.50	0.17	0.04	< 0.001
TTL + MALE + SPM	5	47.11	3.98	0.14	0.03	< 0.001
TTL + MALE + SPM + TTL:SPM	7	48.01	4.88	0.09	0.02	0.176
1 (i.e. intercept-only model)	1	48.47	5.34	0.07	0.02	< 0.001
TTL + MALE + SPM + TTL:MALE	7	48.49	5.36	0.07	0.02	0.107

AICc, Akaike's Information Criterion; *k*, number of parameters; TTL, titillator complexity (none, simple or complex); MALE, male body mass; SPM, spermatophore size; 'X:Y' denotes an interaction term between terms X and Y.
†Difference in AICc between model X and the top model.
‡Calculated as $e^{(-0.5 \times \Delta AIC)}$.
§Calculated as (relative likelihood of model X)/(sum of relative likelihoods of all models).

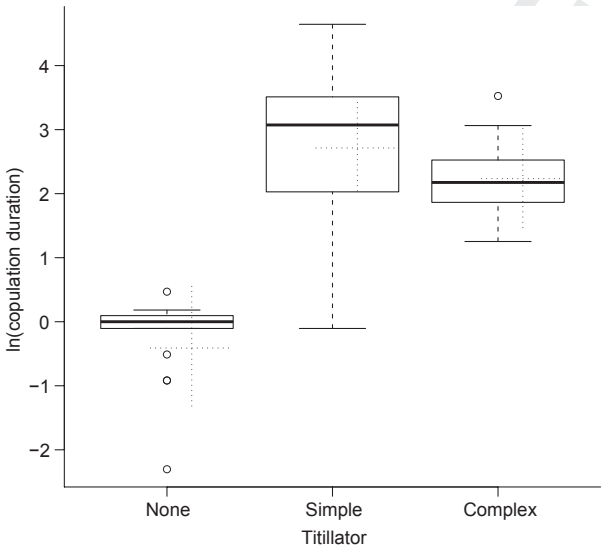

Fig. 2 Copulation duration in bushcrickets with different classes of titillator complexity. Solid boxplots show medians ± IQR (boxes) and 90% quantile (whiskers). Dotted lines show estimated means ± standard errors from the best phylogenetic model, with other predictor variables held at mean values (see text for details).

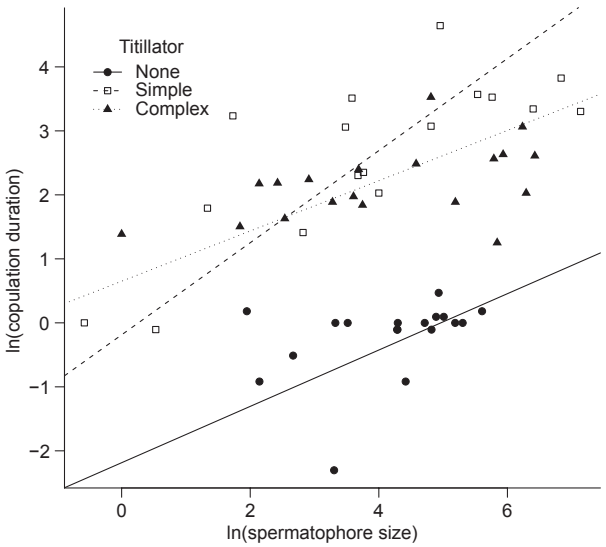

Fig. 3 Relationship between copulation duration and spermatophore size (ln mg) among bushcrickets with different classes of titillator complexity. Regression lines are given from the phylogenetic model.

Table 3 Table of parameter values for (a) top model of copulation duration (see Table 2a) in analysis of full data set and (b) equally supported models of copulation duration (see Table 2b) in analysis of Tettigoniinae. Where appropriate, values are given as mean and SE for each level of titillator complexity. Model terms are abbreviated as in Table 2.

TTL	Intercept	SE	SPM slope	SE	MALE slope	SE
(a)						
None	1.16	0.96	0.44	0.17	−0.53	0.14
Simple	3.15	0.71	0.72	0.17		
Complex	3.99	0.78	0.39	0.19		

Model	TTL	Intercept	SE	SPM slope	SE	SIZE slope	SE
(b)							
15	Simple	1.63	0.37	0.26	0.08		
	Complex	1.16	0.21				
9	Simple	0.34	0.78	0.56	0.18		
	Complex	1.41	0.83	0.20	0.19		
17	Simple	1.34	0.37	0.26	0.08		
	Complex						
13	Simple	0.09	0.86			0.43	0.14
	Complex	−0.70	0.08				

complex titillators, which mirrored the findings of the wider analysis. Within the Tettigoniinae, phylogenetic structure in copulation duration was again weak ($\lambda \sim 0$).

In the Phaneropterinae ($n = 17$), none of which, in our sample, possessed titillators, we fitted PGLS models with only main effects owing to small sample size. Copulation duration was related neither to spermatophore mass (LRT statistic = 0.05, df = 1, $P = 0.82$) nor to male body mass (LRT = 1.12, df = 1, $P = 0.30$); Pagel's lambda could not be statistically differentiated from either 1 or 0.

Discussion

In our study, copulation duration across bushcricket taxa was explained by both spermatophore mass and titillator occurrence, plus their interaction. Copulation duration was longer in taxa with larger spermatophores, which supports the hypothesis that longer copulation durations function as a mate assessment period (Gwynne, 1981; Wedell, 1992; Bateman & Ferguson, 2004) and the prediction that males are expected to be increasingly choosy as male mating costs increase (Bonduriansky, 2001; Simmons, 2001). Although previous studies of individual species with particularly large and costly spermatophores and/or ejaculates have provided evidence that prolonged copulation prior to ejaculate transfer can act as a mate assessment period for males in bushcrickets and other insects (Gwynne, 1981; Wedell, 1992; Wang & Millar, 1997; Bateman & Ferguson, 2004), the present study appears to be the first to provide comparative evidence to support this hypothesis. It should be noted, however, that although male bushcrickets are putatively in control of copulation duration prior to spermatophore transfer because the male's cerci link the pair together and the timing of spermatophore transfer is presumably determined by the male, the possibility still exists that events within the female's body may influence the duration of copulation. This period could therefore also act as a mate assessment phase for the female (Lehmann, 2007). If, however, large spermatophores benefit females nutritionally (see Gwynne, 2001; Voigt *et al.*, 2005, 2006, 2008), it would seem to be in the female's interests to accept spermatophores from all males, rather than to break off copulation prior to spermatophore transfer. Species with larger spermatophores, however, have a longer sexual refractory phase in females and a lower lifetime degree of polyandry, which appears to be induced by substances in the ejaculate (Vahed, 2006, 2007a). Thus, a female that had mated with an unsatisfactory male might be delayed or prevented from finding a better male if she accepted the spermatophore. In such species, the spermatophores are generally very firmly attached and the female must eat her way through the gelatinous spermatophylax before she can interfere with ejaculate transfer (Gwynne, 2001; Vahed, 2007b). Therefore, females might benefit from assessing males prior to spermatophore transfer. Further experiments are required to determine which sex is in control of this phase of copulation in bushcrickets (see Lehmann & Lehmann, 2000a, 2008; Lehmann *et al.*, 2011). It should also be noted that a positive correlation between spermatophore mass and copulation duration prior to spermatophore transfer is expected if we assume that males that invest more into the nuptial gift will also increasingly invest into stimulation of the female via a long copulation to ensure that the female accepts the spermatophore (see Eberhard, 1996).

An alternative interpretation of the positive relationship between copulation duration prior to spermatophore transfer and spermatophore mass is simply that larger spermatophores take longer to form (in tettigoniids, unlike gryllid crickets, spermatophores are only partly formed prior to copulation, Boldyrev, 1928). However, data from the seventeen species of Phaneropterine bushcrickets in the present study go against this explanation: within this subfamily, there was no relationship between spermatophore mass and the duration of copulation prior to spermatophore transfer: spermatophores were transferred after about just 1–2 min of copulation in most species, even those with very large spermatophores. Why phaneropterines should have such short copulations is uncertain: it is possible that in this subfamily, the mate assessment period occurs at an earlier stage of the mating process (Lehmann & Lehmann, 2008) than in subfamilies such as the Tettigoniinae. To determine whether this lack of a relationship between copulation duration and spermatophore size is specific to the Phaneropterinae or general to species without titillators,

more data points from nonphaneropterine species that lack titillators would be required.

Our analysis clearly demonstrates that copulation duration across the bushcrickets was longer in species possessing titillators than those without, although more fine-scale analysis within subfamilies that vary in the occurrence of titillators (e.g. the Phaneropterinae, Pseudophyllinae, Listroscelidinae and the Tribe Agraecini, see Ingrisch, 1998; Rentz, 2001; Naskrecki & Bazelet, 2009; Rentz *et al.*, 2010) could further test the generality of this pattern. This appears to go against the mating efficiency hypothesis – i.e. that more complex genitalia facilitate rapid spermatophore transfer. In apparent contrast, Takami & Sota (2007) found that in a carabid beetle genus, the size of the male's sclerotized 'copulatory piece' correlated negatively with copulation duration. However, we did find that the relationship between copulation duration and spermatophore size in the present study was steeper in species with simple rather than complex titillators, i.e. species with more complex titillators transferred larger spermatophores more rapidly than those with simple titillators. This could be taken as support for the mating efficiency hypothesis.

That copulation duration was longer in species possessing titillators than those without in our study is consistent with the hypothesis that sclerotized genital contact structures are more efficient as anchors, allowing the male to prolong copulation (Edvardsson & Canal, 2006). However, we cannot exclude the possibility that male genitalia may act as copulatory courtship devices to ensure female acceptance of the ejaculate (Eberhard, 1985, 1996): the selection pressures favouring increased stimulation of the female could select for titillators and longer copulation duration in concert. A related possibility is that copulatory courtship might be necessary to induce the female to remain still during prolonged copulation prior to spermatophore transfer. To help disentangle these mechanisms, further knowledge is required as to exactly how titillators contact female tissues during copulation. It could be argued that the observation that titillators move rhythmically within the female's genital chamber (e.g. Boldyrev, 1928) goes against the Anchor hypothesis: if an organ can be partly withdrawn, it seems unlikely that it can act as an anchor (Eberhard, 2011). However, rhythmic movement of the titillators could be compatible with the Anchor hypothesis if, as suggested by Hartely & Warne (1984), their action within the female is rachet-like.

We can summarize that copulation duration across the bushcrickets was longer in species with larger spermatophores and in species possessing titillators than those without. The former result is consistent with the hypothesis that prolonged copulation prior to ejaculate transfer is a mate assessment period, although it is unclear to what extent males or females control the duration of this period. The latter result is consistent with the hypothesis either that complex genitalia are more efficient as anchors, thereby allowing the male to prolong copulation, or that they act as copulatory courtship devices to ensure female acceptance of the ejaculate (Eberhard, 1985, 1996, 2010). Experiments on focal bushcricket species would complement the comparative approach used here and could be designed to distinguish between female and male controls over the copulatory period (see Eberhard, 2011).

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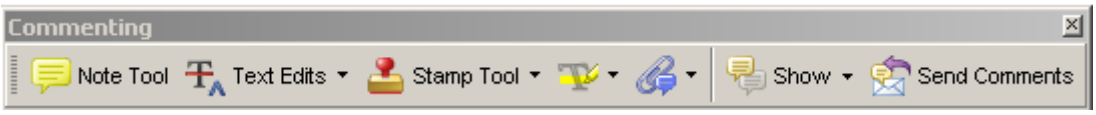

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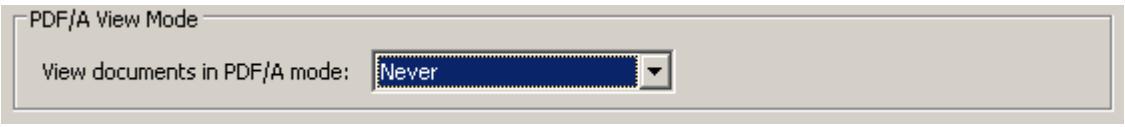

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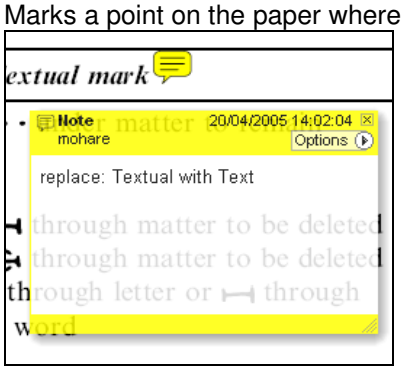

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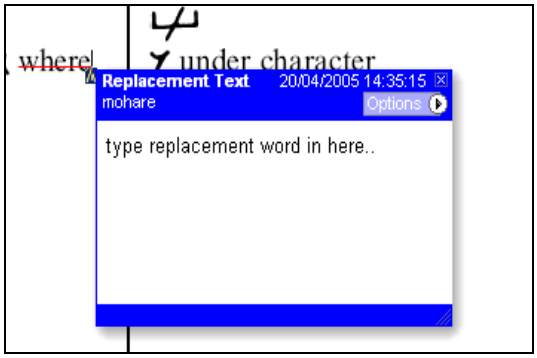

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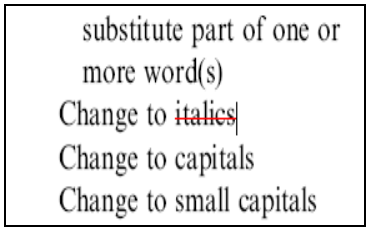

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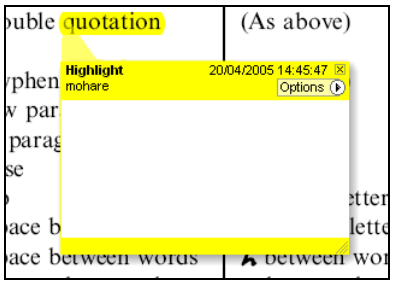

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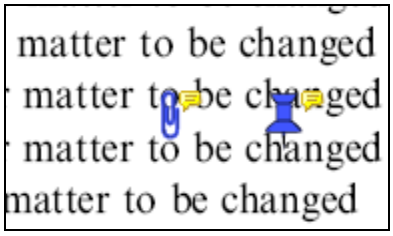

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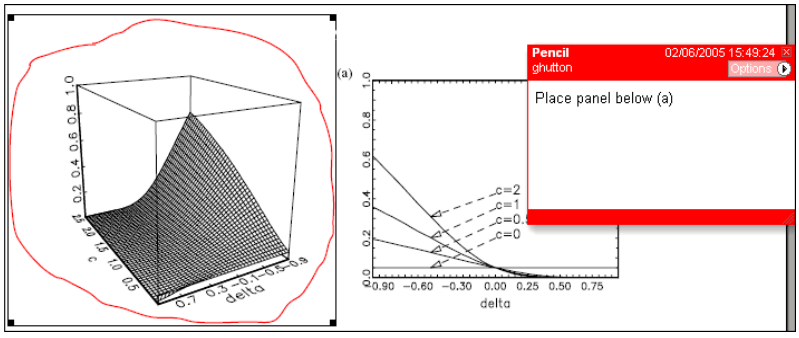

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