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ALLOCATIONS STRATEGIQUES DES RESSOURCES REPRODUCTIVES, GAMETIQUES ET NON-GAMETIQUES, CHEZ DEUX ESPECES D'INSECTES

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SOMMAIRE

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Résumé en français :

Pour les mâles, non seulement la reproduction est une activité particulièrement couteuse, mais les ressources dont ils disposent pour y faire face s'avèrent souvent largement contraintes. En effet, la production de l'éjaculat (sperme et substances non gamétiques) peuvent générer des couts énergétiques substantiels, voire être limités au cours de la vie des mâles. Par ailleurs, les traits impliqués dans l'attraction des femelles ou la compétition entre mâles rivaux peuvent également être liés à d'importantes dépenses énergétiques et/ou des risques de dommages physiques. Enfin, la sélection sexuelle post-éjaculat (compétition spermatique et choix femelle cryptique) peut fortement affecter leurs chances de paternité. Sous ces conditions, il est attendu que les mâles allouent stratégiquement leurs ressources reproductives au regard (i) des opportunités d'accouplement présentes et futures, (ii) de la qualité reproductive de la femelle et (iii) du risque et/ou de l'intensité de la compétition spermatique. Toutefois, la nature des allocations stratégiques chez les mâles et les contraintes qui influencent leur évolution restent encore mal comprises, en particulier parce que les interactions potentielles entre sélection sexuelle pré- et post-copulatoire peuvent soumettre les mâles à des épisodes sélectifs plus complexes que ceux existants chez les femelles.Dans le cadre de ce travail de thèse, j'ai examiné les allocations de ressources reproductives chez les mâles de deux espèces d'insectes : le papillon *Achroia grisella* et la sauterelle *Ephippiger diurnus*. Les mâles de ces deux espèces émettent des chants d'appel sexuels ciblés par les préférences des femelles, et subissent de ce fait d'intenses pressions de sélections pré-copulatoire intra et intersexuelles. En revanche, ces deux modèles diffèrent grandement dans la contribution des mâles aux femelles lors de l'accouplement : tandis que le transfert des mâles *A. grisella* se résume aux spermatozoïdes, les mâles *E. diurnus* transfèrent le produit de leurs glandes accessoires sous la forme d'un large spermatophylax que les femelles consomment à l'issue de l'accouplement. J'ai donc plus spécifiquement étudié les variations d'allocation i) spermatique chez A. *grisella* et ii) du spermatophylax chez *E. diurnus*, au regard de la variance de qualité des femelles et/ou de la compétition inter-mâle pré- et post-copulatoire. Les résultats obtenus dans le cadre de cette étude révèlent des allocations stratégiques complexes et variées au sein des deux espèces étudiées, notamment en réponse aux indices sociaux révélateurs de compétition pré et/ou post copulatoire. En revanche, la qualité des femelles semble avoir un effet marginal sur les variations de réponse sexuelle des mâles *A. grisella* et *E. diurnus*. Nos résultats fournissent un apport empirique intéressant dans la compréhension des facteurs sous-jacents à l'évolution d'allocations stratégiques chez les mâles sous pressions conjointes de sélection pré et post-copulatoire. Ils mettent en lumière que la combinaison des processus sélectifs peut contraindre l'évolution d'allocations stratégiques associées à la qualité de la partenaire. Ces résultats sont ensuite discutés dans le contexte de la théorie de la sélection sexuelle et des rôles sexuels.

Résumé en anglais

Contrasting with a long assumed assumption, reproduction is particularly costly for males. Not only can ejaculate production (sperm and other non gametic materials) induce substantial energetic costs and be drastically limited along males' life, but the traits involved in mate attraction or competition between rival males might further incur energetic cost and/or risks of injuries. In addition, post-copulatory mechanisms such as sperm competition and cryptic female choice might strongly bias male's chances of paternity. Under these conditions, males are expected to strategically allocate their reproductive resources according to i) present and future mating opportunities, ii) the female reproductive quality and iii) the risk or intensity of sperm competition.

However, the means by which males allocate their reproductive resources as well as the factors affecting their evolution remains poorly understood, in particular because potential interactions between pre and post-copulatory sexual selection makes the selective episodes in males often far more complex than those existing in females.

Therefore, during my PhD, I investigated the strategic allocations of male reproductive resources in two insect species: the moth *Achroia grisella* and the bushcricket *Ephippiger diurnus*. In both species, males experience strong precopulatory sexual selection, as males display sexual courtships songs targeted by female choice. However, these two models are contrasting when it comes to the male contribution to females during copulation: while males *A. grisella* appear to transfer only sperm, males *E. diurnus* provide females with the product of their accessory glands under the form of a large spermatophylax, that females readily consumes after mating. Thus, I more specifically investigated the variations of male investment in i) fertilizing sperm in *A. grisella*, and ii) spermatophylax in *E. diurnus*, in regards to female quality and/or pre and post-copulatory intra-sexual competition.

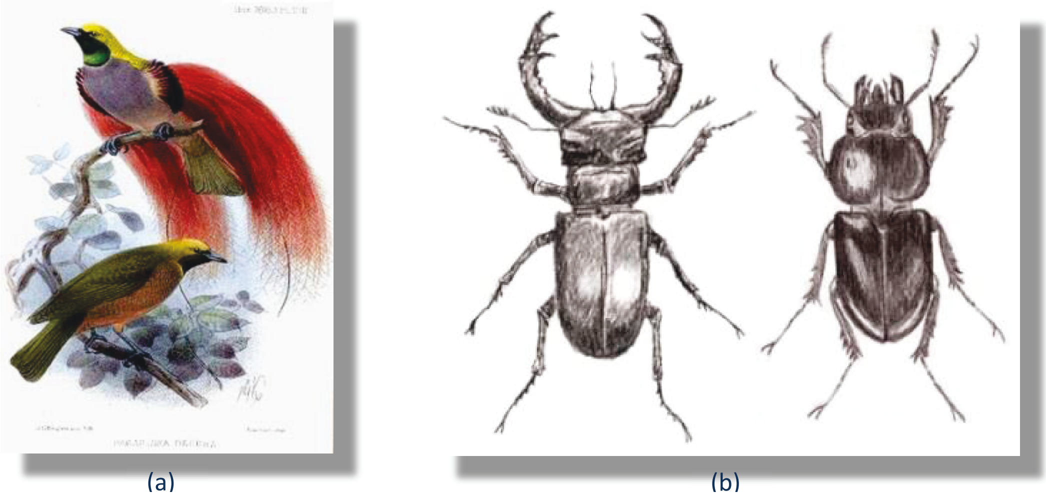
The results obtained in this study revealed complex and multimodal allocations of reproductive resources in the two model species, in particular according to social cues indicative of pre and/or post copulatory competition. However, considering both species, variance in female quality appears to have a marginal effect on males' sexual response.

Our results provide interesting empirical insight on the factors affecting the evolution of male strategic allocation under joint pre and post-copulatory selective pressures. In particular, they shade light on the combination of selective pressure that might constrain the evolution of strategic allocation toward female quality. These results are further discussed within the frame of sexual selection theory and sex roles.

Introduction générale

La science progresse au travers d'idées et d'hypothèses qui sont autant de nouvelles manières de voir le monde. Si ces idées sont confirmées au travers de l'expérimentation, elles constituent alors la vision la plus probable du monde qui nous entoure, et ce jusqu'à ce qu'une nouvelle idée en fournisse une meilleure explication. Ainsi, la théorie de la sélection naturelle publiée par Darwin en 1859 remplace le concept de Théologie Naturelle selon lequel la nature est l'œuvre de Dieu, dont l'Existence est reflétée dans la perfection avec laquelle chaque espèce est adaptée à son environnement (Ray 1691, Paley, 1802). Toutefois, il apparait rapidement à Darwin que la sélection naturelle, dont les mécanismes favorisent les traits avantageux pour la survie et la fécondité, ne permet pas d'expliquer les traits sexuels secondaires extravagants, tels que les ornements vifs et variés, les chants puissants ou encore les armements imposants rencontrés chez les mâles de nombreuses espèces d'oiseaux, d'insectes et de mammifères (Darwin 1959, 1871 ; Figure 1). Ces traits, qui semblent coûteux voire préjudiciables à la lutte pour l'existence (Darwin, 1859, 1871 ; Wallace, 1871), auraient dû au contraire être éliminés par la sélection naturelle. Il propose alors la théorie de la sélection sexuelle, qu'il définit comme **l'avantage de certains individus d'une même espèce et d'un même sexe uniquement à l'égard de la reproduction** (Darwin 1871). Aussi, s'il existe une grande variance dans le succès reproducteur des individus liée à la variation de traits héritables, la sélection sexuelle sera forte, perpétuant les traits de ceux qui se reproduisent le plus.

A l'issue de ses observations, Darwin décrit les rôles sexuels « typiques » et propose deux mécanismes distincts, et non mutuellement exclusifs, par lesquels la sélection sexuelle opère : la **sélection intra-sexuelle** désigne la lutte entre les individus d'un même sexe, en général les mâles, pour l'accès aux individus du sexe opposé, tandis que la **compétition intersexuelle** reflète la sélectivité des membres d'un sexe envers les individus de l'autre sexe et qui caractérise, selon lui, le sexe femelle (Darwin, 1871). Si la compétition intra-sexuelle est relativement bien acceptée par la critique de l'époque (Birkhead, 2010 ; Huxley 1938), l'idée que les femelles puissent être sélectives est en revanche vivement rejetée (Wallace, 1889 ; Huxley 1938).



(a) (b)

Figure 1 : Deux exemples de traits extravagants portés par les mâles. A gauche (a), les mâles du Paradisier de Golgie (*Paradisia decora*) possèdent un plumage flamboyant qu'ils exhibent lors de parades nuptiales complexes pour « séduire » les femelles (Frith et Frith 2009). A droite (b), les Lucanes Cerf-Volant *(Lucanus cervus)* mâles utilisent leurs mandibules hypertrophiées dans les combats entre mâles : contrairement aux apparences, ces dernières sont moins efficaces que celles des femelles quand il s'agit d'infliger des morsures (Paulian et Baraud 1982). Crédit image : (a) J.G Keulemans, (b) M. Thyssen.

D'une manière générale, l'étude de la sélection sexuelle tombe en désuétude pendant plusieurs décades, et c'est plus de 70 ans plus tard que les travaux de Bateman tentent d'apporter une explication aux rôles sexuels décrits par Darwin (Bateman, 1948; Bonduriansky, 2001; Darwin, 1871). En mesurant le succès reproducteur de petits groupes de mâles et de femelles *Drosophila melanogaster* chromosomiquement distincts, il révèle que la variance du succès reproducteur des mâles surpasse celui des femelles, mais aussi que la paternité des mâles augmente linéairement avec le nombre de copulations tandis que la fertilité des femelles augmente peu (voire pas du tout) avec le nombre d'accouplements réalisés. Bateman attribue ces différences à l'**anisogamie**, c'est-à-dire au contraste de taille et de coût de production entre les gamètes des deux sexes. Aussi, parce qu'elles produisent peu de gamètes

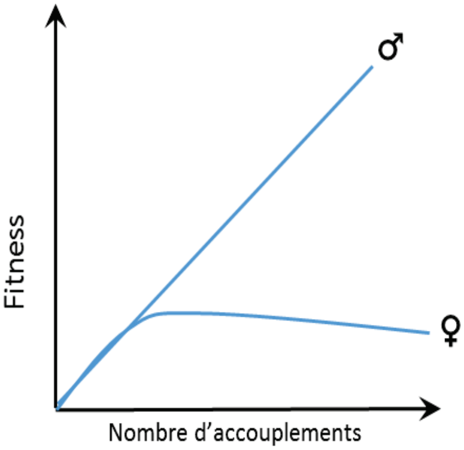


Figure 2 : Ci-dessus, le gradient de Bateman (1948), illustrant que la variance de la fitness des mâles excède celle des femelles.

volumineux et coûteux énergétiquement, le succès reproducteur des femelles est limité par le nombre d'œufs qu'elles peuvent produire et non par le nombre d'accouplements réalisés. Au contraire, le succès reproducteur des mâles ne semble pas restreint par la production de gamètes mais seulement par le nombre de femelles qu'ils parviennent à inséminer avec succès (Bateman 1948, p. 364, Figure 2).

Les idées préliminaires de Bateman feront l'objet de nombreux développements théoriques, notamment par Robert Trivers qui présente un modèle verbal identifiant la relation existant entre l'**investissement parental** (Parental Investment, PI), la disponibilité des partenaires et la sélection sexuelle. Ce modèle prédit que l'investissement parental - c'est-à-dire tout investissement dans la descendance actuelle intervenant aux dépens de la capacité à investir dans la descendance future - conditionne les rôles sexuels en déterminant le temps de récupération associé à chaque épisode de reproduction (Trivers, 1972, 1985). Ainsi, lorsque le sexe ratio des adultes est équilibré et en l'absence de soin parental, l'anisogamie prédit clairement que le nombre de mâles en capacité de se reproduire excède celui des femelles sexuellement disponibles. Ces dernières auraient alors avantage à être sélectives (Trivers, 1972).

Le modèle de Trivers galvanise un véritable regain d'intérêt pour la sélection sexuelle et la sélectivité intersexuelle – décriée un siècle plus tôt – se trouve créditée d'un flot de preuves empiriques et théoriques attestant de son importance non seulement en ce qui concerne l'évolution de traits chez les mâles, mais aussi chez les femelles (Basolo, 1990, 1995; Grafen, 1990a, 1990b; Hamilton & Zuk, 1982; Lande, 1981; O'Donald, 1980; Pomiankowski, 1987; Zahavi, 1975, 1977).

D'autres études s'attachent à trouver des prédicteurs plus facilement mesurables de la force et de la direction de la sélection sexuelle : le sexe ratio opérationnel (Operational Sex Ratio, OSR) et le potentiel reproducteur des individus (Potential Reproductive Rate, PRR) furent définis à cet effet. Le **sexe ratio opérationnel** (OSR), défini comme le quotient du nombre de femelles fécondables sur le nombre de mâles sexuellement actifs, fut tout d'abord proposé comme mesure permettant de traduire la différence de PI entre mâles et femelles et de prédire les rôles sexuels (Emlen & Oring, 1977). Un peu plus tard, il fut admis que le nombre de descendants indépendants que les parents peuvent produire par unité de temps, ou **potentiel reproducteur**

des individus (PRR), constituait l'index clé prédisant le maximum de compétition au sein du sexe ayant le potentiel reproducteur le plus élevé pour le sexe ayant le potentiel reproducteur le plus faible (Clutton-Brock & Parker, 1992; Clutton-Brock & Vincent, 1991). En jargon acronymique, on peut donc résumer les choses sous la forme suivante : le PRR d'un sexe est inversement corrélé à son PI, et le sexe sélectif est celui avec le PRR le plus faible parce que l'OSR se trouve alors biaisé en faveur de l'autre sexe.

Le socle théorique ainsi formé constitue le **modèle classique[1] de la théorie de la sélection sexuelle**, et la majorité des scientifiques y trouve une explication satisfaisante à la nature des rôles sexuels et à l'évolution des traits qui en découlent. Aussi, l'idée largement admise entre la fin des années 70 et le début des années 80 suggère que les coûts liés à la reproduction sont importants pour les femelles, mais insignifiants pour les mâles. Pour ces derniers, il est donc admis que la maximisation de leur fitness dépend avant tout de leur capacité à concrétiser l'acte d'accouplement. Un excellent aperçu de la conception des rôles sexuels à cette période est donné par Richard Dawkins dans son ouvrage influent « Le gène égoïste » (Dawkins 1989, p.76) :

"Since a female produces a limited number of eggs at a relatively slow rate, she has little to gain from having a large number of copulations with different males. A male on the other hand, who can produce millions of sperm every day, has everything to gain from as many promiscuous mating as he can snatch. [...]. A male on the other hand can never get enough copulations with as many different females as possible: the word excess has no meaning for a male."

Toutefois, de nouvelles avancées dans le dernier tiers du 20ème siècle jettent un jour nouveau sur les mécanismes qui influencent le succès reproducteur des mâles et la conception des rôles sexuels. En effet, la découverte de la **compétition spermatique** (Parker 1970, Chapitre 1, Partie 2.2.1 et Encadré 1), suivie un peu plus tard par la mise en évidence que la **production de l'éjaculat est coûteuse et limitée** pour les mâles (Dewsbury, 1982; Nakatsuru & Kramer, 1982), effriteront l'idée selon laquelle il suffit aux mâles de multiplier les accouplements pour accroître leurs chances de paternité. Dès lors, une vaste proportion des recherches s'oriente vers l'étude

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[1] Aussi parfois appelé modèle de Bateman-Trivers ou Darwin-Bateman (Bonduriansky 2005)

des mécanismes permettant aux mâles de faire face aux contraintes reproductives nouvellement mises en lumière.

En effet, **leurs ressources reproductives étant limitées**, et leur succès reproducteur reposant potentiellement sur des **interactions complexes entre épisodes sélectifs pré- et post-éjaculat**, il est attendu que **les mâles allouent stratégiquement leurs ressources de sorte à maximiser leurs retours en terme de fitness.**

Les travaux ainsi menés peuvent être répartis selon deux axes principaux : 1) un considérable effort théorique et empirique concerne les ajustements des ressources spermatiques au regard du risque et de l'intensité de la compétition spermatique (Kelly & Jennions, 2011; Parker & Pizzari, 2010, Encadré 1 et 2) de nombreux travaux examinent les préférences des mâles au regard de la qualité reproductive de la femelle (choix des mâles ; Bonduriansky, 2001; Edward & Chapman, 2011, Partie 3.1).

Ces nouvelles découvertes conduiront à d'importants remaniements de la théorie de la sélection sexuelle. En particulier, l'accumulation d'exemples de sélectivité chez les mâles (Bonduriansky, 2001, 2009 ; Edward & Chapman, 2011 ; Gowaty & Hubbell, 2005 ; Gowaty, Steinichen, & Anderson, 2003), mais aussi de compétitivité chez les femelles (Bro-Jørgensen, 2007; Rillich, Buhl, Schildberger, & Stevenson, 2009; Rosvall, 2008), révèle que la nature des rôles sexuels est plus diverse, plus dynamique, et surtout moins prédictible qu'il ne l'a tout d'abord été supposé (Berglund, Widemo, & Rosenqvist, 2005; Gowaty, Steinichen, & Anderson, 2002). Par ailleurs, les mécanismes par lesquels les mâles allouent leurs ressources reproductives restent encore mal compris, notamment au sein des espèces chez lesquelles les femelles expriment des préférences marquées pour certains partenaires (Johnstone, Reynolds, & Deutsch, 1996; Kokko & Johnstone, 2002) et/ou lorsque les épisodes de sélection auxquels sont soumis les individus sont complexes en intervenant avant et après le transfert de sperme (Chapitre 1, Partie 2). Or, la compréhension de ces mécanismes ainsi que des interactions entre processus sélectifs chez les mâles et femelles est indispensable pour appréhender la coévolution des traits reproducteurs exprimés chez les mâles et les femelles et l'évolution des systèmes d'appariements (Fritzche & Booksmythe, 2013).

Au cours cette thèse, j’ai donc exploré les allocations de ressources reproductives, gamétiques et non gamétiques, au regard de différents facteurs (qualité des femelles, risque de compétition spermatique…) chez deux espèces d’insectes chez lesquelles les mâles subissent d’intenses pressions de sélection pré-éjaculat intra et intersexuelles (Chapitre 1, Partie 4). Les objectifs de ce travail et le plan du présent manuscrit sont détaillés ci-dessous.

Contexte scientifique, objectifs de la thèse et plan du mémoire

Pour les mâles, la reproduction implique souvent d'importants coûts en énergie et en temps, qui se traduisent en une limitation plus ou moins importante de leur potentiel reproducteur (Chapitre 1, Partie 1). Par ailleurs, les épisodes sélectifs conditionnant les chances de paternité des mâles peuvent être complexes et impliquer des mécanismes intervenant avant et/ou après le transfert de sperme (Chapitre 1, Partie 2). **Afin de maximiser leur succès reproducteur total, il est donc attendu que les mâles allouent stratégiquement leurs ressources reproductives au regard des coûts et des bénéfices inhérents à chaque étape de la reproduction** (Chapitre 1, Partie 3).

Toutefois, de nombreuses zones d'ombre subsistent concernant la **nature** des allocations stratégiques (c'est-à-dire *comment* elles sont exprimées), ainsi que sur les **facteurs influençant leur évolution** chez les mâles (*pourquoi* elles sont exprimées). Tout d'abord, l'enchevêtrement complexe de facteurs et de pressions de sélection qui conditionne les chances de paternité des mâles peut amener à des réponses sexuelles flexibles et multimodales, souvent exprimées de façon cryptique[2] (Chapitre 1, Partie 3). De plus, les allocations stratégiques sélectionnées sont celles permettant aux mâles de maximiser leurs chances de paternité à l'issue de l'ensemble des épisodes sélectifs et compétitifs, or les interactions potentielles entre les différents facteurs peuvent rendre difficile l'établissement de prédictions, notamment en ce qui concerne l'évolution de préférences pour la qualité reproductive de la femelle. Par exemple, si au-delà de la variance de qualité des femelles, il existe de la compétition spermatique (Chapitre 1, Partie 2.2.1, Encadré 1), les coûts et bénéfices à choisir les partenaires peuvent être remis en perspective au regard des coûts et bénéfices liés aux allocations stratégiques des ressources reproductives visant à maximiser le taux de fertilisation des gamètes femelles (variation de la quantité de sperme transféré aux femelles, gardiennage post-éjaculat[3] des partenaires sexuelles… ; Kvarnemo &

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[2] C'est-à-dire exprimées lors de l'accouplement, et de ce fait moins facilement observables que les variations de réponse sexuelle exprimées avant l'accouplement.

[3] Ces comportements, qui consistent à rester en contact étroit avec les femelles jusqu'à ce que celles-ci deviennent sexuellement réceptives, peuvent prendre des formes variées comme monter sur le dos de la femelle (Arakaki et al., 2004) ou encore s'accoupler avec elle jusqu'à ce que le transfert de sperme soit possible (Arakaki et al., 2004; Bennett, Smith, & Betts, 2012; Ridley, 1983; Schröder, 2003)

Simmons, 2013). Une autre interrogation réside dans la rareté du choix mutuel au sein du règne animal: bien que les femelles soient souvent de qualité reproductive variable, pourquoi le choix des mâles est-il si rarement observé ? Enfin, bien que ces dernières décades aient vu une nette expansion des travaux étudiant les réponses sexuelles des mâles (Bonduriansky, 2001; Edward & Chapman, 2011; Parker, 1970; Simmons, 2001; Chapitre 1, Partie 2.2.1), ces dernières restent nettement moins étudiées que celles des femelles, appelant à de plus amples investigations.

Ainsi, un important effort de recherche est nécessaire pour mieux comprendre l'évolution des rôles sexuels et i) cerner les paramètres qui affectent le succès reproducteur des mâles ainsi que les allocations stratégiques qui en découlent, et ii) avoir une vision plus globale des différents facteurs en jeu et de leurs potentielles interactions.

Dans cette optique, l'objectif de ce travail de thèse a été d'étudier les allocations stratégiques de ressources reproductives chez les mâles en intégrant l'influence de plusieurs facteurs tels que la qualité des femelles et la compétition spermatique, chez deux espèces d'insectes aux traits d'histoire de vie contrastés. Plus précisément, je me suis intéressée aux allocations de ressources i) **gamétiques** chez le papillon ***Achroia grisella*** (Lepidoptera : Pyraliae), un candidat idéal dans la mesure où la contribution des mâles aux femelles se limite au transfert de sperme et ii) **non gamétiques** chez la sauterelle ***Ephippiger diurnus*** (Orthoptera : Tettigoniidae) où les mâles transfèrent lors de l'accouplement, en plus du sperme, le produit de leurs glandes accessoires sous la forme d'un spermatophylax (Chapitre 1, Partie 4.2), constituant de ce fait un bon modèle pour étudier le partitionnement des ressources non gamétiques. Ces deux espèces partagent **d'importantes limitations en ce qui concerne la disponibilité des ressources reproductives** (Chapitre 1, Partie 4), puisque la production de sperme fertilisant cesse lors de la nymphose chez *A. grisella,* tandis que les mâles d'*E. diurnus* sont fortement contraints dans la fréquence de production et de transfert de leurs spermatophylaxes (Chapitre 1, Partie 4.2). Par ailleurs, les mâles de ces deux espèces sont soumis à de **fortes pressions de sélection intra et intersexuelles pré-éjaculat**, puisqu'ils se regroupent pour émettre des chants d'appel dont la production requiert un investissement énergétique important, et faisant l'objet de préférences marquées et directionnelles de la part des femelles (Chapitre 1, Partie 4).

Le présent mémoire s'articule en quatre chapitres. Au cours du premier chapitre, j'illustre la nature et la diversité des coûts liés à la reproduction ainsi que la complexité des épisodes sélectifs chez les mâles. Au cours de cette partie, j'aborde également les allocations stratégiques des ressources reproductives chez les mâles ainsi que les facteurs connus pour les influencer. Le second chapitre sera consacré à l'étude des allocations gamétiques chez *A. grisella*. Au cours de cette partie, j'ai étudié si, et dans quelle mesure, le risque de compétition affectait la quantité de sperme transmise par des mâles vierges soumis ou non à la présence d'un compétiteur (Chapitre 2, Article 1). Dans un second temps, j'ai souhaité savoir si l'environnement social perçu par les mâles ainsi que la qualité reproductive de la femelle influençaient la quantité de sperme alloué aux femelles et/ou les comportements de gardiennage pré-éjaculat chez des mâles récemment accouplés (Chapitre 2, Article 2). Enfin, j'ai étudié les contraintes liées à la disponibilité en sperme au cours de la vie des mâles, et l'allocation de ces ressources spermatiques au regard de femelles de qualité variables (Chapitre 2, Article 3). Dans le troisième chapitre de ce mémoire, je présente les travaux concernant l'allocation des ressources non gamétiques (spermatophylax) chez *E. diurnus.* Tout d'abord, grâce à des expériences en conditions contrôlées, j'ai quantifié la variation de taille et de composition en acides aminés (libres et protéiques) du spermatophylax au regard de femelles vierges d'âge et de taille variables (Chapitre 3, Article 4, Article 6 en annexe). Ensuite, j'ai mené une étude de terrain visant à mesurer l'existence de préférences chez les mâles en conditions naturelles (Chapitre 3, Article 5). Enfin, dans le dernier chapitre de ce manuscrit, l'ensemble de ces travaux seront discutés et leurs perspectives présentées dans le contexte de la théorie de la sélection sexuelle et des rôles sexuels.

CHAPITRE 1 : Le succès reproducteur des mâles : coûts, contraintes et allocations des ressources

L’une des facettes de la sélection sexuelle : la sélection intersexuelle. Crédit image : John S. Dykes

1. Une reproduction coûteuse pour les mâles

Pendant de longues décades, la théorie de la sélection sexuelle a supposé que les coûts reproductifs des mâles étaient insignifiants en comparaison de ceux des femelles, se résumant à l'élaboration bon marché et virtuellement illimitée de spermatozoïdes (Bateman, 1948; Dawkins, 1989). Cette conception est remise en question au début des années 1980, lorsqu'il apparaît que la **production de sperme est coûteuse pour les mâles**, limitant le nombre d'éjaculats qu'ils peuvent produire au cours de leur vie et/ou la quantité de sperme disponible à un instant donné (Dewsbury, 1982). Depuis, l'accumulation de nombreuses preuves empiriques permet d'affirmer que les coûts de la reproduction des mâles sont loin de se réduire à la production de sperme et peuvent intervenir lors de chacune des étapes de la reproduction (attraction des partenaires, combats entre mâles, production de sperme, soins aux jeunes ; Perry, Sirot, & Wigby, 2013; Scharf, Peter, & Martin, 2013; Schneider & Lubin, 1998; Vahed, 2007; Wedell, Gage, & Parker, 2002).

Pour être coûteux, un trait doit **affecter négativement une composante de la fitness de son porteur** (Kotiaho, 2001; Scharf et al., 2013), c'est-à-dire affecter négativement sa survie et/ou sa capacité future de reproduction. De nombreux travaux ont examiné les coûts reproductifs au travers de mesures directes, en quantifiant la dépense énergétique et/ou les blessures supportées par les mâles au cours de la reproduction (Kotiaho, 2001).Toutefois, une non moins large proportion de ces études aborde ces coûts au travers des *trade-offs,* c'est-à-dire les compromis énergétiques existants entre les ressources nécessaires pour se reproduire, et celles nécessaires aux autres compartiments de la fitness des mâles (Scharf et al., 2013).

1.1. Coûts directs de la reproduction des mâles :

Les **coûts reproductifs directs** définissent les **dépenses énergétiques**[4] faites par les mâles lors des activités de reproduction, ou **l'impact négatif directement mesurable** de la reproduction sur leur fitness (Scharf et al. 2013). Parmi les coûts directs les plus fréquemment documentés, on retrouve les dépenses énergétiques générées par l'émission de chants d'appel

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[4] Mesurées en consommation de CO_2, émission d'O_2, concentration en azote ou en en lactate dans l'hémolymphe, ou encore en perte de poids du mâle (Scharf et al. 2013).

chez les insectes et les amphibiens (Mowles, 2014; Stoddard & Salazar, 2011; Wells, 2010). Par ailleurs, les combats entre mâles ou les comportements de gardiennage des femelles peuvent également occasionner d'importantes dépenses énergétiques, et être à l'origine de blessures ou d'épuisement physique chez les mâles (Benesh, Valtonen, & Jormalainen, 2007; Scharf et al., 2013). Enfin, blessures et épuisement peuvent aussi survenir pendant la recherche de partenaires et/ou la copulation elle-même, et impacter la capacité de survie ou d'évitement des prédateurs (Kuriwada & Kasuya, 2009; Scharf et al., 2013).

1.2. Trade-offs entre reproduction immédiate et reproduction future :

L'existence et l'importance de **trade-offs entre reproduction immédiate et reproduction future** ont été documentées au sein de très nombreux taxa (Scharf et al., 2013; Stearns, 1992). L'exemple le plus flagrant de l'omniprésence de ce trade-off est la diminution - quasi universelle - du nombre de spermatozoïdes au fur et à mesure des accouplements réalisés par les mâles (Kelly & Jennions, 2011; Wedell et al., 2002, mais voir Perez-Staples & Aluja, 2006 pour un contre-exemple). En effet, bien qu'individuellement peu coûteux à produire, les spermatozoïdes sont transférés sous la forme d'éjaculats pouvant contenir des millions de gamètes, et ainsi représenter une ressource limitante pour les mâles (Dewsbury 1982). Les contraintes liées à la disponibilité des ressources spermatiques peuvent être extrêmes au sein des espèces chez lesquelles les mâles émergent avec un stock fini et non renouvelable de spermatozoïdes (Damiens & Boivin, 2005). De plus, les éjaculats ne se résument que très rarement aux seuls spermatozoïdes, et comprennent souvent d'autres produits du métabolisme du mâle (Perry et al., 2013). Or, tout comme le sperme, chacune des substances accessoires transférées concomitamment au sperme peut s'avérer contrainte en quantité, qualité, ou au regard du temps nécessaire à sa production (Dowling & Simmons, 2012; Gwynne, 2001; Marcotte, Delisle, & McNeil, 2005; Vahed, 2007; Wedell et al., 2002). Par exemple, chez de nombreux insectes, les mâles transfèrent le produit de glandes accessoires sous forme de cadeaux nuptiaux lors de l'accouplement (voir Encadré 2). Pour les mâles, l'élaboration de ces donations peut être drastiquement coûteuse en temps et en énergie et entrainer des périodes réfractaires de plusieurs heures à plusieurs jours (Gwynne, 2008; Vahed, 2007).

Par ailleurs, chez les espèces à fertilisation interne, les copulations sont séquentielles puisque les mâles ne peuvent fertiliser qu'une femelle à la fois. Aussi, chaque unité de temps investie dans la reproduction immédiate peut limiter le temps disponible pour la reproduction future, et ainsi conduire à la perte de futures opportunités de reproduction (Scharf et al., 2013). Par exemple, chez les espèces pour lesquelles il existe des comportements de gardiennage des femelles, les mâles doivent souvent « arbitrer » entre persister dans le gardiennage en cours et prospecter pour d'autres opportunités d'accouplement (Alcock, 1994; Saeki, Kruse, & Switzer, 2005). L'importance de ce trade-off s'accroît souvent au cours de la vie des mâles, dans la mesure où la durée de la copulation s'allonge avec le nombre d'accouplements déjà réalisés (Paukku & Kotiaho, 2005; South, Steiner, & Arnqvist, 2009).

1.3. Trade-offs entre reproduction et longévité :

Facilement observable et mesurable, la **diminution de la durée de vie** est souvent utilisée pour mesurer le coût global de la reproduction chez les mâles. En effet, chez de nombreuses espèces, les mâles accouplés voient leur espérance de vie réduite (Scharf et al., 2013), parfois drastiquement (Papadopoulos et al., 2010; Pereira, Sivinski, Teal, & Brockmann, 2010). Par exemple, chez la mouche du fruit *Ceratitis capitata*, la durée de vie des mâles accouplés peut décliner de plus de 60 % (Papadopoulos et al., 2010). Néanmoins, observer une diminution de la longévité ne permet pas toujours de déterminer le ou les mécanisme(s) directement responsable(s), et qui peuvent impliquer de manière non mutuellement exclusive :

i) une **réduction de la capacité immunitaire** des mâles liée à la reproduction. Cette baisse de la capacité immunitaire peut résulter de la transmission de maladies (Knell & Webberley, 2004) ou de parasites (Rolff & Siva-Jothy, 2002) pendant l'accouplement. L'affaiblissement du système immunitaire peut aussi être la conséquence indirecte de l'accroissement de ressources énergétiques dévouées à la reproduction (Himuro & Fujisaki, 2010).

ii) une **augmentation du risque de prédation** : la reproduction, et les activités qui y sont associées, peuvent fortement augmenter le risque de prédation pour les mâles

(Magnhagen, 1991; Scharf et al., 2013). En effet, la recherche de partenaires, les comportements de parade ou les chants peuvent attirer l'attention de prédateurs (Gwynne, 2001; Koga, Backwell, Christy, Murai, & Kasuya, 2001), et l'accouplement lui-même peut augmenter les risques dans la mesure où la copulation peut être voyante et où les individus impliqués sont moins mobiles et vigilants (Miettinen, Kaitala, Smith, & Ordóñez, 2006).

iii) une **réduction du temps ou de l'énergie disponible pour d'autres activités essentielles** à la fitness des mâles. En effet, les activités de reproduction peuvent intervenir aux dépens d'autres activités telles que la recherche de nourriture ou la défense du territoire. Toutefois, il existe très peu de démonstrations empiriques de l'existence de ce trade-off, en particulier entre reproduction et activité de fourragement (Scharf et al., 2013).

Simple sur le papier, la mesure des coûts reproductifs et l'identification des mécanismes sous-jacents sont pourtant plus ardues qu'il n'y parait. En effet, lorsque les coûts sont mesurés sous la forme de trade-offs, il est parfois difficile de déterminer les processus mécanistiques à l'origine de la diminution de fitness chez les mâles (Scharf et al., 2013). Au contraire, la mesure directe des dépenses énergétiques ne permet pas toujours de déterminer si, et dans quelle mesure, ces dépenses induisent une réduction de fitness pour les mâles (Kotiaho, 2001; Paukku & Kotiaho, 2005). Une autre difficulté réside dans la prise en compte des investissements « diffus », c'est-à-dire qui ne diminuent pas au fur et à mesure des accouplements successifs (et ne peuvent donc pas être partitionnés entre les partenaires), mais peuvent néanmoins affecter positivement la reproduction des mâles aux dépens d'autres compartiments de leur fitness (Bonduriansky, 2001; Johnson & Burley, 1998). C'est par exemple le cas des phéromones, des colorations et ornements, ou encore de la compétition mâle-mâle pour l'acquisition et la défense de territoires (Bonduriansky, 2001; Johnson & Burley, 1998).

En résumé, les coûts reproductifs des mâles sont nombreux et variés, et peuvent potentiellement intervenir à **toutes les étapes de la reproduction** : i) **avant l'éjaculation**, impliquant la production du sperme et/ou d'autres substances accessoires transférés concomitamment dans l'éjaculat ; ou les dépenses énergétiques associées à l'acquisition de partenaires (e.g. chants, parades nuptiales, combats entre mâles, gardiennage) ; ii) **pendant l'accouplement** notamment au travers des risques accrus de prédation, de blessures et/ou de contamination par des pathogènes et iii) **après l'accouplement**, s'il existe des comportements de gardiennage post-éjaculat ou des soins aux jeunes qui peuvent occasionner des coûts additionnels en temps et en énergie.

Bien entendu, tous les mâles ne sont pas impliqués dans les activités détaillées ci-dessus, et les coûts reproductifs varient d'une espèce à l'autre, ainsi que selon de nombreux facteurs intrinsèques et extrinsèques aux mâles, telles que leur condition physiologique, l'OSR, et la présence de prédateurs.

2. Sélection sexuelle et épisodes sélectifs : le cas complexe des mâles.

Des travaux de Darwin jusqu'au milieu des années 1970, il était admis que les chances de paternité des mâles reposaient presque uniquement sur leur capacité à accéder aux femelles (Bateman, 1948; Darwin, 1871; Trivers, 1972). En définitive, l'existence de mécanismes post-éjaculat complexifie souvent fortement les épisodes de sélection auxquels les mâles sont soumis (Andersson & Simmons, 2006; Parker, 1970). Comme précédemment développé, la reproduction des mâles peut s'avérer coûteuse au cours de chacune de ses étapes. Pour bien comprendre quand et comment ces coûts peuvent entrainer des conséquences sur le succès reproducteur des mâles, il est nécessaire de se pencher sur la nature des épisodes sélectifs et comment ils s'articulent entre eux. C'est l'objet de cette seconde partie.

La sélection sexuelle chez les mâles peut impliquer deux épisodes sélectifs : la sélection sexuelle **pré-éjaculat** et la sélection sexuelle **post-éjaculat**. Aux origines de la théorie de Darwin, les mécanismes post-copulatoires sont encore inconnus, et les termes « pré- » et « post-*copulatoire* » ne font leur apparition dans la littérature que dans le dernier tiers du 20ème siècle (Parker, 1970). Toutefois, dans le cadre de ce manuscrit, je leur préfère les termes de pré- et post-*éjaculat* dans la mesure où le terme de *copulatoire* est particulièrement mal adapté chez les espèces où il n'existe pas, à proprement parler, de copulation (amphibiens, poissons) (Roberts & Byrne, 2011; Taborsky, 1998). Par ailleurs, l'accouplement est un phénomène parfois complexe dont les fonctions peuvent dépasser le simple transfert de sperme (Simmons, 2001; Thornhill & Alcock, 1983): par exemple, l'accouplement peut constituer une forme de gardiennage avant ou après l'éjaculation (García-González & Gomendio, 2004; Greenfield & Coffelt, 1983; Ridley, 1983; Schöfl & Taborsky, 2002) et ne pas toujours aboutir à une éjaculation : aussi il est préférable de distinguer les épisodes sélectifs intervenant avant le début et pendant/après le transfert de sperme.

2.1. La sélection sexuelle pré-éjaculat

Comme son nom l'indique, la sélection sexuelle pré-éjaculat désigne l'ensemble des mécanismes sélectifs prenant place avant le transfert du sperme par les mâles. Il s'agit de la **compétition intra-sexuelle** et de la **compétition intersexuelle**[5] (Darwin 1871).

2.1.1. La compétition intra-sexuelle:

La compétition intra-sexuelle réfère à la compétition entre les mâles pour l'accès aux femelles. Les exemples les plus emblématiques de la compétition entre mâles sont retrouvés chez les cervidés, chez lesquels les mâles entrechoquent leurs ramures dans des combats parfois mortels (Clutton-Brock, 1989). Facilement observable et conceptuellement simple, l'accès privilégié des vainqueurs aux femelles vaut à la compétition intra-sexuelle directe d'être très tôt reconnue comme moteur de sélection (Darwin 1871, Huxley 1938, Birkhead 2010). Toutefois, la compétition intra-sexuelle ne se limite pas aux affrontements directs : chez certaines espèces, les mâles peuvent utiliser des tactiques alternatives aux combats frontaux pour accéder aux femelles. Ces stratégies alternatives[6] permettent aux compétiteurs inférieurs de s'accoupler en évitant ou en minimisant les combats conventionnels avec les meilleurs compétiteurs directs (Gross, 1996; Oliveira, Taborsky, & Brockmann, 2008). La stratégie alternative au combat la plus communément rencontrée est de se faufiler discrètement jusqu'aux femelles, comme c'est le cas chez les mâles scarabées du genre *Onthophagus* désavantagés par la taille de leur corps ou de leurs « cornes » (Cook, 1990; Emlen, 1994 ; Figure 3).

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[5] Sensu Darwin 1871. La compétition spermatique (compétition intra-mâle post-éjaculat, est traitée ci-dessous en partie 2.2.1).

[6] Combats directs et stratégies alternatives peuvent être assimilés à la compétition par interférence et à la compétition par exploitation, respectivement. Dans le cas des stratégies alternatives, la compétition se fait au travers de la diminution des ressources (ici les femelles sexuellement réceptives) disponibles pour les rivaux sans impliquer de rencontre directe avec ces derniers (Andersson 1994, Danchin et al. 2005).

2.1.2. La compétition intersexuelle:

La sélectivité des femelles pour les mâles est un important mécanisme sélectif si les femelles s'accouplent préférentiellement avec certains mâles plutôt que d'autres. Aussi, les mâles porteurs des traits ayant la préférence des femelles (parades, chants, plumages…) auront une plus grande probabilité de s'accoupler que leurs rivaux (Andersson, 1994; Darwin, 1871). Proposée par Darwin dès 1871, la sélectivité des femelles reste controversée jusque dans le début des années 1980, avant d'être empiriquement et théoriquement validée comme force majeure de l'évolution de caractères sexuels secondaires chez les mâles (Andersson & Simmons, 2006; Kirkpatrick, 1982; Lande, 1981).

2.2. La sélection sexuelle post-éjaculat:

La sélection sexuelle post-éjaculat désigne l'ensemble des mécanismes qui influence le succès reproducteur des mâles pendant ou après le transfert de sperme aux femelles, et regroupe la **compétition spermatique** et le **choix femelle cryptique**.

2.2.1. La compétition spermatique:

La compétition spermatique correspond à la compétition entre le sperme de plusieurs mâles pour la fertilisation des gamètes d'une femelle (Ball & Parker, 1998; Parker, 1970). Découverte par Geoff Parker chez la mouche *Scatophaga stercoraria*, beaucoup de chercheurs y voient tout d'abord un mécanisme marginal cantonné aux insectes (Ball & Parker, 2007). Pourtant, il s'avère rapidement qu'il s'agit d'une force évolutive majeure et ubiquiste au sein du règne animal (Simmons, 2001; Smith, 1984), retrouvé même chez les espèces à fertilisation externe telles que les amphibiens ou les poissons (Roberts & Byrne, 2011; Taborsky, 1998). La présence et l'intensité de la compétition spermatique peuvent fortement biaiser les chances de paternité d'un mâle (Parker 1970, Simmons 2001; voir Encadré 1).

2.2.2. Le choix femelle cryptique:

Le choix femelle cryptique désigne l'utilisation non aléatoire par les femelles du sperme lors de la fertilisation de leurs gamètes (Eberhard, 1996; Simmons, 2001). Par exemple, chez la poule domestique (*Gallus gallus domesticus*) les femelles éjectent différentiellement le sperme des mâles au regard de leur statut de dominance sociale (Birkhead & Pizzari, 2002; Pizzari & Birkhead, 2000; Pizzari, Froman, & Birkhead, 2002; Pizzari, Cornwallis, Løvlie, Jakobsson, & Birkhead, 2003). La mise en évidence du choix femelle cryptique et de ses mécanismes potentiels est parfois ardue : par exemple, il n'est pas toujours possible d'affirmer que les biais de paternité observés résultent d'une réelle sélection femelle au niveau gamétique plutôt que de la mortalité différentielle des combinaisons de gamètes les moins compatibles (Birkhead, 2000; Simmons, 2001). En toute logique, la découverte de l'importance des mécanismes post-éjaculat s'est accompagnée de la mise en lumière de la promiscuité des femelles au sein de nombreux taxa, parfois même au sein d'espèces longtemps considérées comme monogames (Arnqvist & Nilsson, 2000; Griffith, Owens, & Thuman, 2002; Jennions & Petrie, 2000). C'est le cas notamment chez de nombreux oiseaux, où la polyandrie était perçue comme rare (Lack 1968) jusqu'à ce que l'usage de marqueurs moléculaires révèle qu'il s'agit du système d'appariement le plus répandu (Griffith et al., 2002).

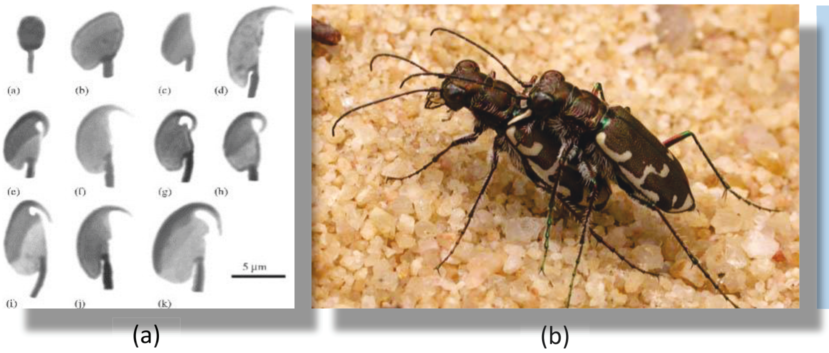
Ainsi, chez les espèces à fertilisation interne, le succès reproductif pré-éjaculat repose sur l'ensemble des facteurs permettant aux mâles d'être en position de *transférer* leurs gamètes (accès aux femelles), tandis que le succès reproducteur des mâles post-éjaculat est conditionné par l'ensemble des mécanismes permettant aux gamètes mâles de *fertiliser* les gamètes des femelles. Selon qu'il existe de la compétition spermatique ou non, les chances de paternité des mâles reposent donc seulement sur leur capacité à accéder aux femelles sexuellement disponibles, ou sur la combinaison de leur succès au cours de **deux épisodes sélectifs séquentiels et dépendants**. On notera également que si la catégorisation des épisodes sélectifs en compétition versus sélection, pré- versus post-éjaculat etc., est confortable pour formaliser et appréhender les mécanismes en œuvre, il est parfois difficile de classer certaines observations dans l'une de ces catégories. Par exemple, les femelles topi

(*Damaliscus lunatus*) marquent leur préférence pour certains mâles par des comportements compétitifs plus intenses (e.g. Bro-Jørgensen, 2007), une situation où sélection intersexuelle et compétition intra-sexuelle sont confondues et par conséquent impossibles à démêler.

Leurs ressources reproductives étant limitées, il est attendu que les mâles les allouent stratégiquement de sorte à maximiser leur succès reproducteur à l'issue de l'ensemble des épisodes sélectifs pré- et post-éjaculat. Les allocations stratégiques des mâles au regard de la reproduction feront l'objet de la troisième partie de ce manuscrit.

Encadré 1 : **LA COMPETITION SPERMATIQUE**

Lorsque les femelles s'accouplent de manière multiple, il peut exister une rivalité entre le sperme de mâles différents pour la fertilisation des gamètes de la femelle : c'est la **compétition spermatique**. Depuis la découverte de son existence dans la seconde moitié du 20$^{\text{ème}}$ siècle, de nombreux travaux attestent de la prééminence de la compétition spermatique comme force évolutive majeure (Simmons 2001). Aussi, l'ensemble des traits impliqués dans la compétition spermatique ou son évitement, sont soumis à une sélection rapide et divergente par le biais de fortes variances dans le succès reproducteur des mâles. Les adaptations des mâles en réponse à la compétition spermatique sont illustrées au travers de l'évolution de traits variés qui peuvent être morphologiques (structure des organes reproducteurs ou du sperme des mâles), physiologiques (composition chimique de l'éjaculat) ou encore comportementaux (comportements de gardiennage ; Simmons 2001, Figure E1).



(a) (b)

Figure E1 : Exemples d'adaptations ayant évolué sous la pression de la compétition spermatique. (a) Variance de la morphologie de la tête des spermatozoïdes chez les rongeurs Muridés (Figure issue de (Gomez Montoto et al., 2011). (b) Gardiennage post-éjaculat chez *Cicindela repanda*. Crédit photo : P. Cotinis).

La compétition spermatique a fait l'objet de nombreuses études théoriques et de tests empiriques visant en particulier à déterminer l'optimisation du nombre de spermatozoïdes produits et transférés aux femelles (Parker, 1990, 1993; Parker, Ball, Stockley, & Gage, 1996, 1997). Bien que les chances de paternité des mâles augmentent avec la quantité de spermatozoïdes transmis, les coûts et limitations associés à la production de sperme (Dewsbury, 1982; Nakatsuru & Kramer, 1982; Olsson, Madsen, & Shine, 1997), ces modèles prédisent une allocation prudente des ressources gamétiques et l'optimisation des chances de fertilisation de chaque spermatozoïde (Parker & Pizzari, 2010; Simmons, 2001; Wedell et al., 2002). La modélisation du nombre optimal de spermatozoïdes attendu en situation de compétition spermatique est généralement abordée sous l'angle des Stratégies Evolutivement Stables (ESS)[7], puisque, pour un mâle, la balance coûts/bénéfices d'une stratégie dépend de celle adoptée par les mâles rivaux (Parker & Pizzari 2010).

Les prédictions de l'allocation stratégique optimale diffèrent selon que l'on considère i) le **risque de compétition spermatique** (SCR), c'est-à-dire la **probabilité** que le sperme du mâle soit en compétition avec le sperme d'un ou plusieurs rivaux, et ii) **l'intensité de la compétition spermatique** (SCI), qui définit le **nombre d'éjaculats** effectivement en compétition pour la fertilisation des œufs d'une femelle. En effet, si les mâles sont capables de déterminer le risque de compétition associé à un épisode reproducteur, il est

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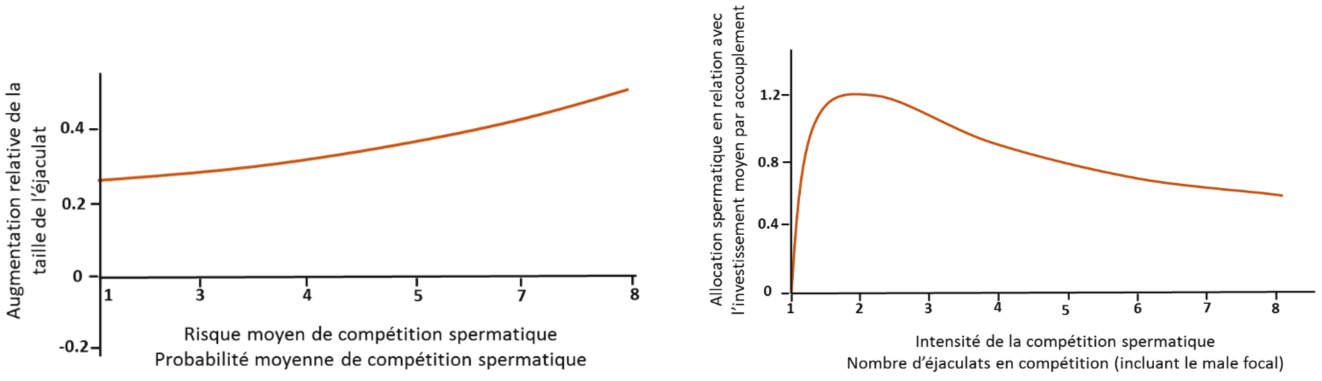
[7] Une stratégie donnée est une ESS si, lorsque toute la population a adopté cette stratégie, aucune stratégie déviante ne peut envahir la population (Smith & Price 1973).

prédit que ces derniers allouent une plus large proportion de leurs ressources gamétiques dans les situations où le risque de compétition est le plus élevé. En revanche, les ajustements gamétiques attendus en réponse à l'intensité de la compétition spermatique sont plus complexes : il est attendu que les mâles réduisent la quantité de leur éjaculat lorsque qu'aucun compétiteur n'est présent, et investissent de façon maximale lorsqu'un seul rival est présent. Toutefois, dès lors que le nombre de compétiteurs excède deux mâles, les mâles devraient au contraire investir une quantité de sperme inversement proportionnelle au nombre de compétiteurs impliqués. En effet, les probabilités de fertilisation d'un spermatozoïde déclinent avec le nombre de spermatozoïdes rivaux présents (Engqvist & Reinhold, 2005; Parker, 1990, 1993; Parker et al., 1996, 1997, Figure E2).



(a) (b)

Figure E2 : Allocations stratégiques des ressources gamétiques attendues au regard (a) du risque de compétition spermatique et (b) de l'intensité de la compétition spermatique. Figure adaptée à partir d' Engqvist and Reinhold 2005.

Par ailleurs, les mécanismes par lesquels les spermatozoïdes sont en compétition peuvent fortement influencer l'issue de la compétition spermatique. Dans la situation la plus simple, tous les spermatozoïdes ont une probabilité identique de fertiliser les œufs de la femelle, on parle alors de **loterie équitable** (*fair raffle*). Dans ce cas, les chances de paternité d'un mâle sont proportionnelles à la quantité de son sperme relativement au sperme des autres mâles (Parker et al. 1990). Toutefois, de nombreux facteurs peuvent affecter la probabilité que le sperme d'un mâle donné féconde avec succès les œufs de la femelle tels que i) l'espace disponible pour la compétition spermatique (maximale chez les espèces à fertilisation externe et fortement contrainte chez les espèces à fertilisation interne ; Parker & Pizzari, 2010) ii) les inégalités pouvant exister entre les spermes de différents mâles dans leur capacité compétitrice (par exemple liées à une différence de mortalité), ou encore iii) si et comment le sperme se mélange à l'intérieur du tractus génital de la femelle et l'ordre d'utilisation du sperme par les femelles (qui varie selon la morphologie des espèces ; Parker & Pizzari, 2010). Lorsqu'il existe des iniquités entre le sperme de différents mâles au regard des probabilités de fertilisation des œufs de la femelles, on parle de **loterie biaisée** (*loaded raffle*).

Ces différents facteurs peuvent conduire à des chances de paternité différentielles selon l'ordre d'accouplement des mâles : par exemple, si le sperme est stratifié au sein du tractus génital de la femelle, le premier, ou au contraire le dernier sperme transmis est le premier à être utilisé pour féconder les œufs de la femelle (e.g. Lessells & Birkhead, 1990). On parle alors de **précédence spermatique** pour le premier/le dernier sperme, et de rôle favorisé ou défavorisé pour les mâles. Enfin, l'issue de la compétition spermatique peut être influencée par l'existence de sélection intersexuelle post-éjaculat. Par exemple, les femelles peuvent différentiellement neutraliser ou éliminer le sperme provenant de certains mâles (voir 2.2.2), et ainsi favoriser les chances de paternité de mâles particuliers (e.g. Ball & Parker, 2003; Eberhard, 1996).

3. Allocations stratégiques des ressources reproductives chez les mâles

En l'absence de coûts ou de limitations associés à la reproduction, les mâles peuvent invariablement maximiser leur investissement dans les accouplements successifs, et ce tout au long de leur vie. Toutefois, comme je l'ai abordé précédemment, les coûts reproductifs auxquels font face les mâles sont souvent considérables et composites (Chapitre 1, Partie 2). Dans ce cas, **il est attendu que les mâles allouent stratégiquement leurs ressources – c'est-à-dire ajustent leur réponse sexuelle – de sorte à maximiser leur succès reproducteur au regard des contraintes et bénéfices associés.** Les stratégies complexes auxquelles peuvent recourir les mâles pour améliorer leur fitness, ou **allocations stratégiques**, font l'objet de cette troisième partie.

L'investissement des mâles dans la reproduction est loin d'être constant, et la littérature regorge d'exemples montrant que les mâles, tout comme les femelles, optimisent, plutôt que maximisent le nombre et la qualité de leurs partenaires. Les décisions auxquelles sont confrontés les mâles concernent tout d'abord **la décision de s'engager ou non dans un épisode de reproduction**, puis **quelle quantité de ressources y investir** (Edward & Chapman 2011, Wedell et al. 2002). Aussi les variations de réponses sexuelles des mâles peuvent être nombreuses, et être exprimées lors de chacune des séquences de la reproduction. Tout d'abord, les mâles peuvent **ajuster leur effort pour l'accès aux femelles**, en modifiant le temps et l'énergie alloués aux comportements dont dépendent les probabilités de rencontre avec les femelles. Par exemple, les mâles peuvent augmenter les temps de recherche des partenaires, l'intensité des comportements visant à attirer les femelles, tels que les chants ou les parades nuptiales, ou se montrer plus agressifs vis-à-vis des compétiteurs ou des femelles elles-mêmes (Jia et al. 2001, Convoy & Gray 2014, Kim & Velando 2014). L'effort pour l'accès aux femelles peut également se manifester au travers de variations de l'intensité ou de la fréquence de comportements de gardiennage pré-éjaculat[8], par lesquels les mâles

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[8] Ces comportements, qui consistent à rester en contact étroit avec les femelles jusqu'à ce que celles-ci deviennent sexuellement réceptives, peuvent prendre des formes variées comme monter sur le dos de la femelle (Arakaki et al., 2004) ou encore s'accoupler avec elle jusqu'à ce que le transfert de sperme soit possible (Arakaki et al., 2004; Bennett, Smith, & Betts, 2012; Ridley, 1983; Schröder, 2003).

empêchent les femelles gardées de s'accoupler avec d'autres mâles (Grafen & Ridley, 1983; Jormalainen, 1998). Par ailleurs, les mâles peuvent également **accepter ou refuser** les opportunités d'accouplement qui se présentent à eux, et ainsi n'allouer leurs ressources que lorsque les retours potentiels en termes de fitness sont les plus avantageux (Chapitre 1, Partie 3).

Chez un grand nombre d'espèces, les variations de la réponse sexuelle des mâles sont exprimées **durant l'accouplement**, par le biais d'ajustements dans la quantité ou la qualité des transferts gamétiques et/ou non gamétiques à la femelle (Bonduriansky, 2001; Edward & Chapman, 2011; Gwynne, 2001; Simmons, 2001; Wedell et al., 2002). Ces ajustements sont souvent dits **cryptiques**, dans la mesure où leur observation est moins aisée que celle des comportements pré- ou post-éjaculat, raison pour laquelle ils sont longtemps restés inaperçus.

Enfin, les variations de la réponse sexuelle des mâles peuvent prendre place **après le transfert de sperme**, au travers d'ajustements de l'intensité des soins parentaux (apport de nourriture, protection…) ou des comportements de gardiennage post-éjaculat des femelles (Elias, Sivalinghem, Mason, Andrade, & Kasumovic, 2014; Parker, 1970; Simmons, 2001).

Ces différentes possibilités ne sont pas mutuellement exclusives et les ajustements des mâles dans la reproduction peuvent être exprimés selon plusieurs modalités au sein d'une même espèce (e.g. Xu & Wang, 2009).

Facteurs influençant l'évolution des allocations stratégiques chez les mâles

Si le prérequis à l'évolution des allocations stratégiques chez les mâles est **l'existence de coûts liés à la reproduction**, de nombreux facteurs peuvent influencer l'évolution et la nature des variations de réponse sexuelle observées chez les mâles.

3.1. Variance de la qualité de la femelle

Lorsque les femelles varient dans leur potentiel reproducteur, les mâles peuvent maximiser leur fitness en allouant stratégiquement leurs ressources au regard des femelles de meilleure qualité : on parle dans ce cas de **choix** ou de **préférences**. Plus la variance dans la qualité des femelles est grande, plus les bénéfices potentiels à être sélectif sont importants (Johnstone et al., 1996). Pourtant, les évidences empiriques de l'expression par les mâles de préférences liées à la qualité de la partenaire ont longtemps été considérées comme des exceptions aux rôles sexuels conventionnels, probablement parce que leur nature souvent cryptique complique leur observation. Néanmoins, les choix des mâles apparaissent en définitive être largement répandus au sein du règne animal (Bonduriansky 2001, Edward & Chapman 2011). Le plus souvent, les mâles choisissent leurs partenaires sur la base d'indices révélateurs de leur **fécondité** ou de leur **proximité à le devenir** (Berglund, Rosenqvist, & Bernet, 1997; Bonduriansky, 2001; Edward & Chapman, 2011; Jones, Walker, & Avise, 2001). Chez de nombreux taxa, la taille des femelles s'avère un indicateur fiable de leur fécondité, aussi la préférence des mâles pour les femelles les plus grosses est retrouvée au sein d'un grand nombre d'espèces (Bonduriansky, 2001; Dosen & Montgomerie, 2004; Honek, 1993; Roff, 1982). A titre d'exemple, les mâles transfèrent plus de sperme aux femelles les plus grosses chez de nombreux insectes, crustacés (Wedell et al. 2002), ou encore…chez l'humain (Baker & Bellis, 2014; Baker & Bellis, 1993). Les mâles peuvent également discriminer les femelles les plus fécondes sur la base d'autres indices, tels que leur âge, leur statut reproducteur (MacLeod & Andrade, 2014)), leur statut de dominance (Berglund & Rosenqvist, 2001), ou encore de leur faible charge parasitaire (Mazzi, 2004; Partridge et al., 2009; Rosenqvist, Johansson, 1995).

Par ailleurs, les mâles peuvent également choisir les femelles en fonction de leur compatibilité génétique (Roth, Sundin, Berglund, Rosenqvist, & Wegner, 2014; Tigreros, Mowery, & Lewis, 2014) ou avec lesquelles ils ne se sont pas préalablement accouplés (Bro-Jørgensen, 2007; Saether, Fiske, & Kålås, 2001). Souvent, les préférences exprimées et les indices utilisés peuvent êtres complexes : ainsi, chez le poisson tube *Syngnathus typhle*, les mâles ayant accès à des indices visuels s'accouplent préférentiellement avec les femelles les plus grosses, mais choisissent les femelles les plus compatibles génétiquement sur la base d'indices olfactifs (Roth et al. 2014). Comme pour les femelles, les variations de réponse sexuelle liées à des préférences chez les mâles peuvent être variées et impliquer tout type d'ajustements intervenant avant, pendant ou après le transfert de sperme (acceptation/rejet de la femelle, variation de la taille de l'éjaculat, des soins aux jeunes, etc. ; Chapitre 1, Partie 3).

3.2. Opportunités d'accouplement présentes et futures

L'évolution et la nature des allocations stratégiques exprimées par les mâles reposent aussi fortement sur leurs **probabilités présentes et futures de s'accoupler**. A ce titre, le **sexe ratio opérationnel** joue un rôle fondamental, puisqu'il conditionne **la disponibilité des femelles** sexuellement réceptives dans la population ainsi que **l'intensité de la compétition inter-mâles**. En effet, lorsque le sexe ratio opérationnel est biaisé en faveur des mâles, il peut s'ensuivre une augmentation de l'intensité de la compétition intra-sexuelle (ainsi que de la sélection sexuelle post-éjaculat, ce cas est traité Chapitre 1, Partie 2.2). Sous ces conditions, les mâles peuvent accroitre leurs opportunités d'accouplement en maximisant leur effort pour l'accès aux femelles, par exemple en intensifiant leurs comportements de cour (Jia, Greenfield, & Collins, 2001) ou de compétition au regard de la présence et du nombre de mâles rivaux (Kim & Velando, 2014; Le Boeuf & Peterson, 1969). L'effort pour l'accès aux femelles peut également se manifester au travers de variations dans l'intensité ou la fréquence des comportements de gardiennage pré-éjaculat. Cette dernière stratégie est notamment rencontrée chez certains crustacés amphipodes ou certains insectes chez lesquels la réceptivité des femelles est réduite à une courte fenêtre temporelle, biaisant fortement l'OSR en faveur des mâles (Arakaki et al., 2004; Bennett et al., 2012; Grafen & Ridley, 1983; Jormalainen, 1998; Schröder, 2003).

Par ailleurs, si les probabilités d'accouplements futurs sont faibles, les mâles ont intérêt à investir de manière maximale et indiscriminée dans chaque opportunité d'accouplement qui se présente à eux ; dans ce cas, même en cas de grande variabilité dans la qualité reproductive des femelles, l'évolution de préférences n'est pas attendue (Edward & Chapman, 2011; Johnstone et al., 1996 ; Partie 3.2). Au contraire, un sexe ratio opérationnel biaisé en faveur des femelles peut favoriser l'évolution de choix chez les mâles (Gwynne, 1985) : tout d'abord parce que la compétition entre mâles peut s'en trouver relaxée mais aussi parce que le risque lié au rejet des partenaires de moindre qualité est moins susceptible de se traduire par une perte nette d'opportunité d'accouplement. Enfin, le choix de partenaires est facilité s'il existe la possibilité de choisir entre femelles présentes simultanément plutôt que séquentiellement (Jennions & Petrie, 1997).

Comme vu en partie 2, les opportunités de reproduction obtenues par les mâles sont souvent fonction de leur attractivité auprès des femelles (Amundsen & Forsgren, 2003 ; Andersson, 1994), ce qui génère des biais parfois conséquents en faveur des mâles préférés. Une illustration extrême de ces biais est retrouvée dans le cadre des systèmes d'appariement **en leks** (voir Encadré 2), où les choix prononcés des femelles se traduisent par la monopolisation quasi-totale des accouplements par les quelques mâles les plus attirants (Höglund & Alatalo, 1995). Aussi, si cela leur est possible, il est attendu que les mâles maximisent leur investissement dans les traits ciblés par les choix des femelles (chants, parades…). Toutefois, les traits préférés par les femelles peuvent être dépendants de la qualité génétique des mâles ou de leur condition intrinsèque (voir ci-dessous), auquel cas il est physiologiquement impossible aux mâles de «tricher».

3.3. Condition physiologique du mâle

Tous les mâles ne sont pas égaux face à la reproduction : au sein d'une même espèce, la **condition physiologique** des mâles peut être très variable, et conduire à des **différences dans la quantité de ressources reproductives dont disposent les individus**. Dans ce cas, **se reproduire est proportionnellement plus coûteux pour les mâles en mauvaise condition physiologique** que pour leurs rivaux mieux dotés (Engqvist & Sauer, 2001; Martel, Damiens, & Boivin, 2008). Les mâles en mauvaise condition physiologique devraient donc, théoriquement, retirer de plus amples bénéfices, en termes de fitness, à allouer stratégiquement leurs ressources et ainsi rentabiliser au mieux chacun de leurs investissements (Engqvist & Sauer, 2001). C'est le cas par exemple chez la panorpe *Panorpa cognata* : les mâles en mauvaise condition physiologique montrent des préférences marquées en ce qui concerne la qualité de la femelle, tandis que les individus de meilleure qualité se montrent moins discriminants (Engqvist & Sauer, 2001).

Bien que les ressources acquises au cours du développement jouent souvent un rôle prépondérant dans la capacité reproductive des individus adultes (Cook, 1990; Emlen, 1994), la condition physiologique des mâles peut être influencée par des facteurs génétiques, leur âge ainsi que leur statut reproducteur (Scharf et al., 2013). En effet, il est fréquent que les mâles ayant déjà préalablement investi dans la reproduction disposent de moins de ressources (temps, énergie, gamètes…) pour les accouplements subséquents (Scharf et al., 2013). Dans ce cas, il est attendu que les mâles allouent de plus en plus stratégiquement leurs ressources à mesure que celles-ci diminuent (Wedell et al., 2002) jusqu'à un possible investissement terminal maximal (Williams, 1966). La condition physiologique des mâles peut aussi dépendre de nombreux facteurs environnementaux, tels que la disponibilité en nourriture ou les conditions climatiques locales. Ces notions sont abordées ci-dessous (Chapitre 1, Partie 3.2).

Ainsi, moins les mâles ont de ressources à allouer, et plus ils devraient les allouer stratégiquement. Dans les faits, ce n'est pas aussi simple, notamment si la condition physiologique des mâles affecte leur capacité compétitrice ou leur attractivité auprès des femelles, une situation qui peut amener les mâles en mauvaise condition à n'avoir que de très rares opportunités d'accouplement. Ce cas particulier devrait notamment contre-sélectionner l'évolution de **préférences** : puisque les mâles se retrouvent alors dans une situation équivalente à celle d'un OSR biaisé en leur faveur (Chapitre 1, Partie 3.2). Ils devraient alors maximiser leur effort pour accéder aux partenaires plutôt que de se montrer discriminant au regard de la qualité reproductive de la femelle ou du risque de sélection post-éjaculat rencontré.

Lorsque les différences de condition physiologique au sein d'une espèce sont extrêmes, les mâles peuvent accéder à la reproduction en adoptant des stratégies plus en adéquation avec leurs capacités intrinsèques (e.g. Convoy & Gray 2014). Par exemple, les mâles que la condition physiologique désavantage dans la compétition intra-sexuelle peuvent augmenter leurs chances de paternité en fertilisant les femelles à l'insu de leurs rivaux. Si ces stratégies sont payantes, une **sélection disruptive** peut amener à l'évolution de **stratégies alternatives**, c'est-à-dire une distribution discontinue des traits ou de comportements permettant aux mâles de maximiser leurs chances de paternité (Gross, 1996; Taborsky, 1998 ; Figure 3). Par exemple, chez de nombreux poissons, les mâles les plus gros adoptent une stratégie bourgeois[9] et investissent intensément dans les traits leur permettant d'accéder aux femelles[10]. En revanche, les petits mâles ont avantage à adopter une stratégie parasitique, en exploitant les investissements des mâles bourgeois pour féconder les œufs déposés par les femelles (Taborsky 1998).

Figure 3: Exemple de distribution discontinue des traits liée à l'évolution de stratégies alternatives. Chez les scarabées du genre *Onthophagus* (en haut : *O. taurus*, en bas : *O. nigriventris*), les mâles en moins bonne condition physiologique possèdent une petite taille corporelle et des armements (cornes) peu développés. Ces derniers évitent les combats directs avec les mâles cornus qui gardent l'entrée des tunnels dans lesquels se trouvent les femelles, en se faufilant jusqu'à elles par des tunnels détournés (Emlen 1994, Cook 1990). Sur la photo, seuls les mâles sont représentés. Crédit photo : S. Valena & A.P. Moczek.

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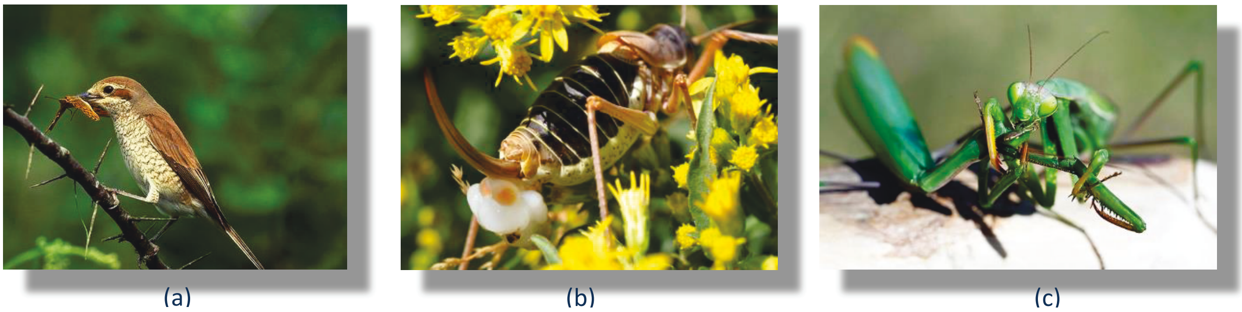
[9] *Sensu* Taborsky 1998: la stratégie bourgeois fait référence aux males investissant dans l'accès aux femelles pour accéder aux œufs, et monopolisant la ressource « femelles », tandis que la stratégie parasitique consiste à fertiliser les œufs pondus suite aux efforts des mâles bourgeois par des moyens détournés (Taborsky 1998)

[10] Par exemple au travers de comportements de défense des femelles, de comportements de cours intenses, d'investissement dans les caractères sexuels secondaires ou encore la production de phéromones (Taborsky 1998, 1999).

Encadré 2 : **LES CADEAUX NUPTIAUX**

Les **cadeaux nuptiaux** sont définis comme tout type de substances ingérables[11] transférées par les mâles aux femelles[12] lors de l'accouplement (Vahed, 1998). Ces donations peuvent prendre de multiples formes: tout ou partie de proies capturées par les soins du mâle, sécrétions glandulaires plus ou moins complexes, et même tout ou partie du corps du mâle (Lewis & South, 2012; Vahed, 1998). Les cadeaux nuptiaux sont particulièrement répandus chez les insectes et les araignées, mais on trouve aussi des exemples au sein d'autres groupes tels que les oiseaux (Yosef, 1992 ; Lorek 1995) ou les mammifères comme…les humains (Cronk & Dunham, 2007).



(a) (b) (c)

Figure E3 : Quelques exemples d'espèces au sein desquelles les mâles transfèrent des cadeaux nuptiaux aux femelles. (a) Chez la Pie-grièche grise (*Lanius excubitor*), les mâles capturent et offrent des proies aux femelles, probablement pour inciter ces dernières à accepter des appariements extra-couple (Tryjanowski & Hromada, 2005). (b) Chez la sauterelle *Ephippiger diurnus*, les mâles transfèrent aux femelles le produit de leurs glandes accessoires sous la forme d'un spermatophore (voir Chapitre 1, Partie 4.2). (c) En revanche, chez les mantes religieuses (*Mantis religiosa*), c'est le mâle lui-même qui sert d'offrande nutritive aux femelles à l'issue de l'accouplement. Crédit photos (a) M. Szcepanek, (b) S. Dourlot, (c) O. Koemmerling.

Deux hypothèses, non mutuellement exclusives, ont été avancées pour expliquer l'évolution et le maintien des cadeaux nuptiaux (Vahed, 1998; 2007). Tout d'abord, l'hypothèse de **l'investissement parental** (*parental effort*) propose que ces donations contiennent des substances nutritives améliorant la fitness des femelles, par exemple en augmentant le nombre et/ou la qualité de leurs œufs ou leur durée de vie. Loin d'être altruiste, ce comportement serait également bénéfique aux mâles donneurs puisqu'il maximise le nombre et/ou la qualité de leurs descendants. En revanche, l'hypothèse de **l'investissement pour la reproduction** (*mating effort*) propose que la fonction des cadeaux nuptiaux est de maximiser les chances de paternité des mâles en augmentant les chances de fertilisation du sperme du mâle donneur, par exemple en protégeant le sperme de la consommation par la femelle, ou en augmentant la durée de l'intervalle entre deux accouplements de celle-ci. Dans ce dernier cas, la manipulation de la réceptivité sexuelle peut notamment représenter une défense efficace contre la compétition spermatique (Gwynne, 2008; Vahed, 1998; Wedell, 1994; voir Encadré 1).

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[11] Sarah Lewis et Adam South (2013) en donnent toutefois une définition beaucoup plus large et considèrent comme cadeau nuptial toute substance non gamétique transférée par l'un des partenaires à l'autre lors de l'accouplement.

[12] Il existe toutefois des exceptions! Par exemple chez le Scarabée de Zeus (*Phoreticovelia spp*), ce sont les femelles qui nourrissent les mâles de leurs sécrétions glandulaires (Arnqvist, Jones, & Elgar, 2003).

Encadré 3 : LES LEKS

Chez certaines espèces, les mâles se regroupent pour parader et attirer les femelles. Les groupes ainsi formés, appelés **leks**, sont visités par les femelles qui choisissent alors librement un partenaire parmi les mâles présents (Höglund & Alatalo, 1995). Les leks sont notamment retrouvés chez certains oiseaux, insectes, poissons ou amphibiens et les parades consistent le plus souvent en des vocalisations ou, dans le cas des oiseaux, à l'exhibition de plumages élaborés (Höglund & Alatalo, 1995; Kirkpatrick & Ryan, 1991).

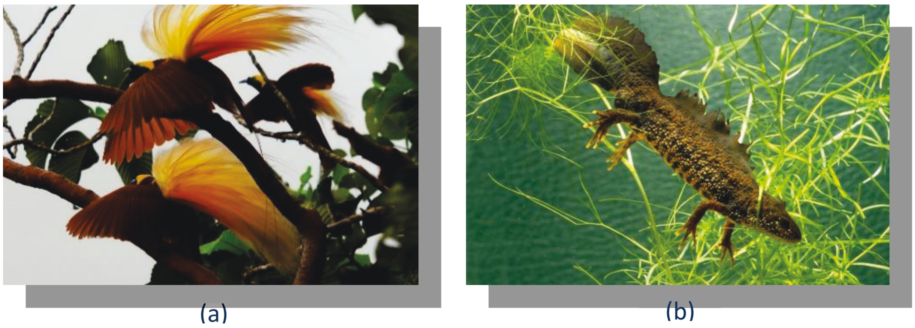
(a) (b)

Figure E4 : Deux exemples d'espèces où les mâles forment des leks pour attirer les femelles. A droite (a), trois oiseaux de Paradis mâles (*Paradisea minor*) exhibent leur plumage à l'attention des femelles (Beehler & Pruett-Jones, 1983). A gauche (b), chez le grand triton à crête (*Triturus cristatus*), les femelles choisissent les mâles sur la base de phéromones et de la taille de leur crête dorsale (Malmgren & Enghag, 2008). Crédit photo : (a) R. Eime, (b) S.F. Marijnissen.

Ces systèmes sont en particulier caractérisés par l'existence de préférences femelles marquées et directionnelles, ou le succès reproducteur des mâles peut être biaisé à l'extrême puisque quelques mâles acquièrent la majorité des accouplements (Höglund & Alatalo, 1995; Kirkpatrick & Ryan, 1991). Par ailleurs, la contribution des mâles se résume à la transmission de sperme, et les femelles ne reçoivent aucun bénéfice direct (nourriture, soins parentaux) à l'issue de leur choix (Höglund & Alatalo, 1995; Kirkpatrick & Ryan, 1991).

Le système d'appariement en lek est également à l'origine de l'une des questions les plus déroutantes de la théorie de la sélection sexuelle : le paradoxe du lek (Höglund & Alatalo, 1995; Kirkpatrick & Ryan, 1991; Kotiaho, 2001; Tomkins, Radwan, Kotiaho, & Tregenza, 2004). En effet, la sélection directionnelle imposée par les préférences des femelles devrait éroder la variance génétique des traits préférés chez les mâles, et, par là même, éroder l'existence de bénéfices à choisir pour les femelles. En d'autres termes, pourquoi les femelles continuent-elles à choisir des mâles sur la base de bénéfices génétiques si ces choix dépriment la variance génétique à l'origine de leur choix ? Ce paradoxe a fait l'objet de nombreux et vifs débats (Hoffman, Forcada, Trathan, & Amos, 2007; Kotiaho, 2007) et de près de 200 publications au cours des dernières décades, dont certaines remettent l'existence même du paradoxe en question (Kotiaho & Puurtinen, 2007). Aujourd'hui, il semblerait qu'il n'existe pas une, mais plusieurs solutions à ce paradoxe, permettant la persistance de la variance génétique sous forte sélection intersexuelle. Parmi ces solutions, il a été proposé que le choix femelle augmente la variabilité en favorisant le taux de mutation au sein des loci déterminant la viabilité et l'expression des traits sexuels (Petrie & Roberts, 2007). Mais la question est encore loin d'être élucidée.

3.4. Présence et intensité de mécanismes post-éjaculat

Si les femelles sont **polyandres**, les chances de paternité des mâles peuvent être fortement biaisées lorsque leur sperme est en compétition avec celui de rivaux pour la fertilisation des œufs de la femelle. Lorsqu'il existe de la **compétition spermatique**, la nature des allocations stratégiques exprimées par les mâles dépend avant tout du pattern d'utilisation du sperme par les femelles, c'est-à-dire si l'ordre « de passage » des mâles est important dans la détermination de leurs chances de paternité (Simmons 2001, voir Encadré 1).

Dans de très nombreux cas, les allocations stratégiques des mâles consistent en des ajustements de la quantité de spermatozoïdes produite et transférée aux femelles (Moatt, Dytham, & Thom, 2014; Parker & Vahed, 2010; Simmons, 2001). Différents modèles théoriques présentent des prédictions complexes abordées dans l'encadré 1). Les ajustements peuvent également concerner la composition qualitative ou quantitative des substances non gamétiques transférées conjointement aux gamètes. Par exemple, s'il existe un avantage au dernier sperme, les mâles peuvent augmenter leurs chances de paternité en transférant plus de substances accessoires réduisant la réceptivité sexuelle des femelles et/ou augmentant la durée de leur période réfractaire entre deux accouplements (Moatt et al., 2014; Perry et al., 2013; Simmons, 2001). Un excellent exemple de la plasticité de la taille et de la composition de l'éjaculat en réponse au risque de compétition spermatique est retrouvé chez la drosophile (*Drosophila melanogaster*) où les mâles soumis à un risque de compétition spermatique élevé produisent et transfèrent plus de sperme, mais aussi maximisent la quantité de substances accessoires transférées aux femelles (Moatt et al., 2014; Perry et al., 2013).

L'évitement de la compétition spermatique peut par ailleurs être manifesté au travers de comportements de gardiennage par lesquels les mâles empêchent physiquement les femelles de se réaccoupler (Elias et al., 2014; Parker, 1970; Simmons, 2001). Dans la grande majorité des cas, ces comportements prennent place après l'accouplement, mais il a été proposé que le gardiennage puisse évoluer de manière pré-copulatoire au sein des espèces chez lesquelles il existe un fort avantage au premier sperme (Parker 1974). Enfin, les mâles peuvent également refuser de s'accoupler avec les partenaires qui présentent les risques de compétition spermatique les plus élevés. Là encore, les traits des femelles induisant le rejet des mâles varient selon le pattern d'utilisation du sperme : par exemple, les mâles peuvent préférer les femelles vierges si il existe un avantage au premier sperme, ou contraire les femelles les plus âgées ou déjà accouplées lorsque c'est le sperme du dernier mâle qui est avantagé (Bateman & Ferguson, 2004; Parker & Vahed, 2010; Simmons, 2001; Wedell et al., 2002 ; Figure 4a).

Au-delà du risque de compétition spermatique inhérent au statut reproducteur de la femelle, les mâles peuvent ajuster leur réponse sexuelle sur la base d'indices sociaux, comme la présence et l'abondance de compétiteurs (Engqvist & Reinhold, 2005; Wedell et al., 2002). Par exemple, chez le crénilabre ocellé (*Symphodus ocellatus*), les mâles refusent parfois les femelles indépendamment de leur qualité si un ou plusieurs mâles sont présents aux alentours du nid, et réduisent ainsi le risque de voir les œufs de la femelle fertilisés par un rival (Alonzo & Warner, 1999 ; Figure 4b).

(a) (b)

Figure 4 : Chez la sauterelle *Acanthoplus discoidalis*, le risque de compétition spermatique est moindre chez les femelles vierges. Aussi, les mâles s'accouplent préférentiellement avec les femelles vierges bien qu'elles soient par ailleurs plus petites et portent des œufs moins développés (Bateman & Ferguson 2004). Crédit photo : D. et S. Solomon. (b) Le crénilabre ocellé (*Symphodus ocellatus* ; voir texte ci-dessus). Crédit image : http://maltafishingforum.com/.

3.5. Autres facteurs environnementaux

Les allocations stratégiques peuvent également être affectées par de nombreux facteurs environnementaux biotiques et abiotiques. En premier lieu, la **disponibilité en nourriture** peut influencer la condition physiologique des mâles et des femelles, et conduire à des variations dans le partitionnement des ressources reproductives aux femelles (Ritchie, Sunter, & Hockham, 1998). En particulier, si le manque de ressources alimentaires affecte le potentiel reproducteur des mâles, ces derniers peuvent tirer avantage à n'allouer leurs ressources qu'au regard des partenaires de meilleures qualités (Kvarnemo & Simmons, 1998; Martel et al., 2008). Par ailleurs, si **l'environnement fluctue de manière imprévisible** et affecte la mortalité des adultes, le partitionnement des ressources entre différentes partenaires et la fertilisation d'un plus grand nombre de femelles peut être bénéfique à la fitness du mâle (Rittschof, Hilber, Tudor, & St Mary, 2011 ; Figure 5), i) en réduisant le risque que les chances de descendance des mâles disparaissent avec la mortalité des femelles, mais aussi ii) en augmentant la diversité génétique et donc les chances de survie d'une partie de leur descendance (Ala-Honkola et al., 2015), une hypothèse qui jusqu'à présent n'a été que rarement testée empiriquement.

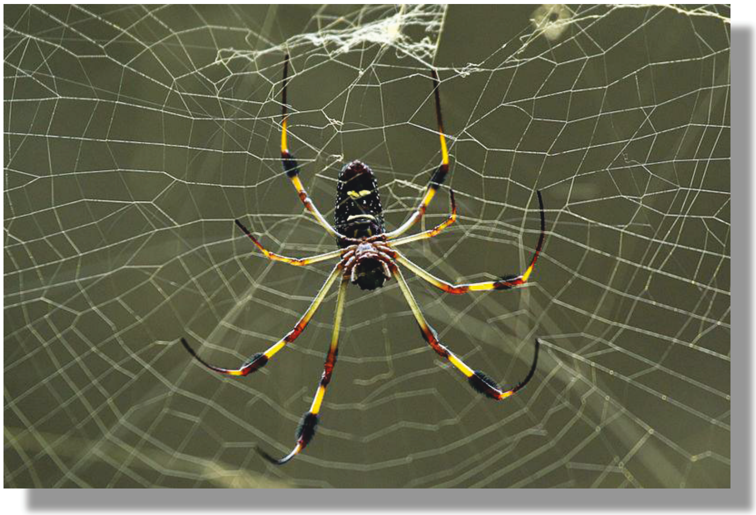

Figure 5 : Chez l'araignée *Nephila clavipes*, les mâles sont avantagés en s'accouplant de façon multiple si le risque de mortalité des femelles avant la ponte est élevé (Rittschof et al. 2011). Crédit photo : C. Jsharp.

Pour les mâles, si se reproduire est important, survivre l'est tout autant. Toutefois, les activités liées à la reproduction peuvent entrainer un risque accru de prédation notamment lorsque les mâles paradent, recherchent les femelles ou s'accouplent. Pour cette raison, lorsque le risque de prédation est fort, les mâles peuvent être avantagés s'ils ajustent leurs investissements au regard du risque de prédation (Kelly & Godin, 2001; Lucas, Howard, & Palmer, 1996).

3.6. Interactions entre facteurs et allocations stratégiques

La partie précédente résume les facteurs principaux affectant l'évolution d'allocations stratégiques chez les mâles ainsi que la nature des variations de réponse sexuelle qu'ils sont théoriquement susceptibles d'induire. Pourtant, dans les faits, la complexité des contraintes reproductives et des épisodes sélectifs rencontrés par les mâles (Chapitre 1, Partie 1 et 2 ; Figure 6) implique souvent l'influence conjointe de plusieurs facteurs, une situation dans laquelle les variations de réponse sexuelle des mâles peuvent être difficiles à prédire.

Tout d'abord, les opportunités d'accouplement d'un mâle dépendent à la fois de l'OSR de la population, mais également du potentiel attractif et compétitif des mâles, qui peut lui-même varier avec le nombre de mâles rivaux présents et leur propre condition physiologique (Chapitre 1, Partie 3.2 et 3.3). Sous ces conditions, la mesure du sexe ratio opérationnel seule n'est souvent pas suffisante pour prédire l'évolution des mécanismes sélectifs et compétitifs au sein des deux sexes.

Ensuite, le succès d'une allocation stratégique donnée ne peut être évalué qu'à l'aune de l'ensemble des facteurs impliqués. A titre d'exemple, les choix des mâles pour les femelles de meilleure qualité reproductive peuvent parallèlement induire un risque de compétition spermatique plus important chez les femelles préférées (Wedell et al. 2002). Dans ce cas, la préférence pour les femelles les plus fécondes s'avère plus coûteuse, et ne devient profitable qu'aux mâles capables de faire face avec succès au risque de compétition spermatique subséquent : c'est-à-dire ceux disposant d'importantes ressources spermatiques et/ou d'un bon potentiel compétitif s'il existe du gardiennage pré-copulatoire (Knox & Scott, 2006). De façon similaire, l'évolution du choix chez les mâles peut être fortement conditionnée par l'existence et l'intensité de sélection inter- et intra-sexuelle (préférences des femelles et/ou compétition intra-mâles), puisque seuls les mâles les plus attirants ou les meilleurs compétiteurs rencontreront suffisamment de femelles pour tirer avantage de leur sélectivité. Sous ces conditions, les mâles dont les chances de succès sont moindres (c'est-à-dire les moins attirants, ou les moins bons compétiteurs) peuvent optimiser la balance des coûts/bénéfices reproductifs en ciblant les partenaires de moins bonne qualité (Mautz & Jennions, 2011). Ce cas de figure peut aboutir à l'évolution **d'appariements assortis** (*assortative mating*), au cours desquels les mâles de meilleure qualité s'accouplent avec les femelles de meilleure qualité, tandis que les mâles de moindre qualité s'apparient avec les femelles de qualité similaire. Ainsi, chez l'araignée *Zygiella x-notata*, la compétition intra-sexuelle a conduit à l'appariement assorti des individus en fonction de leur taille : les plus gros mâles s'accouplent majoritairement avec les femelles les plus grosses (plus fécondes) tandis que les compétiteurs les plus petits s'apparient avec les femelles les plus petites (Bel-Venner & Venner, 2008). Enfin, les variations de réponse sexuelle des mâles peuvent également fluctuer au cours de leur vie au regard des variations de leur condition physiologique et/ou des ressources disponibles pour la reproduction (Edward & Chapman, 2013).

4. Biologie des modèles d'étude

4.1. *Achroia grisella*

4.1.1. Généralités

Figure 6 : *Achroia grisella* mâle (en dessous) et femelle (au-dessus). Crédit image : A. Jarrige.

La petite fausse teigne *Achroia grisella* (Pyralidae : Galleriinae, Figure 6) est un symbionte des abeilles à miel (*Apis mellifera*) (Milum 1935). C'est une espèce nocturne, dont la distribution géographique cosmopolite épouse celle de son espèce hôte. Les individus étudiés dans le cadre de cette thèse proviennent de l'élevage en laboratoire d'une population issue de la collecte de plusieurs centaines d'individus dans le département de l'Indre et Loire, France, en Octobre 2007.

L'espérance de vie des adultes est relativement brève : les mâles vivent en moyenne 7 jours, et les femelles 14 jours, au laboratoire (Greenfield & Coffelt, 1983; Ligout, Munier, Marquereau, Greenfield, & Tregenza, 2012). Les adultes possèdent des pièces buccales atrophiées qui ne leur permettent pas de s'alimenter, aussi la totalité des ressources dont disposent les individus pour le maintien somatique et la reproduction reposent sur le capital acquis au stade larvaire (Greenfield & Coffelt, 1983). Le développement est holométabole et le cycle de développement, mesuré d'oviposition à oviposition, dure en moyenne de 45 à 60 jours, variant avec les conditions de température et de nutrition auxquelles sont soumises les larves (Kunike 1930 ; Greenfield & Coffelt 1983, Figure 7). Les générations sont chevauchantes et au laboratoire les individus sont présents toute l'année. Dans les heures qui suivent leur émergence, les jeunes adultes se mettent en quête d'un partenaire sexuel. Après l'accouplement, les femelles pondent à l'intérieur de la ruche, où les larves se développent en se nourrissant de cire, de miel, voire même du couvain des abeilles (Kunike 1930).

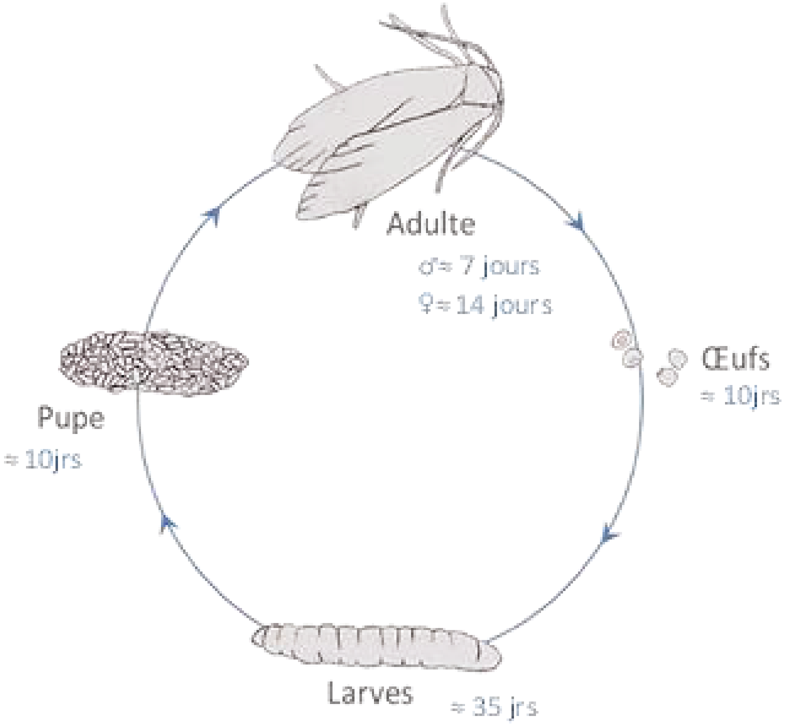

Figure 7 : Cycle de vie d'*Achroia grisella.* Crédit image : A. Jarrige.

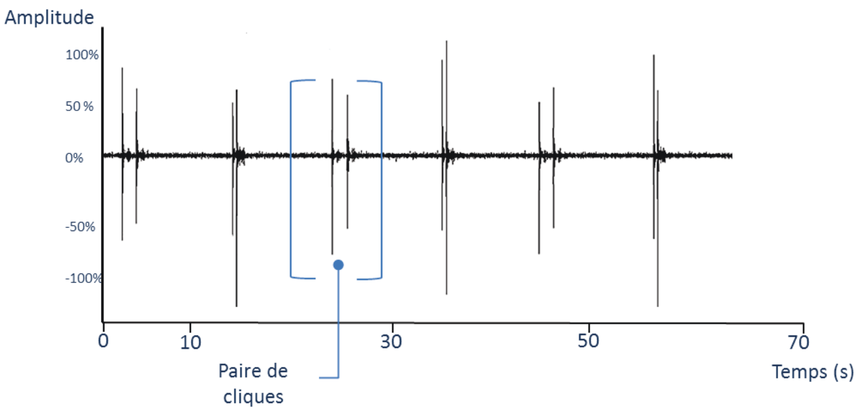

Figure 8 : Oscillogramme d'un chant de mâle d'*A. grisella* appartenant à la population d'Indre et Loire. Crédit image : V. Party.

4.1.2. Attraction des femelles et copulation :

Les mâles sont polygynes, tandis qu'approximativement 10 à 20 % des femelles se réaccouplent une seconde fois[13] avec un mâle différent (Cordes et al. 2014). Bien qu'en apparence faible, ce taux de réaccouplement génère un risque de compétition spermatique estimé à plus de 30 % (Leif Engqvist et al., 2014). Les activités de reproduction se déroulent à l'intérieur même de la ruche ou dans ses alentours immédiats (Greenfield & Coffelt, 1983). Cette espèce est caractérisée par un comportement reproducteur peu commun : pendant 6 à 10 heures chaque nuit, les mâles se regroupent en lek et émettent des chants d'appel ultrasoniques pour attirer les femelles.

Les chants sont produits par le battement des ailes des mâles : chaque mouvement ascendant et descendant entraine la déformation de minuscules structures acoustiques situées à la base des ailes et responsables de la production du son : les timbales. Le chant des mâles se compose donc d'une succession de paires de cliques ultrasoniques (voir figure 8).

Les femelles réceptives situées dans un rayon d'un mètre s'approchent en courant des mâles restés immobiles (Greenfield & Coffelt, 1983; Spangler, 1984). Une fois la femelle arrivée à proximité du mâle, l'accouplement prend place après quelques contacts antennaires, lorsque le mâle monte sur le dos de la femelle et introduit son appareil copulatoire (aedeagus) dans le tractus génital de la femelle (Eaton, 1988).

Les femelles choisissent les mâles sur la base de leur chant, et de précédentes études montrent que les préférences des femelles sont notamment influencées par trois caractéristiques : l'amplitude, le nombre de cliques par unité de temps, ainsi que la longueur des intervalles silencieux entre les paires de cliques successifs (Jang & Greenfield, 1996; Jang & Greenfield, 1998; Limousin & Greenfield, 2009 ; Figure 8). Ces chants sont coûteux à produire et entraînent d'importantes dépenses métaboliques (Reinhold, Greenfield, Jang, & Broce, 1998). Les signaux acoustiques s'accompagnent également de la libération d'une phéromone produite par une paire

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[13] Toutefois, les femelles se réaccouplent rarement plus de deux fois (une femelle sur 130, Jarrige, observation personnelle, 2014).

de glandes située ventralement sous les ailes antérieures, et dont la fonction serait l'attraction des femelles à courte distance (Finn 1967). Lors de l'accouplement, le mâle transfère un spermatophore à la femelle, sorte de sac séminal contenant les spermatozoïdes. A l'instar de nombreux lépidoptères, la spermatogénèse est dichotomique et deux types de sperme sont conjointement transférés aux femelles i) les spermatozoïdes eupyrènes (nucléés), transmis sous forme de bundles (paquets) de 256 spermatozoïdes (Figure 9) et ii) les spermatozoïdes apyrènes (anucléés) transmis sous forme libre (Fernandez-Winckler & da Cruz-Landim, 2008). Seuls les spermatozoïdes eupyrènes sont fertilisants, et le rôle des spermatozoïdes apyrènes est encore mal compris (Silberglied, Shepherd, & Dickinson, 1984; Friedländer et al. 2005). Enfin, il est important de noter que la spermatogenèse eupyrène s'interrompt irrémédiablement lors de la nymphose. De ce fait, les mâles adultes émergent avec un stock de spermatozoïdes eupyrènes défini qu'ils ne peuvent renouveler au cours de leur vie (Friedlander, 1997; Friedländer, Seth, & Reynolds, 2005).

Au sein des leks, les femelles choisissent librement leur partenaire (Encadré 3), aussi l'accès des mâles aux femelles repose sur leur investissement dans les chants d'appel et peut donc être fortement biaisé en faveur des mâles les plus attractifs. Il a notamment été montré que des mâles en présence de compétiteurs accélèrent temporairement le rythme des paires de cliques de leur chant (Jia et al., 2001).

Figure 9: Bundles de spermatozoïdes eupyrènes d'*A. grisella* colorés au DAPI. Crédit image : A. Jarrige.

4.2. *Ephippiger diurnus*

4.2.1. Généralités

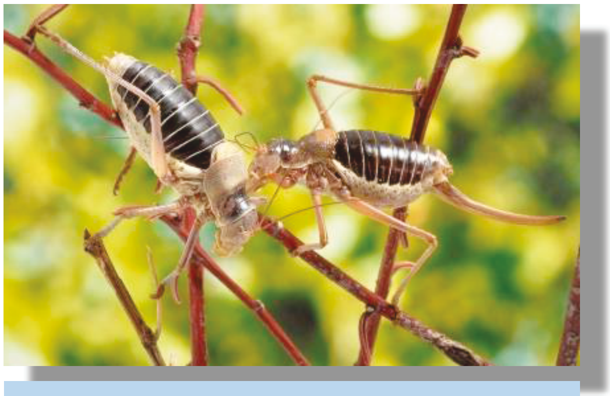

Figure 10 : *Ephippiger diurnus* mâle (à gauche) et femelle (à droite). Crédit image : S. Dourlot.

Ephippiger diurnus (Orthoptera : Tettigoniidae : Bradyporinae) est une sauterelle commune trouvée principalement dans la garrigue et les habitats perturbés du pourtour Méditerranéen (Duijm, 1989 ; Oudman et al. 1990). Du fait de sa dispersion limitée (les individus sont aptères), cette espèce présente une distribution géographique disjointe, ainsi qu'une grande variabilité morphologique et comportementale à l'origine de nombreuses études et révisions taxonomiques et phylogéographiques (Ritchie, Kidd, & Gleason, 2001). Par exemple, les chants sexuels des mâles présentent des caractéristiques variables selon les populations permettant selon certains auteurs l'identification de sous-espèces (Ritchie, Racey, Gleason, & Wolff, 1997; Spooner & Ritchie, 2006). La population étudiée dans le cadre de cette thèse provient du Col de Mantet (42°28´N, 2°18´E), à 1700 m d'altitude dans la région des Pyrénées Orientales, France, et préalablement désignée comme *E. cunii* Bolivar et *Ephippiger ephippiger* (Harz, 2012; Kidd & Ritchie, 2006; Spooner & Ritchie, 2006).

En conditions naturelles ainsi qu'au laboratoire, les adultes de cette population ne sont présents que pendant une période relativement courte, qui varie sensiblement d'une année sur l'autre selon les conditions météorologiques. Les œufs éclosent de la fin du printemps jusqu'au début de l'été, tandis que les premiers adultes émergent au début du mois de Juillet (Jarrige, 2014, observation personnelle). La saison de reproduction culmine généralement entre mi-Aout et mi-Septembre. Sur le terrain, la durée de vie des adultes est probablement d'environ deux mois, et n'excède qu'exceptionnellement trois mois puisque les individus succombent aux premières gelées survenant, à cette altitude, dès le mois d'Octobre (Jarrige, 2014, observation personnelle). Les générations ne sont pas chevauchantes : seuls les œufs passent l'hiver dans le sol et éclosent entre un et trois[14] ans après avoir été pondus (Hartley & Dean, 1974). Au laboratoire, l'espérance de vie des adultes est notablement plus longue, puisqu'ils vivent en moyenne trois mois et qu'il n'est pas rare de voir des individus vivre jusqu'à 4 mois (Jarrige, 2014, observation personnelle).

4.2.2. Attraction des femelles et copulation :

Lorsque les conditions d'ensoleillement et de température sont favorables, les mâles se placent généralement en hauteur sur un buisson ou une plante et produisent un chant d'appel du lever du soleil jusqu'au milieu de la journée (Berg & Greenfield 2005), créant ainsi des chorus où chaque mâle chante en alternance avec ses rivaux (Greenfield & Snedden 2003, Greenfield et al. 2004). Les chants d'appel sont produits par le frottement l'une contre l'autre de deux rangées de dents cuticulaires portées par les élytres (ailes vestigiales) (Dumortier 1963, 1972), et les femelles sexuellement réceptives s'orientent vers les signaux acoustiques ainsi émis. Lorsque qu'une femelle est suffisamment proche (environ 60 cm) (Stiedl & Kalmring 1989), le mâle cesse de chanter et les individus manifestent parfois un comportement vibratoire, appelé trémulation, en faisant trembler rapidement leur corps sur le substrat[15] (Busnel 1955, Keuper et al. 1985). Il semblerait que ce comportement ait un rôle dans l'orientation à courte distance des partenaires l'un vers l'autre (Stiedl & Kalmring 1989).

Une fois à proximité, mâle et femelle se font face et se contactent réciproquement avec leurs antennes. Puis, le mâle effectue un demi-tour de manière à présenter la partie postérieure de son abdomen à la femelle. Après en avoir examiné l'extrémité à l'aide de ses palpes maxillaires, la femelle grimpe sur le dos du mâle. Ce dernier recourbe son abdomen de sorte à amarrer ses cerques aux sillons copulatoires présents au niveau de la plaque subgénitale de la femelle (Dujim

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[14] Une diapause obligatoire étant nécessaire à leur développement, à laquelle s'ajoute(nt) une ou deux seconde(s) diapause(s) facultative(s) (Hartley & Dean 1976, Jarrige, observation personnelle 2014).

[15] Généralement une branche d'un arbuste (Jarrige, observation personnelle 2014).

et al. 1983). Les tittilateurs, sortes de dents internes présentes au niveau de l'appareil génital du mâle, agrippent alors la femelle en position de copulation. Pour cette raison, la durée de l'accouplement et la nature du transfert semblent sous le contrôle du mâle (Dujim et al.1983, Vahed 2007), toutefois, l'un des deux partenaires peut interrompre la séquence en cours et prospecter pour un autre partenaire durant chaque étape précédant le début de la copulation.

Ephippiger diurnus est une espèce polygame. L'accouplement dure environ 20 minutes, pendant lesquelles le mâle transfère à la femelle le produit de ses glandes accessoires sous la forme d'un spermatophore externe (e.g. Wedell 1994, Wedell 2004). Ce spermatophore est constitué de deux parties distinctes : l'ampulla, qui contient les spermatozoïdes, et le spermatophylax, masse gélatineuse composée principalement d'eau, de protéines (4% à 27 % de la masse humide) et d'une très faible proportion de glucides et de lipides (Wedell 1994, Heller et al. 1998, Gwynne 2003), que la femelle consomme après l'accouplement pendant que le sperme contenu dans l'ampulla migre dans son tractus génital.

Cette espèce se distingue par la taille remarquable du spermatophore transféré aux femelles, qui peut atteindre jusqu'à 40% du poids du mâle (Wedell 2004, Figure 12). Pour les mâles, la production d'une telle donation représente donc un investissement considérable obligatoirement suivie d'une période réfractaire de 48 à 72 heures pendant laquelle ils ne chantent ni ne s'accouplent (Busnel & Durmortier 1955). Par ailleurs, il a été montré que les effets de l'âge tendent à augmenter la taille et la qualité du spermatophore, tandis que le nombre d'accouplements a l'effet inverse en réduisant la quantité de nutriments et le nombre de spermatozoïdes transférés (Wedell & Ritchie 2004).

De nombreux travaux documentent la sélectivité des femelles pour certaines caractéristiques du chant des mâles, et il a été mis en évidence que les femelles préfèrent les chants des mâles de leur population native (Ritchie 1991), les chants les plus intenses et/ou qui possèdent un nombre de syllabes légèrement supérieur à la moyenne (Ritchie 1992, 1996, Brunel 2012, Figure 13), ainsi que les mâles dont les chants sont émis avec une légère avance temporelle de phase sur ceux de leurs voisins (Greenfield & Snedden 2003, Greenfield et al. 2004). Il a également été montré que les femelles discriminent par contre le chant des mâles âgés, dont la structure est altérée par la perte ou l'usure des dents de l'appareil stridulatoire (Ritchie et al. 1991).

Par ailleurs, les chances de paternité d'un mâle sont biaisées en faveur du dernier mâle à s'être accouplé avec une femelle, lié à un important effet de précédence du dernier sperme (Hockam et al. 2004).

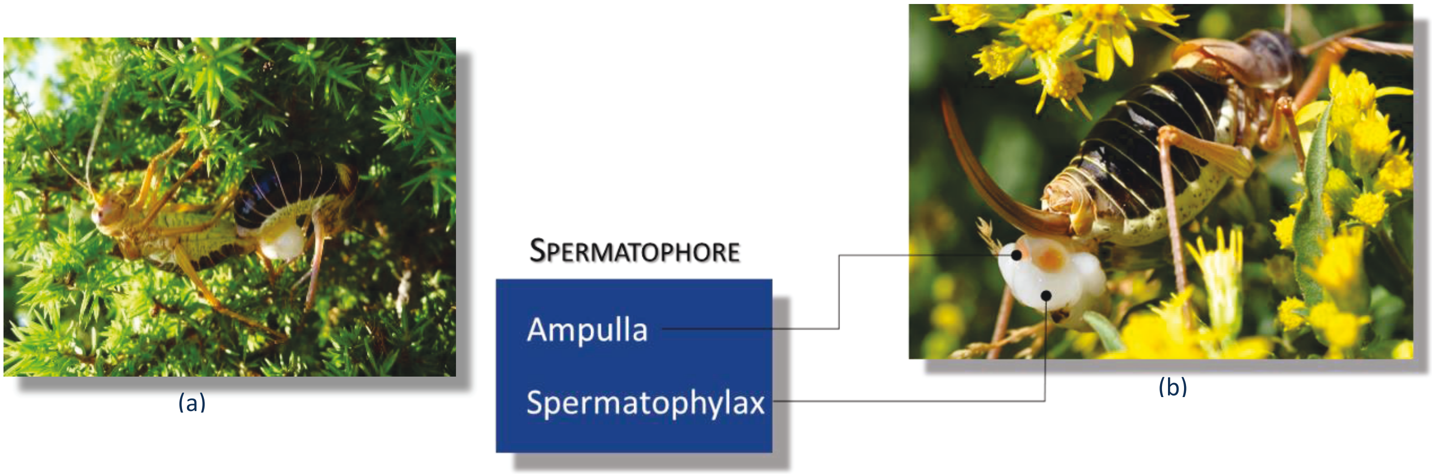

Figure 11 : Accouplement (a) et détail d'un spermatophore (b) chez *Ephippiger diurnus*. Crédit image : (a) A. Jarrige, (b) S. Dourlot.

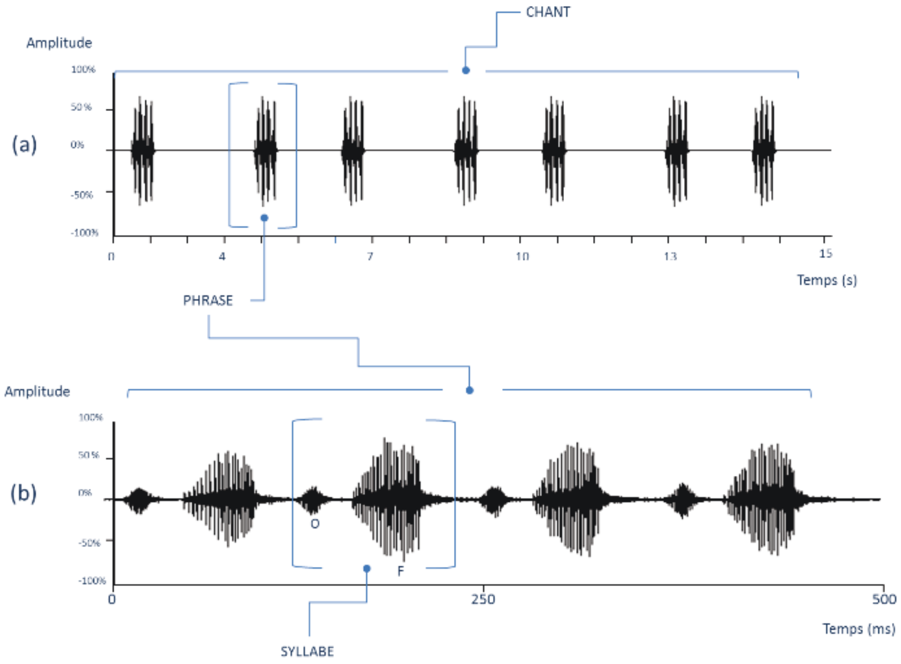

Figure 12 : Oscillogramme d'un chant de mâle *Ephippiger diurnus* de la population du Col de Mantet (a) et détail d'une phrase (b). Chaque syllabe consiste en une composante courte produite par l'ouverture des élytres (O), et d'une composante longue produite par la fermeture des élytres (F). Le chant des mâles de la population du Col de Mantet possède en général 4 à 5 syllabes.

CHAPITRE 2 : Allocation des ressources gamétiques chez *Achroia grisella*.

Mâle allouant ses ressources spermatiques à une femelle. Crédit image : A. Jarrige

Chapitre 2. Allocation des ressources spermatiques chez *Achroia grisella*.

Bien qu'il soit avéré que les spermatozoïdes soient individuellement moins couteux à produire que les ovules, le transfert par les males d'éjaculats contenant les milliers de spermatozoïdes génère souvent des coûts substantiels pour les mâles, limitant parfois drastiquement leur potentiel reproducteur (Dewsbury, 1982; Nakatsuru & Kramer, 1982). Par ailleurs, le timing de formation des gamètes peut fortement contraindre le nombre de spermatozoïdes dont disposent les mâles tout au long de leur vie, ou le nombre pouvant être transféré à un moment donné (Boivin, Jacob, & Damiens, 2005). Les contraintes liées à la disponibilité des ressources spermatiques peuvent s'avérer particulièrement drastiques chez les espèces prospermatogéniques, ou la spermatogénèse cesse chez l'adulte et où, par conséquent, les mâles disposent d'une quantité de gamètes définie qu'ils devront partitionner entre leurs différents épisodes reproducteurs (Boivin et al., 2005).

Sous ces conditions, **il est attendu que les mâles allouent prudemment leurs ressources spermatiques au regard i) des opportunités d'accouplement présentes et future ii) de la qualité reproductive de la femelle et iii) de la présence et de l'intensité de mécanismes post-éjaculat**, notamment sous la forme de compétition spermatique (Edward & Chapman, 2011; Wedell, Gage, & Parker, 2002).

Toutefois, les facteurs affectant les chances de fertilisation des gamètes mâles ne constituent cependant que la dernière étape sélective à laquelle sont soumis les mâles. En effet, toute opportunité de fertilisation repose au préalable sur la capacité des mâles à concrétiser l'acte d'accouplement, et donc de leur succès préliminaire à l'égard des mécanismes de sélection intra-sexuelle et intersexuelle impliqués (Chapitre 1, Partie 2). Pour cette raison, l'établissement de prédictions concernant les allocations des ressources gamétiques s'avère souvent difficile, notamment du fait d'interactions potentielles complexes entre les facteurs pré et post-éen jeu (Chapitre1, Partie 2). Aussi, un important effort de recherche reste nécessaire pour comprendre les variations de l'allocation gamétique aux femelles lorsque les mâles doivent répondre à des contraintes variées.

Au cours de ce chapitre, je me suis intéressée aux allocations des ressources gamétiques chez *A. grisella*. Chez cette espèce, les mâles se regroupent en leks pour émettre des chants d'appels ciblés par les préférences des femelles, conduisant à **d'intenses pressions de sélection pré-copulatoire intra et intersexuelle**. Par ailleurs, **l'ensemble des ressources dont disposent les mâles pour la reproduction et le maintien somatique s'avèrent fortement contraintes** puisque les individus ne se nourrissent pas à l'état adulte et que la spermatogénèse cesse lors de la nymphose (Chapitre 1, Partie 4.1).

Dans un premier temps, j'ai examiné l'influence du risque de compétition spermatique sur les allocations de mâles placés en présence d'un compétiteur durant les premiers jours de leur vie adulte (Article 1). Puis, j'ai souhaité étudié l'effet de l'environnement social des mâles et de la qualité des femelles sur 1) l'allocation spermatique des mâles et 2) l'intensité des comportements de gardiennage pré-copulatoire (Article 2). Enfin, dans une troisième étude, j'ai examiné l'influence de l'histoire reproductive des mâles sur leurs investissements gamétiques. Les résultats obtenus au cours de cette dernière étude suggérant l'existence deux phénotypes distincts en ce qui concerne le comportement reproducteur des mâles et le transfert de sperme aux femelles, j'ai ensuite examiné les préférences des mâles pour la qualité reproductive de la femelle au sein des deux phénotypes (Article 3).

ARTICLE 1

Strategic sperm allocation in response to perceived sperm competition risk in a lekking insect

Alicia Jarrige, Dennis Riemann, Marlène Goubault, & Tim Schmoll

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ABSTRACT

Sperm competition has been demonstrated to be a strong selective force shaping male mating behaviours and ejaculate traits particularly in highly polyandrous species. Its effect, however, is expected to be limited in lek mating systems, where female mating decisions appear relatively unconstrained and thus female remating rate is generally low. Hence, testing for male adaptations to sperm competition in lek-mating species is of particular importance in order to understand whether and how sperm competition, as a selective force, may have shaped male phenotype also in such systems. Here, we investigated copulation behaviour and strategic sperm allocation in the lekking moth *Achroia grisella*, a well-established model for studying pre-copulatory sexual selection as males display elaborate ultrasonic courtship songs targeted by female choice. Recent evidence suggests that females do sometimes remate, thus creating a selective potential for sperm competition. In order to test the hypothesis that sperm competition selects for male adaptations in ejaculate traits also in lek mating systems, we staged experimental matings after males had experienced either a competitive (one competitor present), or a non-competitive (no competitor present) social environment during early adulthood. Males increased sperm transfer rate and thereby allocated higher proportions of available sperm reserves during virgin copulations when experiencing the elevated sperm competition risk environment. Our study provides first evidence of strategic sperm allocation in relation to sperm competition risk in a lekking insect, demonstrating that sperm competition might represent an important selective force in such mating systems, too.

KEYWORDS

Achroia grisella; competitive environment; ejaculate size; lek mating system; Lepidoptera; post-copulatory sexual selection, sperm competition risk; sperm economy; strategic ejaculation.

INTRODUCTION

Sperm competition occurs when spermatozoa from more than one male compete for a defined set of ova (Parker 1970; Simmons 2001; Parker & Pizzari 2010). Under sperm competition, male reproductive success will not only depend on the number and quality of females a male can mate with, but also on its ability to fertilize the eggs of the females it successfully copulated with. As a male's share of paternity usually increases with relative ejaculate size (Martin, Reimers, Lodge, & Dziuk, 1974; Parker & Pizzari 2010), the question of strategic investment in sperm production and/or strategic sperm allocation at a given mating have been central to sperm competition models. In particular, models predict that males should respond differently to sperm competition risk (i.e. the probability that at least one rival ejaculate is present) compared to sperm competition intensity (i.e. the number of ejaculates in competition). While male ejaculate expenditure, both in terms of sperm production and sperm allocation, is predicted to increase with higher sperm competition risk, males are expected to reduce the number of sperm allocated when sperm of more than one competitor is involved (Parker, Ball, Stockley, & Gage, 1996; Parker, Ball, Stockley, & Gage, 1997; Engqvist & Reinhold 2005; Kelly & Jennions 2011).

In lekking species, males gather in a display arena where they compete for females while females visit the lek in order to choose freely the highest quality male to mate with (Kirkpatrick & Ryan 1991; Höglund & Alatalo 1995). Lek mating systems are well known to exhibit, in exemplary fashion, the distinctive Darwinian sex roles of pronounced female choosiness and strong male-male competition (Darwin 1871; Höglund & Alatalo 1995). For decades, it has been assumed that strategic allocation of male reproductive resources was of minor importance in these systems. First, because reproductive investment of lekking males consists solely in courtship display and sperm delivery and does not involve male parental care, males were expected to mate rather indiscriminately with as many females as possible. The benefit of male mate choice is therefore unlikely to compensate for the cost of losing mating opportunities (Trivers 1972; Deutsch & Reynolds 1995; Johnstone, Reynolds, & Deutsch, 1996). Second, because females do not derive direct fitness benefits from mate choice and can express unconstrained mating preferences, female remating behaviour and thus the risk of sperm competition was thought to be rare or absent (Avery 1984; Birkhead, Atkin, & Møller, 1987), which in turn would decrease the prospect of male adaptations to sperm competition (Parker 1990 a,b; Hunter, Harcourt, Wright, & Davis, 2000).

However, this classical view of what constitutes male sex role in a lek has been recently questioned, because sperm production is costly and might considerably limit male reproductive rate through permanent or temporary sperm depletion (Dewsbury 1982; Galvani & Johnstone 1998; Boivin 2012). In addition, courtship might be energetically demanding (Höglund, Kålås, & Fiske, 1992; Anderson 1994). For both reasons, strategic allocation of male reproductive resources, including strategic sperm allocation, may well be expected and it has indeed been demonstrated, for example, that males of lekking species adjust sperm transfer to female reproductive quality in birds and mammals (Saether, Fiske, & Kålås, 2001; Bro-Jørgensen 2007). Moreover, although sperm competition is often predicted to be low or absent, evidence for multiple mating (and multiple paternity) in leks suggests that sperm competition could play a non-negligible role also in these systems (Petrie, Hall, Halliday, Budgey, & Pierpoint, 1992; Lank et al. 2002; Hess, Dunn, & Whittingham, 2012). Yet, despite the tremendous amount of theoretical and empirical work on sperm competition and strategic sperm allocation, and the fact that the potential for post-copulatory processes to affect male fitness has recently been emphasized in lekking species (Leftwich, Edward, Aphey, Gage, & Chapman, 2012; Sardell & DuVal 2014), no study has yet directly addressed the evolution of strategic sperm allocation in response to sperm competition in a lek mating system.

The moth *Achroia grisella* (lesser wax moth, Lepidoptera: Pyralidae) is a well-established model system for studying pre-copulatory sexual selection due to its remarkable mating system where males gather in leks and broadcast ultrasonic courtship calls (Greenfield & Coffelt 1983; Spangler, Greenfield, & Takessian, 1984; see also Methods below). Given that approximately 20% of females remate with another male (Greenfield & Coffelt 1983; Engqvist, Cordes, Schwenniger, Bakhtina, & Schmoll, 2014), which creates a sperm competition risk of more than 30% (Engqvist et al. 2014), we may expect selection for male adaptations to sperm competition.

Females that remate do so only very rarely more than once (one female out of 130, Jarrige, personal communication, 2014), thus competition between sperm of more than two males is very rare and both average sperm competition risk and intensity are relatively low, which constitutes prerequisites to fit sperm competition risk models (Parker 1990a; Parker et al. 1997; Engqvist & Reinhold 2005 and see above). Indeed, because manipulation of perceived sperm competition risk might also affect male perception of sperm competition intensity, clear predictions for sperm allocation would be difficult in more strongly promiscuous systems as sperm competition risk and sperm competition intensity are predicted to have opposite effects on ejaculate size (Engqvist & Reinhold 2005). Moreover, adult males emerge with a fixed amount of eupyrene (i.e. fertilizing) sperm (spermatogeny index of 1) (Friedländer 1997; Boivin 2005; Friedländer, Seth, & Reynolds, 2005) aggregated in bundles of 256 spermatozoa (Fernandez-Winckler & da Cruz-Landim 2008). Thus sperm production (in the larval stage) and sperm allocation (in the adult) are separated in time and can be singled out by suitable experimentation, rendering *A. grisella* an ideal model system to investigate strategic sperm allocation in response to sperm competition risk.

In the present study, we investigated whether male mating behaviour and sperm allocation in *A. grisella* is affected by experimentally manipulated sperm competition risk by exposing males to the presence of a competitor during 72 hours prior to and during virgin mating. We predict that males experiencing social cues indicative of sperm competition risk (i.e. with one competitor present) transfer a greater proportion of their sperm reserves to females during their first copulations compared to males of the same age experiencing no such cues (no competitor present, control). In order to test this prediction, we compared i) copulation duration ii) number of eupyrene sperm transferred at virgin copulations and iii) number of eupyrene sperm retained in the male genital tract between the two groups.

MATERIAL AND METHODS

Study species

The lekking moth *Achroia grisella* is a cosmopolitan symbiont of the western honeybee *Apis mellifera* in whose colonies the moth larvae feed on honeycomb and other organic material (Kunike 1930). Both male and female *A. grisella* adults have atrophied mouthparts and neither feed nor drink. Hence, all resources for somatic maintenance and reproduction are entirely acquired at the larval stage (Greenfield & Coffelt 1983). Adults have a markedly short lifespan as males and females typically only live for seven or ten to 14 days in the laboratory, respectively. Mating activities of moths usually take place in the vicinity of their natal honeybee colony. For six to ten hours every night, males gather in leks and broadcast ultrasonic courtship calls by wing-fanning while remaining stationary on the substrate (Greenfield & Coffelt 1983; Spangler et al. 1984). Receptive females in the vicinity orient and move towards singing males up to one meter distant, usually by running. Female pre-copulatory mating preferences based on evaluation of male song traits have been studied in several populations, showing that females prefer songs delivered with greater power (expressed as the product of mean amplitude by calling rate), greater peak amplitude and amplitude fluctuations (Jang & Greenfield 1996; Jang & Greenfield 1998; Limousin & Greenfield 2009). Males respond to surrounding males by temporarily accelerating the pulse-pair rhythm in their songs (Jia, Greenfield, & Collins, 2001). Males exhibit the dichotomous spermatogenesis typical for Lepidoptera by producing eupyrene (nucleate) and apyrene (anucleate) spermatozoa (Friedländer 1997; Friedländer et al. 2005). Both types of sperm reach the female spermatheca, but only eupyrene sperm fertilize eggs while the function of apyrene spermatozoa is still to be clarified (Silberglied, Shepherd, & Dickinson, 1984; Friedländer et al. 2005).

Laboratory stock population and experimental animals

We maintained a stock population of *A. grisella* derived from several hundred individuals collected in Departement of Indre et Loire, France, in October 2007. Moths were reared in an environmental chamber at 24 ± 2°C, 45% relative humidity and under a 12:12 hour light:dark photoperiod. Larvae were cultured on a standard *ad libitum* diet containing wheat, corn and rye flour, water, glycerol, nutritional yeast, honey and beeswax (modified from Dutky, Thompson, & Cantwell, 1962, see appendix table A1). These laboratory conditions closely mimic natural conditions for this species. Under these conditions, generation times as measured from oviposition to oviposition range from 45 to 55 days. In each generation, individuals were bred such that close inbreeding (full-sib matings) was avoided and a large population size was maintained to minimize the loss of genetic variation. To do so, every one to two weeks, 20 to 25 virgin females originating from different broods were randomly paired with the same number of non-sibling virgin males also originating from different broods. To further control for close relatedness between the individuals used during the tests, all experimental animals were F1 offspring from pairs of partners which were randomly sampled from the stock population. The stock population was maintained at the Institut de Recherche sur la Biologie de l'Insecte in Tours and the experimental F1 pupae were transferred to Bielefeld University to conduct the experiment in November and December 2014. From second larval instar onwards, experimental individuals were held individually in 28 ml plastic cups to ensure virginity and standardize social conditions. Emergence was monitored twice a day, ensuring that individuals were 0 to 16 h old when entering the experiment.

Experimental setup

On the day of emergence (defined as day zero), focal males were weighed to the nearest mg with a Kern 770 electronic scale (Kern & Sohn GmbH, Balingen, Germany) and randomly assigned to either the "competitor" treatment level (sperm competition risk) or "no competitor" control level, by placing the focal male into a 17.5 x 11.5 x 7.5 cm plastic box either together with one competitor male or alone (control). Both focal and competitor males were placed into small net cages made of metallic mosquito net preventing direct physical contact between males but allowing acoustic and olfactory communication. After experiencing the assigned treatment for 72 h, males were weighed again and experimentally mated with a female zero to two days old for which body mass was recorded on the day of mating. Experimental mating trials were staged by introducing the female directly into the focal male's net cage. Previous competitive conditions were maintained: the plastic box still contained the competitor male in his separate net cage or not (control). Copulation duration was measured to the nearest second with a stopwatch.

Experimental mating trials took place under red light in an environmental chamber at 24 ± 2 °C during the initial six hours of the scotophase when generally mating activities are peaking.

Dissection of experimental animals and eupyrene sperm bundle counts

Immediately after copulation had ended naturally, males and females were dissected and the number of eupyrene sperm bundles was counted as follows.

Number of bundles transferred to the female

Within three minutes after copulation had ended naturally, females were chilled in the freezer for two minutes and then killed by decapitation. The bursa copulatrix was removed by ventral opening of the female abdomen, and the spermatophore was carefully isolated in a drop of 5 µl of 10^{-4} M DAPI (4',6'-diamidino-2-phenylindole, Sigma-Aldrich) placed on a microscopic slide. In *A. grisella*, the spermatophore transferred by males consists of a seminal sac containing both solitary apyrene sperm and eupyrene sperm, the latter aggregated in bundles of 256 spermatozoa (Fernandez-Winckler & da Cruz-Landim 2008). The seminal sac was ruptured with a fine needle tip to release the bundles and to allow full dispersion. After gentle homogenization with a needle tip, eupyrene bundles were counted under UV light microscopy at 500 x magnification.

Number of bundles remaining in the male genital tract

As for females, males were chilled immediately after copulation for two minutes in the freezer and decapitated. The genital tract of the males consists of a pair of fused testes loosely attached to the dorsal region of the abdomen. From each testis an upper vas deferens originates and leads to one seminal vesicle each. Lower vasa deferentia start from the seminal vesicles and end in the cuticular duplex. The duplex arms then merge to form the simplex that meets the dorsal surface of the aedeagus (Eaton 1988). The whole reproductive tract was removed after ventral opening and placed into a drop of 5 µl of 10^{-4} M DAPI on a microscopic slide. Subsequently, parts of the reproductive tract were gently unfolded with two needle tips. Testes, seminal vesicles and duplex were then transferred to separate drops of DAPI and carefully ruptured. After gently stirring the drop contents with a needle tip, eupyrene bundles were counted under UV light microscopy at 500 x magnification.

The total number of sperm bundles available at the time of virgin copulation was calculated by summing the number of bundles transferred to females and the number of bundles remaining in the male genital tract. The proportion of eupyrene sperm bundles transferred was obtained by dividing the number of bundles transferred to the female by the total number of spermatozoa available. Finally, eupyrene sperm bundle transfer rate was calculated as the number of bundles transferred per minute copulation duration.

Ethical Note

We used 284 individuals in this study: 113 females, 116 males in the competitor treatment (58 focal males and 58 competitors), and 55 males in the control treatment. All individuals were handled with care and handling time was kept to an absolute minimum. Subjects submitted to dissection were chilled in a freezer prior to decapitation.

Statistical analysis

We tested for effects of treatment level on sperm expenditure and copulation behaviour using linear mixed effects models (LME). As explanatory fixed effects we included presence of a competitor during early adult life (yes/no) as a two-level factorial variable and male mass at emergence and female mass at mating as continuous covariates. Furthermore, we included male sibship identity as a random effect to account for the non-independence due to shared genotypes among full brothers.

Significance of fixed effects was determined by removing the focal terms from a maximum likelihood fit of the model. P-values in the context of LME analyses always refer to the increase in model deviance when a term is removed from a model compared against a χ^2 distribution using a likelihood ratio test. All models were fitted in R 3.0.1 (R-Core-Team, 2014) using the function *lmer* from the package *lme4* (Bates, Maechler, Bolker, & Walker, 2014). All statistical tests were two-tailed and we rejected the null hypothesis at $p < 0.05$. To normalize residuals we used log-transformed values when using copulation duration as a dependent variable. Sample sizes for analyses below vary slightly due to partial lack of data for example because males failed to achieve copulation or because dissections of males failed subsequent to successful copulations (see Table 1 below).

There was a trend for males facing a rival to be heavier at emergence than control males (competitor present: $N = 52$; control: $N = 53$; LME: $\chi^2 = 2.49$, d.f. = 1, $p = 0.11$). Furthermore, there was a trend for females assigned to the competitor treatment to be lighter at mating ($N = 52$; $N = 53$; $\chi^2 = 2.66$, d.f. = 1, $p = 0.10$). We therefore first report results based on the effect of treatment alone and subsequently when controlling for male emergence mass and female mating mass in a multiple regression model to control potentially confounding effects of the two latter variables (due to lack of emergence mass for one male assigned to the competitor treatment level, sample sizes between both types of analyses consistently differ by one). Note that male mass at mating must not be used as a covariate in the abovementioned multiple regression models as it has to be considered as a post-treatment variable representing an intermediate outcome (Gelman, 2007).

RESULTS

Male mass loss during the treatment period

Male mass loss between the day of emergence (day zero) and the day of experimental mating (day three) was similar for males with a competitor present and control males (competitor present: $N = 58$; control: $N = 55$; LME: $\chi^2 = 1.20$, d.f. = 1, $p = 0.27$; for descriptive statistics see Table 1).

Table 1.

	Control	Competitor
Male emergence mass (mg)	11.54 ± 2.54; $N = 55$	11.83 ± 2.26; $N = 58$
Male mating mass (mg)	9.35 ± 2.21; $N = 55$	9.52 ± 1.85; $N = 58$
Male mass loss (mg)	2.18 ± 0.60; $N = 55$	2.30 ± 0.62; $N = 58$
Female mating mass (mg)	25.63 ± 4.44; $N = 49$	24.51 ± 4.46; $N = 58$
Copulation duration (min)	12.65 ± 1.83; $N = 44$	12.92 ± 1.25; $N = 49$
Number of bundles transferred to females	58 ± 22; $N = 50$	69 ± 25; $N = 51$
Percentage bundles transferred to females	40 ± 9; $N = 49$	51 ± 10; $N = 50$
Total number of bundles available	142 ± 45; $N = 49$	138 ± 52; $N = 50$

Table 1. Mean values ± SD of analysed traits displayed in relation to experimentally manipulated sperm competition risk (competitor present and control).

Sperm expenditure

The number of sperm bundles transferred to females ($N = 51$; $N = 48$; LME: $\chi^2 = 5.71$, d.f. = 1, p = 0.02) as well as the total number of bundles available ($N = 51$; $N = 48$; LME: $\chi^2 = 3.47$, d.f. = 1, p = 0.06) was positively related to male emergence mass. Males with a competitor present transferred an approximately 24% higher proportion of their eupyrene sperm bundles compared to control males ($N = 51$, $N = 48$; LME: $\chi^2 = 29.9$, d.f. = 1, $p < 0.001$; Figure 1a, Table 1), a robust finding unaffected when controlling for male emergence mass and female mating mass in a multiple regression model ($N = 50$, $N = 48$; $\chi^2 = 30.1$, d.f. = 1, $p < 0.001$). The total number of sperm bundles available was similar across treatment levels ($\chi^2 = 0.10$, d.f. = 1, p = 0.75; Figure 1b, Table 1; controlling for male emergence mass and female mating mass led to the same conclusion: $\chi^2 = 0.69$, d.f. = 1, p = 0.41). However, males with a competitor present transferred approximately 17% more eupyrene sperm bundles during virgin copulations than control males ($\chi^2 = 5.30$, d.f. = 1, p = 0.02; Figure 1c; Table 1). When controlling for male emergence mass and female mating mass, this effect was slightly weaker and marginally non-significant ($\chi^2 = 3.53$, d.f. = 1, p = 0.06).

Copulation behaviour and sperm transfer rate

Males with a competitor present copulated for a similar time compared to control males ($N = 47$, $N = 40$; $\chi^2 = 0.07$, d.f. = 1, p = 0.79; Figure 2a, Table 1; controlling for male emergence mass and female mating mass led to the same conclusion: $N = 46$, $N = 40$; $\chi^2 = 0.03$, d.f. = 1, p = 0.87). Furthermore, the number of sperm bundles transferred was not significantly related to copulation duration ($N = 49$, $N = 43$; $\chi^2 = 0.70$, d.f. = 1, p = 0.40; Table 1; controlling for male emergence mass and female mating mass led to the same conclusion: $N = 48$, $N = 43$; $\chi^2 = 0.78$, d.f. = 1, p = 0.38). However, males with a competitor present transferred sperm bundles at an approximately 16% higher rate compared to control males ($N = 47$, $N = 40$; $\chi^2 = 3.83$, d.f. = 1, p = 0.05; Figure 2b, Table 1). When controlling for male emergence mass and female mating mass, this effect was less pronounced and not significant ($N = 46$, $N = 40$; $\chi^2 = 2.67$, d.f. = 1, p = 0.10).

Figure 1

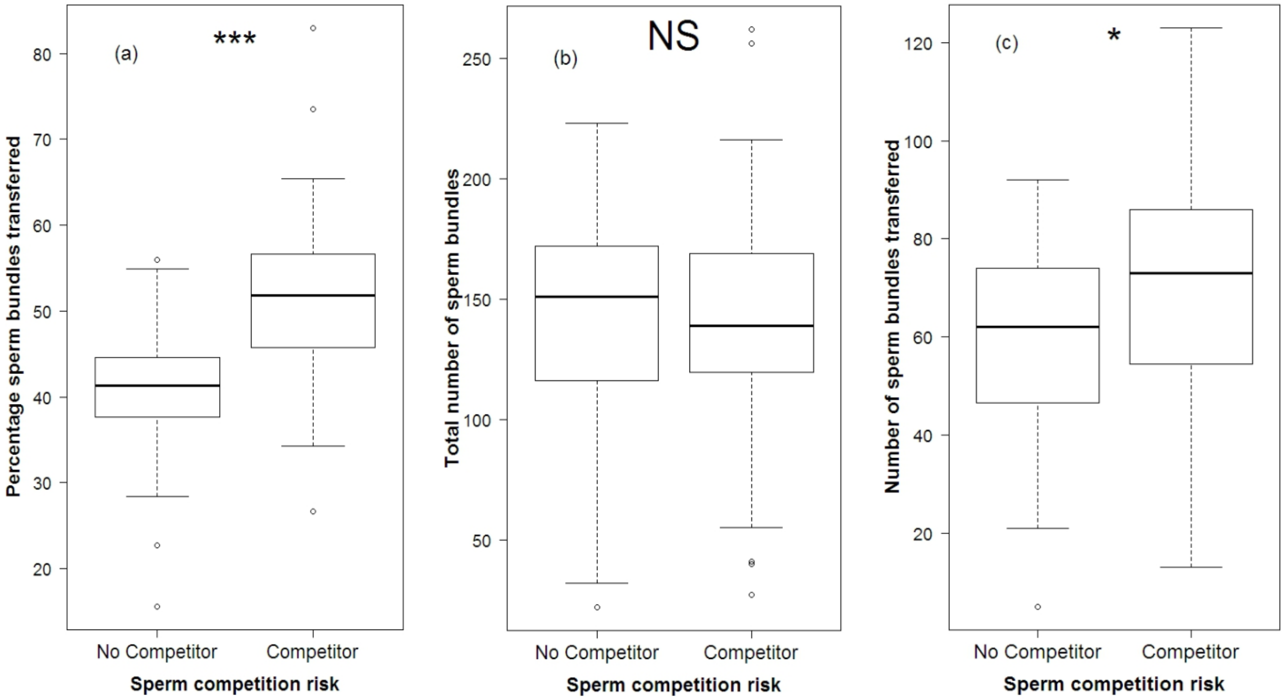

Figure 2

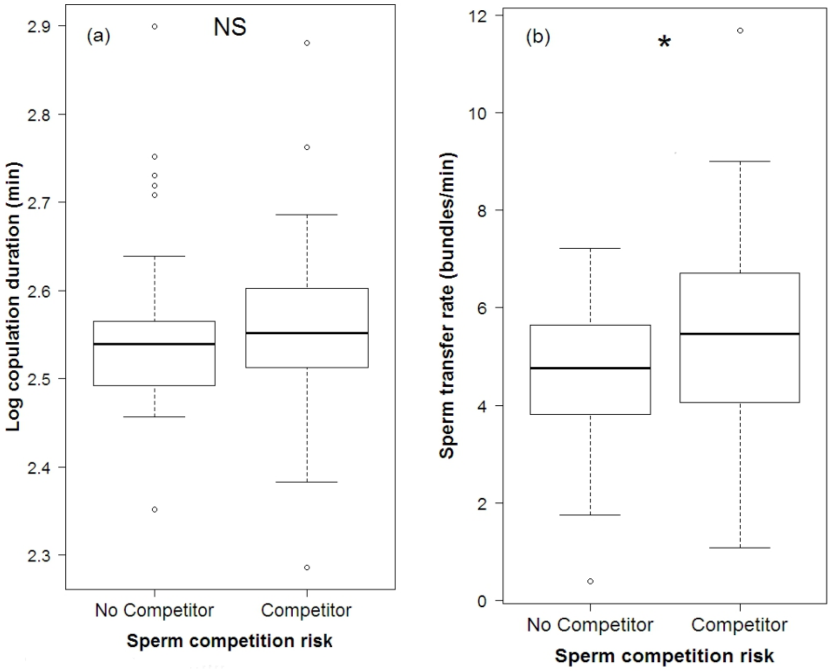

Figure 1. Sperm expenditure of male lesser wax moths, *A. grisella*, in relation to experimentally manipulated sperm competition risk (competitor present: $N = 51$; control: $N = 48$). (a) Percentage of eupyrene sperm bundles transferred to females during virgin copulations, (b) total number of sperm bundles available and (c) number of sperm bundles transferred to females. Plots show medians, interquartile ranges (box), data within 1.5 times the interquartile ranges (whiskers), and dots data outside this range. Asterisks denote significance level (*$p < 0.05$, **$p < 0.01$,***$p < 0.001$).

Figure 2. Copulation behaviour of male lesser wax moths, *A. grisella,* in relation to experimentally manipulated sperm competition risk. a) Copulation duration (competitor present: $N = 49$; control: $N = 43$) and b) sperm transfer rate ($N = 47$; $N = 40$). Plots show medians, interquartile ranges (box), data within 1.5 times the interquartile ranges (whiskers), and dots data outside this range. Asterisks denote significance level (*$p < 0.05$, **$p < 0.01$,***$p < 0.001$).

DISCUSSION

Lekking species are characterized by an intense female directional preference for male precopulatory ornamental traits, such as conspicuous coloration, vocalizations or displays (Kirkpatrick & Ryan 1991). Thus, male variance in mating success appears to be highly skewed as a few males often monopolize females (Kirkpatrick & Ryan 1991; Kokko et al. 1999). Besides, females are generally assumed to remate rarely (if at all) because they are largely unconstrained in exerting female mate choice as informed by pre-copulatory cues of male quality and because direct (material) benefits of mate choice are generally absent (Avery 1984; Birkhead et al. 1987). Therefore, studies investigating lek mating systems have essentially focused on male and female precopulatory reproductive traits. However, recent emphasis on female mating behaviour led to the suggestion that polyandrous mating could be more common in lekking species than previously assumed (Petrie et al. 1992; Lank et al. 2002; Hess et al. 2012; Engqvist et al. 2014). For example, in the ruff, *Philomachus pugnax*, female commonly copulate with multiple males, resulting in 59 % of the clutches sired by more than one male (Lank et al. 2002). Thus, in polyandrous lekking species, mechanisms of post-copulatory sexual selection might play a significant role for male reproductive success, but this has so far received only little attention (Leftwich et al. 2012; Sardell & DuVal 2014). In the present study, we investigated sperm allocation of males according to the presence/absence of a competitor in the lekking moth *A. grisella*.

We demonstrate that male wax moths respond to the presence of a competitor during early adult life by allocating a larger proportion of available eupyrene sperm reserves to virgin copulations when compared to control males experiencing a non-competitive social environment (Figure 1a; Table 1). We interpret this behaviour as an adaptive response to perceived sperm competition risk, which is in line with predictions from sperm competition theory (Parker et al. 1996; Parker et al. 1997) and with empirical evidence across a wide range of taxa showing strategic sperm allocation in response to variation in sperm competition risk or intensity (Kelly & Jennions 2011). The 24% difference in eupyrene sperm allocation documented here integrates well into recent findings suggesting a moderate female remating rate of approximately 20% in *A.*

grisella, which corresponds to an overall sperm competition risk of roughly 30% (Engqvist et al. 2014).

However, our results contrast with established conceptions of lek mating systems and, to the best of our knowledge, represent the first report of strategic sperm allocation in response to sperm competition risk in a lekking insect. Together with earlier, more indirect evidence (Cordes, Yigit, Engqvist, & Schmoll, 2013), these results suggest an important role for sperm competition in shaping male mating behaviour of *A. grisella* with potentially interesting implications for the interaction of pre- *versus* post-copulatory episodes of sexual selection in this species. For example, female remating propensity may vary with the degree of sperm depletion of a female's first mating partner (discussed in Engqvist et al. 2014) and males presented daily with a new virgin female mated up to 13 times, and the number of sperm bundles sharply decreased over successive copulations (Jarrige, pers. communication, 2014). Thus it is precisely the males with the most attractive ornamental traits (ultrasonic songs in the case of *A. grisella*) that would not only suffer most from sperm depletion due to their greater success in lek-based pre-copulatory mate choice but also from the highest risk of sperm competition if females choosing those males are more likely to remate. A similar situation has been documented in parasitoids, where the most successful males are the most susceptible to be sperm depleted (Boivin 2012; Boulton, Collins, & Shuker, 2015). The long term maintenance of additive genetic variance in lekking species, also referred to as the "paradox of the lek", has been one of the most puzzling problems in evolutionary biology over the past decades (Taylor and Williams 1982; Kirkpatrick and Ryan 1991). Indeed, strong female selection for "good genes" should erode the genetic variability among males, together with benefits of female choice. In this respect, permanent or temporary sperm depletion of the most attractive males could promote alternative routes to equal fitness and thus support the maintenance of genetic variance among lekking males.

Previous studies demonstrated that *A. grisella* males experiencing rivalry raise their signal rate (Jia et al. 2001; Alem, Clanet, Party, Dixsaut, & Greenfield, 2015) and thus invest more heavily in their costly calling songs representing the first sequential step on which the male reproductive success depends (Reinhold, Greenfield, Jang, & Broce, 1998). From the present study, it appears that in lekking species where females mate multiply, investment into sperm competitiveness might also be highly important for male paternity share and thus fitness. Under natural conditions, the intensity of pre-copulatory competition for mate attraction and risk of post-copulatory (i.e. sperm) competition are likely to covary. Thus, resource allocation trade-offs would be of central importance for male overall reproductive success, even in synspermatogenic species that produce sperm along adult life (Boivin, Jacob, & Damiens, 2005). Such trade-offs have been demonstrated in non-lekking species such as the domestic fowl, *Gallus gallus domesticus* where sperm quality declines in males that dominate competitive interactions with other males (Pizzari, Cornwallis, & Froman, 2007). Examining the dynamics of allocation between somatic maintenance, investment in pre-copulatory expenditure to acquire mates, ejaculate expenditure (sperm number and/or quality) as well as males chances of paternity under differential environmental conditions would therefore represents worthwhile future studies.

Instead of adjusting relative ejaculate size to perceived sperm competition risk, males could also have speeded up spermiogenesis (i.e. sperm maturation) during exposure to a competitor in order to have a higher number of eupyrene sperm bundles available during their virgin copulations. However, we found no significant difference between treatment levels in the total number of eupyrene sperm bundles (the sum of bundles transferred to the female during virgin copulation plus the number of bundles retained within the male) (Figure 1b, Table 1). This indicates that the experimental treatment did not trigger a differential response in spermiogenesis (note that eupyrene spermatogenesis irreversibly terminates during pupation and differential total eupyrene sperm production therefore cannot explain the differences between treatment levels in our experiment (Friedländer 1997; Friedländer et al. 2005).

An alternative hypothesis for the increased sperm allocation of males facing one competitor would be that males might have perceived the local sex ratio as highly biased towards males, and thus a low availability of virgin females in the environment. Under this scenario, males would adjust their sperm expenditure according to the likelihood to attract future mates and maximize their investment when future mating opportunities appear to be low. In the present study, however, this hypothesis appear less plausible, because males in both treatments eventually experienced the same female encounter rate, and we would have expected a similar response in control males just by being deprived of females.

The proximate mechanisms allowing the transfer of a relatively larger ejaculate under perceived sperm competition risk were not the main focus of our study, but our analysis of copulation behaviour does allow some inference. Although previous work has demonstrated a positive (although weak) relationship between copulation duration and the number of eupyrene sperm bundles transferred (Cordes et al. 2013), this pattern was absent in our study and we also found no difference in copulation durations between treatment levels (Figure 2; Table 1). Instead of copulating longer, males facing a competitor appear to transfer more eupyrene sperm bundles per copulation time unit in order to transfer a larger ejaculate during their virgin copulations. In our experiment, males facing a competitor had approximately 72 hours to perceive the presence (and maybe assess the quality) of a competitor and to prepare a differential allocation decision with respect to eupyrene sperm expenditure. Varying the duration and timing of exposure to a competitor may represent useful future experiments to shed more light on the proximate mechanisms that allow focal males to transfer the observed larger proportion of available eupyrene sperm bundles during virgin copulations under sperm competition risk.

In the present study, males were submitted to zero *versus* one rival to avoid possibly confounding effects with sperm competition intensity and allow unequivocal predictions (a decrease in sperm expenditure is predicted in competitive situations involving more than one competitor, see Engqvist & Reinhold 2005). While our experimental set-up may not reflect the most commonly encountered situation under natural conditions, our results clearly indicate that mechanisms for ejaculate size adjustment exist in *A. grisella,* and further investigations of sperm expenditure involving more than a single rival would be valuable to better understand male strategies under complex selective pressure as those existing in lek mating systems.

To conclude, our results provide strong evidence that sperm competition can select for male adaptations to sperm competition in male mating behaviour and strategic sperm allocation in lekking species. In addition to previous evidence of male mate choice and female-female competition on leks (Saether et al. 2001; Werner & Lotem 2003, 2006), our result demonstrate that an apparently low female remating rate can induce a risk of sperm competition sufficient to trigger adjustment in male sperm expenditure also in lekking species. Sperm competition might have been underappreciated in lek mating systems, and the study of potential trade-offs between traits under pre-copulatory *versus* post-copulatory sexual selection clearly deserves further attention.

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APPENDIX:

Table A1.

	Control	Competitor
Male emergence mass (mg)	11.54 ± 2.54; *N* = 55	11.83 ± 2.26; *N* = 58
Male mating mass (mg)	9.35 ± 2.21; *N* = 55	9.52 ± 1.85; *N* = 58
Male mass loss (mg)	2.18 ± 0.60; *N* = 55	2.30 ± 0.62; *N* = 58
Female mating mass (mg)	25.63 ± 4.44; *N* = 49	24.51 ± 4.46; *N* = 58
Copulation duration (min)	12.65 ± 1.83; *N* = 44	12.92 ± 1.25; *N* = 49
Number of bundles transferred to females	58 ± 22; *N* = 50	69 ± 25; *N* = 51
Percentage bundles transferred to females	40 ± 9; *N* = 49	51 ± 10; *N* = 50
Total number of bundles available	142 ± 45; *N* = 49	138 ± 52; *N* = 50

Table A1. Ingredients constituting the standard rearing diet of *A. grisella.*

ARTICLE 2

Males of a lek-mating insect intensify pre-copulatory mate guarding but not sperm expenditure under male competition

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ABSTRACT

If, in many species, pre-copulatory mate guarding has evolved as a response to the limited availability of female gametes, in the lek-mating lesser wax moth, *Achroia grisella*, such behaviour would result from a constraint on the release of male ejaculate. After a recent mating, males monopolize a female until they are physiologically able to transfer a new spermatophore. In the present study, we investigated how perceived male competition and female mass affected pre-copulatory mate guarding as well as eupyrene sperm allocation in this species. In a first experiment, both males and females of a pair were either exposed to the playback of a courtship song (competitive environment) or to a silent environment (control) prior to and during the second mating of the male with a virgin female of various body mass. To disentangle male *versus* female contribution in the observed behaviours, we conducted a second experiment where either the male or the female was exposed to the playback. In both experiments, pairs involving males exposed to playback showed shorter mating latencies and longer copulation durations compared to control level pairs, while the sum of both durations was statistically indistinguishable between groups. We found no difference between treatment levels or according to female size in the number or proportion of sperm transferred during second copulations. Differential mating latencies in both experiments thus demonstrate a male – not female – behavioural response to perceived male competition. Similar total durations across treatment levels suggest similar spermatophore production times and the differences in copulation durations may simply represent a by-product of differential mating latencies. Together, these results suggest that plastic pre-copulatory mate-guarding may be adaptive as it allows males to maximise their reproductive success by securing mates under intense male competition and simultaneous sperm depletion, a context particularly virulent in lek-mating systems.

KEYWORDS

Achroia grisella, competitive environment, lek mating system, Lepidoptera, mate guarding, precopulatory sexual selection, strategic ejaculation.

INTRODUCTION

Until recently, it was assumed that male reproductive costs were trivial and solely consisted in the production of cheap and unlimited sperm (Bateman 1948, Dawkins 1976). This conception has since been challenged and it is now well established that reproduction is limited for males and induces substantial costs (Schneider and Lubin 1998, Wedell et al. 2002, Perry et al. 2013, Scharf et al. 2013). Sperm, as well as other ejaculate components, are often constrained in quantity, quality, and timing of production (Damiens and Boivin 2005, Perry and Rowe 2008, Perry et al. 2013). Besides, courtship behaviours and fights with other males can result in important energy losses, increased injuries and/or predation risk, and copulation itself can be associated with the transmission of pathogens (Knell and Webberley 2004, Stjernholm and Karlsson 2006, Benesh et al. 2007). In response, complex mechanisms have evolved allowing males to allocate optimally their finite reproductive resources and thereby maximize their lifetime reproductive success (Wedell et al. 2002, Edward and Chapman 2011).

In particular, an extensive body of literature has examined how males adjust their mating behaviours by investing in mate guarding strategies, by which males prevent females from copulating with other males. Post-copulatory mate guarding takes place after female insemination and is thought to be one of the most reliable defences against sperm competition (Parker 1970, Alcock 1994, Simmons 2001, Elias et al. 2014). Conversely, pre-copulatory mate guarding consists of relatively long and intimate associations between the two sexes prior to sperm transfer (Ridley 1983, Jormalainen 1998). Early game theoretic models predicted the evolution of pre-copulatory mate guarding in situations where female encounter rate is low or unpredictable, and when female receptivity is restricted to a brief and predictable time window (Parker 1970, Ridley 1983). Pre-copulatory mate guarding behaviour could also be advantageous in polyandrous species with pronounced first male sperm precedence (Parker 1974): it is particularly common in crustaceans, where males guard immature females until these latter ones become sexually receptive (Ridley 1983, Jormalainen 1998). Analogous behaviours have been documented in amphibians (Bowcock et al. 2008), spiders (Bel-Venner and Venner 2006, Hoefler 2007, Elias et al. 2014), rotifers (Schröder 2003) and insects (Arakaki et al. 2004, Parker and Vahed 2010, Bennett et al. 2012).

Other empirical evidence suggests that pre-copulatory guarding behaviour can also result from male reproductive constraints; however, this unusual behaviour has so far received only little attention (Greenfield and Coffelt 1983, Parker and Vahed 2010). For example, temporary depletion of ejaculate materials (i.e. sperm and other ejaculatory substances) can result in obligatory sexual refractory periods between two successive copulations, which may last from a few minutes to several days (Gwynne 2001, Wedell et al. 2002, Vahed 2007). Under male-biased operational sex ratio or stochastic female encounter rate, mating opportunities can be rare and/or unpredictable. Thus, males encountering, during their refractory period, a receptive mate could increase their reproductive success by guarding it until they are ready to transfer an ejaculate again, rather than losing a mating opportunity, as observed in *Gryllus bimaculatus* (Parker and Vahed 2010).

The rate of successful matings can also be strongly influenced by male competition and female mate choice (Andersson 1994). In lek-mating species, for instance, males gather in groups to display secondary sexual traits (plumage, vocalizations) which are the target of strong directional female preferences (Darwin 1872, Kirkpatrick and Ryan 1991, Höglund and Alatalo 1995). In these highly competitive systems, mate attraction can be strongly biased towards a few males (Höglund and Alatalo 1995). Therefore, any male refractory periods would directly lead to the loss of mating opportunities for those most attractive males, and such a pressure may favour the evolution of pre-copulatory mate guarding. This behaviour could also and concomitantly favour males during post-copulatory episode of sexual selection. For example, extended guarding duration could allow males to adjust their ejaculate expenditure according to the risk of sperm competition, and maximise the male chances of paternity if the transfer of larger ejaculates increases the male fertilisation success or reduce the female propensity to remate (Parker 1974, Simmons 2001).

Despite the above benefits, pre-copulatory mate guarding can be costly in terms of energy loss, injury, or loss of mating opportunities as males involved in guarding cannot attract or approach other mates (Grafen and Ridley 1983, Jormalainen et al. 2001, Komdeur 2001, Plaistow et al. 2003, Benesh et al. 2007). Thus, male investment in guarding should be adjusted according to various factors affecting its pay-off, such as the number and quality of females currently available or the presence and density of rival males (Rondeau and Sainte-Marie 2001, Oku 2009). Here, we investigated the effect of female size and the perception of male competitors on pre-copulatory mate guarding in the lesser wax moth *Achroia grisella* (Lepidoptera: Pyralidae) males.

In this species, males gather in leks and display ultrasonic courtship songs to attract females (see Material and Methods). Upon female approach, male readily attempt to cease her into copula. Copulations involving virgin males usually take ten to 20 minutes (Greenfield and Coffelt 1983). In contrast, males that have mated recently (within an hour) remained in copula for extended periods of time (up to 7h in the Florida population, Greenfield and Coffelt 1983). This prolonged copulation duration would reflect the time necessary for the male to produce a new ejaculate, as the transfer of the spermatophore only occurs during the last 20 minutes of the pairing (Greenfield and Coffelt 1983). Surprisingly, males resume singing shortly after mating and are therefore able to attract females within half an hour (i.e. long before the end of their reproductive refractory period).

To study how male competition and female size affects pre-copulatory mate guarding behaviour in *A. grisella,* we manipulated the male perceived competitive environment by either exposing them to the playback of a male's ultrasonic courtship songs (competitive environment) or a silent environment (control), prior to and during a second mating with a virgin female of various sizes. We predicted that males experiencing cues indicative of competition increase the frequency and/or intensity of guarding behaviours (measured by the latency and duration of the pre-copulatory guarding). A similar pattern is expected when males encounter larger, more fecund females (Honek 1993). In addition, in order to determine whether pre-copulatory mate guarding could concomitantly allow males to increase their competitiveness in case of sperm competition, we measured the number and proportion of fertilizing (eupyrene) sperm transferred in the two groups. Finally, to disentangle male *versus* female contribution in the observed behaviours, we conducted a second experiment similar to the first one except that only the recently mated male or the virgin female was exposed to male song playback.

MATERIAL AND METHODS

Study species

The lesser wax moth *A. grisella* is a symbiont of the western honeybee *Apis mellifera,* in whose colonies the larvae feed on wax, stored pollen, honey and brood (Kunike 1930). Adults possess atrophied mouthparts and neither feed nor drink. Resources for somatic maintenance and reproduction are thus entirely acquired during development (Greenfield and Coffelt 1983). Adults have a short lifespan: in the laboratory, females live for ten to 14 days while male lifespan averages seven days (Greenfield and Coffelt 1983, Ligout et al. 2012). For six to ten hours per night, males gather in small leks and advertise to females with ultrasonic courtship calls. Males produce such songs by fanning their wings while remaining stationary on the substrate. Receptive females in the vicinity orient and run towards singing males (Greenfield and Coffelt 1983, Spangler et al. 1984). Laboratory playback experiments and choice tests revealed that a male's attractiveness and mating success relies mainly on three song characters: amplitude, pulse-pair rate, and the length of silent gaps within pulse pairs (Jang and Greenfield 1996, Jang and Greenfield 1998, Limousin and Greenfield 2009). Males also respond to surrounding males by temporarily accelerating the pulse pair rate of their song (Jia and Greenfield 1997).

Stock population

We used individuals from a stock population of *A. grisella* derived from several hundred individuals collected in the Departement of Indre et Loire, France, in October 2007. The population was bred in the laboratory using a protocol of randomized pairing of males and females in order to prevent close inbreeding (fullsib matings), maintain a large population size and minimise the loss of genetic variation. To do so, every one to two weeks, 20 to 25 virgin females originating from different clutches were randomly paired with the same number of non-full-sibling virgin males emerging from different clutches.

Moths were reared in an environmental chamber at 24 ± 2°C and 45% relative humidity under a 12:12 light:dark photoperiod. Larvae were cultured on a standard *ad libitum* diet containing wheat, corn and rye flours, water, glycerol, nutritional yeast, honey and beeswax

(Dutky 1962, see also Jarrige et al. 2015). These laboratory conditions closely mimic natural conditions for this species. Under these conditions, generation times as measured from oviposition to oviposition range from 45 to 55 days. To ensure virginity and standardized social conditions, pupae were kept individually in 28-ml plastic cups, where they emerged. For the experiments described below, we only used virgin males within the 16 hours following their emergence, and virgin females between 0 to 48h old.

Experimental design

On the day of experimental matings, males and females were weighed to a precision of 0.1 mg with a AX105 DeltaRange balance (Mettler Toledo, Columbus, USA). Males were subsequently transferred in small net cages (1.5 cm diameter, 2 cm height; see Jia et al. 2001) and placed in individual acoustic foam alcoves that kept them acoustically insulated from each other. Females were kept individually in an acoustically confined area where they could not perceive male songs. Males were allocated at random to treatment levels and males of different treatment levels were tested in an alternating fashion. Experiments took place in an acoustically insulated room at 24 ± 2 °C during the initial 6h of the scotophase, which corresponds to the period where most matings occur in nature (Greenfield and Coffelt 1983).

Male first copulation: In both experiments, after a 30 minute acclimatization period in the experimental room, males were mated within a silent environment to a first virgin female of standardized body mass (> 26 and < 33 mg). The duration of this first copulation was measured with a stopwatch.

Experiment A: Immediately following the natural termination of the first copulation, the female was removed and males were either exposed to a courtship song playback (competitive social environment) or a silent environment (control). Song playbacks were broadcasted with one loudspeaker placed at 1 m from the males (see below for details). After 30 minutes, a second virgin female was introduced in the male's net cage. To test the effect of female size on males' mating behaviour, experimental females were taken from a wider range of body mass: between 11 and 43 mg. The treatment conditions (playback of a song or not) were maintained during this second mating. Male mating behaviour, measured as the latency to copula (time between the introduction of the second female and the beginning of the copulation) and the duration of the second copulation were monitored to a precision of 5 minutes.

Experiment B: To determine whether any difference in mating behaviour or sperm expenditure in relation to the perceived social environment was a response of the male and/or the female, we conducted a further experiment in which only one of the partners was exposed to courtship song playback. Thus immediately after the first copulation, either the recently mated male or the second virgin female (body mass comprised between 17 and 32 mg) were exposed to the courtship song playback for 30 minutes. Subsequently, the female was introduced in the male cage and the pair remained within a silent environment. Mating latency and copulation duration were monitored as described in experiment A.

Courtship song playbacks

We created playback stimuli from a recorded song that had average properties for the population (see Limousin and Greenfield 2009 for details). We used BatSound 4.0 (Pettersson Elektronik, Uppsala, Sweden) to digitally repeat the song resulting in a 180 seconds sound file. During the experiment, we constantly looped this sound file on a personal computer, converted the digital signal with an input output card (DAQcard 6062E, National Instruments, Austin Texas USA), and sent the analogue signal at 214.285 samples·s−1 to the loudspeaker (model UltraSoundGate Player BL/Pro 35 70115, Avisoft Bioacoustics, Berlin, Germany) amplified by a mono-channel power amplifier (Avisoft Bioacoustics). The peak amplitude of the loudspeaker was adjusted to 80 dB peSPL (peak equivalent Sound Pressure Level) by measuring the loudest pulse of the stimulus signal at the centre of the arena using a sound pressure level (SPL) meter (model CEL-430/2, Casella, Kempston, UK). This peSPL value was chosen to mimic the song of a male *A. grisella* displaying in a distance of 7-10 cm (Limousin and Greenfield 2009). The method of peak equivalent was implemented by relating the millivolt output of a continuous 20 kHz broadcast, as measured by a condenser ultrasound microphone (model CM16/CMPA, Avisoft Bioacoustics) to the SPL of the 20 kHz broadcast as registered by the SPL meter. We then noted the millivolt output of the male synthetic song broadcasted as measured by the microphone, and we adjusted the gain on the amplifier until this millivolt output was equivalent to 80 dB peSPL (see Jang and Greenfield 1996).

Dissection of experimental animals and eupyrene sperm bundle counts

Immediately after termination of copulations, females were frozen at -80°C in 1.5 ml Eppendorf tubes until dissection. Similarly, males were stored at -80°C immediately after their second copulation. Prior to dissections, individuals were defrosted at ambient temperature for three minutes, and the number of eupyrene sperm bundles was then counted as described below:

Number of bundles transferred to the female:

To count the number of eupyrene sperm bundles transferred to females, each female was ventrally opened to remove the bursa copulatrix. After successful copulation, this organ contains a spermatophore which consists of a seminal sac filled with solitary apyrene sperm and eupyrene sperm aggregated in bundles of 256 spermatozoa (Fernandez-Winckler and da Cruz-Landim 2008). The spermatophore was carefully taken out and placed in a drop of distilled water on a microscopic slide. It was then ruptured with a fine needle tip to release the bundles and allow their full dispersion. After gentle homogenization, eupyrene sperm bundles were counted under dark phase microscopy at 500 x magnification.

Number of bundles remaining in the male genital tract:

To count eupyrene sperm bundles remaining in the male genital tract after the second copulation, males were ventrally opened and the whole reproductive tract was removed (see Jarrige et al. 2015 for a detailed description of its structure) and transferred into a drop of distilled water on a microscopic slide. The different parts of the reproductive tract were carefully isolated, such that testes, seminal vesicles and duplex were placed in separate drops of distilled water. After being ruptured, their contents were gently stirred with a needle tip for homogenization. Eupyrene bundles were then counted under dark phase microscopy at 500 x magnification.

The total number of sperm bundles available at the time of the first copulation was calculated by summing the number of bundles transferred to first and second females with the number of bundles remaining in the male genital tract. For the number of bundles available at the second mating, only the number of bundles transferred to the second females and those remaining in the male tract were summed. The proportion of eupyrene sperm bundles transferred at a particular mating was obtained by dividing the number of bundles transferred at that mating by the total number of spermatozoa available at that time.

Ethical Note

We used 255 individuals in this study: 171 females and 85 males. All individuals were handled with care and handling time was kept to an absolute minimum.

Statistical analysis

We used linear models to test for the effects of courtship song playback on mating behaviour and sperm expenditure. As explanatory effects we included as two-level factorial variables i) the playback of a courtship song to experimental pairs (no/yes) prior to and during male second matings for experiment A or alternatively ii) the song playback broadcasted prior to the male second matings to either a pair's male or female partner for experiment B. We included male mass as a continuous covariate in all models to control for potentially confounding effects of male body size. We also included female mass as a continuous covariate in all models to test for potential effects of female quality on mating behaviour and sperm expenditure. Male and female body mass were mean-centred to obtain biologically meaningful intercept estimates. In order to compare effect sizes between experiments A and B, we tested for the significance of the interaction term between experiment and treatment considering equivalent the treatment levels *pairs not exposed* to playback (experiment A control level) and *females exposed* (experiment B control level) as well as *pairs exposed* (experiment A treatment level) and *males exposed* (experiment B treatment level). In addition, we used Cohen's *d* as implemented in the R package *effsize* (version 0.5.4, Torchiano 2015) to obtain standardized effect sizes for experiments A and B including their 95% confidence intervals. Kolmogoroff-Smirnoff tests on the residuals of our linear models indicated in no case a significant deviation from a normal distribution and respective dependent variables were therefore modelled on their original scale. In addition, we investigated male remating rate using Generalized Mixed Models (GLM, function *glm*) with a Binomial family, and Pearson's product moment correlations to analyse the relationships between male first and male second copulation durations and between behavioural traits and sperm expenditure. All models were fitted in R 3.2.0. All statistical tests were two-tailed and we rejected the null hypothesis at $p < 0.05$.

RESULTS

For both experiments, mating behaviour and sperm expenditure of males at their first mating are given in supplementary online material (OSM, see supplementary results and Tables S2, S3).

Male and female body mass

There were no differences between treatment levels in male or female mass with the exception of female mass at male second mating in experiment A (see Table S1 in OSM).

Experiment A: Pair exposed or not exposed to playback prior to and during male second mating

Mating behaviour

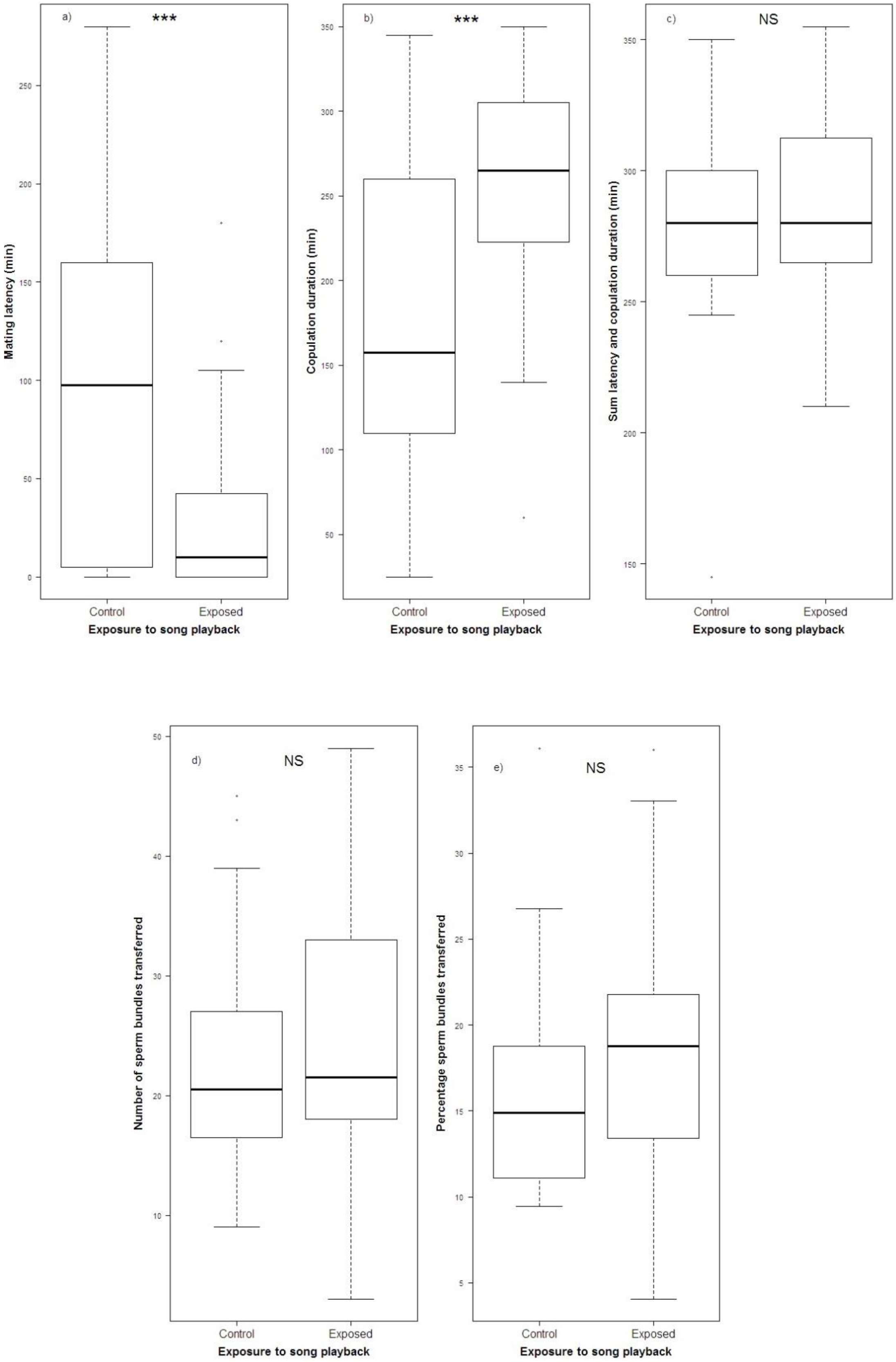
Overall, 49 out of 56 (87.5%) males to which a female was offered shortly after their first copulation engaged in a second copula but there were no differences between treatment levels ($G_1 = 42.82$, $p = 0.24$). Overall, males' second copulations were on average more than 20 times longer than their first copulations (paired $t = 17.1$, $df = 48$, $p < 0.001$). There was no correlation between males' first and second copulation durations (Pearson $r = -0.05$, $t = -3.04$, $df = 47$, $p = 0.74$). Mean mating latencies to second copulations were roughly one hour and a half shorter for pairs exposed to playback compared to control pairs (Table 1a, Figure 1a). They were unaffected by male body mass but were shorter when males mated with larger females (Table 1a). Furthermore, mean second copulation durations were roughly one hour and a half longer for exposed pairs compared to control pairs (Table 1a, Figure 1b), resulting in similar sums of mating latency and copulation duration for second mating across treatment levels (Table 1a, Figure 1c).

Table 1

		(a) Experiment A					**(b) Experiment B**				
Male second mating		**Estimate**	**SE**	**t**	**df**	**p**	**Estimate**	**SE**	**t**	**df**	**p**
Mating latency (min)											
	Intercept	107.58	14.95	–	–	–	166.67	23.29	–	–	–
	Treatment	-84.50	20.84	-4.05	1	**<0.001**	-137.78	32.60	-4.23	1	**<0.001**
	Male mass	0.25	3.60	0.07	1	0.94	5.50	4.61	1.19	1	0.25
	Female mass	-2.90	1.39	-2.08	1	**0.04**	2.31	1.97	1.17	1	0.26
Copulation duration (min)											
	Intercept	171.50	17.35	–	–	–	120.08	26.99	–	–	–
	Treatment	89.87	24.18	3.72	1	**<0.001**	117.34	37.78	3.11	1	**0.006**
	Male mass	0.64	4.17	0.15	1	0.88	-3.85	5.34	-0.72	1	0.48
	Female mass	2.52	1.62	1.56	1	0.13	0.14	2.28	0.06	1	0.95
Sum latency and duration (min)											
	Intercept	279.08	8.64	–	–	–	286.75	15.56	–	–	–
	Treatment	5.37	12.05	0.45	1	0.66	-20.44	21.78	-0.94	1	0.36
	Male mass	0.89	2.08	0.43	1	0.67	1.65	3.08	0.54	1	0.60
	Female mass	-0.38	0.80	-0.47	1	0.64	2.45	1.32	1.86	1	0.08
Number of bundles available											
	Intercept	144.2	11.68	–	–	–	161.88	12.31	–	–	–
	Treatment	-2.33	16.3	-0.14	1	0.89	-18.08	18.47	-0.98	1	0.34
	Male mass	5.15	2.65	1.94	1	0.06	9.07	2.46	3.68	1	**0.002**
	Female mass	0.18	1.07	0.17	1	0.87	-0.54	1.08	-0.50	1	0.62
Number of bundles transferred											
	Intercept	22.76	2.69	–	–	–	28.25	3.79	–	–	–
	Treatment	2.9	3.75	0.77	1	0.44	-6.55	5.69	-1.51	1	0.27
	Male mass	0.28	0.61	0.46	1	0.65	0.29	0.76	0.38	1	0.70
	Female mass	-0.05	0.25	-0.20	1	0.84	-0.37	0.33	-1.1	1	0.29
Proportion of bundles transferred											
	Intercept	16.31	9.70	–	–	–	18.84	2.31	–	–	–
	Treatment	2.31	2.35	0.99	1	0.33	-2.95	3.45	-0.85	1	0.41
	Male mass	-0.37	0.38	-0.97	1	0.34	-1.22	0.46	-2.64	1	**0.02**
	Female mass	-0.08	0.15	-0.55	1	0.59	-0.17	0.20	-0.82	1	0.43
Delta proportion of bundles transferred											
	Intercept	8.68	1.51	–	–	–	5.67	1.62	–	–	–
	Treatment	-3.82	2.08	-1.84	1	0.07	1.55	2.42	0.64	1	0.53
	Male mass	0.31	0.35	0.88	1	0.38	1.02	0.32	3.16	1	**0.006**
	Female mass	-0.08	0.12	-0.63	1	0.53	-0.37	0.15	-2.54	1	**0.02**

Table 1. Results from linear models analysing the effects of *A. grisella* courtship song playback on mating behaviour and sperm expenditure at second mating for (a) experiment A, where experimental pairs were exposed to playback prior to and during male second mating, and (b) experiment B, where either a pair's male or female partner was exposed to playback prior to male second mating. Note that male and female body mass are mean-centred and the intercept estimates represent the predicted effects for males of average mass from the control treatment level (experiment A) or for males of average mass from the treatment level where females had been exposed to playback (experiment B), respectively, paired to females of average mass in both cases. Significant results are highlighted in bold.

Figure 1



a)
***
Mating latency (min)
0
50
100
150
200
250
Control
Exposed
Exposure to song playback
b)
***
Copulation duration (min)
50
100
150
200
250
300
350
Control
Exposed
Exposure to song playback
c)
NS
Sum latency and copulation duration (min)
150
200
250
300
350
Control
Exposed
Exposure to song playback
d)
NS
Number of sperm bundles transferred
10
20
30
40
50
Control
Exposed
Exposure to song playback
e)
NS
Percentage sperm bundles transferred
5
10
15
20
25
30
35
Control
Exposed
Exposure to song playback

Figure 1. Mating behaviour and sperm expenditure of lesser wax moths, *A. grisella,* in relation to perceived male competition in experiment A, where experimental pairs were exposed to playback of courtship song prior to and during male second mating. *Mating behaviour*: pair exposed N = 27; or not exposed to courtship song playback: N = 22. a) Latencies to forming male second copula, b) time in second copula and c) sum of latency to and time in second copula. *Sperm expenditure*: pair exposed N = 24; or not exposed N = 20. d) Number of sperm bundles transferred and e) percentage of sperm bundles transferred during second matings. Plots show medians, interquartile ranges (box) and data within 1.5 times the interquartile ranges (whiskers) and circles data outside this range. *** $P < 0.001$. NS = not significant.

Sperm expenditure

For 20 control and 24 treatment level replicates, eupyrene sperm bundles were successfully prepared from the females of both matings and from the male.

Across treatment levels , the number of bundles as well as the proportion of available bundles transferred decreased from first to second matings within individual males (Paired-*t* = -9.60, df = 43, $p < 0.001$ and Paired-*t* = -6.45, df = 43, $p < 0.001$ respectively, see Figure S3 in OSM). Indeed, males transferred only half the number of bundles (Figure S3a in OSM) and the percentage of available bundles transferred dropped to roughly two thirds in second compared to first matings (Figure S3b in OSM). Furthermore, the number as well as the proportion of sperm bundles transferred was positively correlated with males identity (Pearson tests, r = 0.63, t = 5.26, df = 42, $p < 0.001$ and r = 0.54, t = 4.12, df = 42, $p < 0.001$ respectively, Figures S3 c,d in OSM).

The number of eupyrene sperm bundles available to males in second matings was not influenced by treatment level or by female body mass (Table 1a, Figure S1a in OSM). Heavier males, however, showed a tendency to have a higher number of sperm bundles available (Table 1a). Neither the number of bundles (Table 1a, Figure 1d) nor the proportion of available sperm bundles transferred (Table 1a, Figure 1e) during second matings differed between treatment levels or according to male and female body mass. Furthermore, the within-male difference between first and second matings in the proportion of sperm bundles transferred was also statistically indistinguishable between treatment levels (Table 1a, Figure S1b in OSM).

There was no correlation between mating latency or copulation duration of second matings and the number or proportion of sperm bundles transferred during second matings (all $p > 0.1$).

Experiment B: Male or female partner exposed to playback prior to male second mating

Mating behaviour

Overall, 23 out of 24 males engaged in a second copula but there were no differences between treatment levels ($G_1 = 6.89$, $p = 0.57$). The males' second copulations were on average more than 17 times longer than their first copulations (paired t = 8.14, df = 22, $p < 0.001$). Furthermore, there was a significant positive correlation between the durations of males' first and second copulations (Pearson r = 0.50, t = 2.65, df = 21, p = 0.01). Mean mating latencies to male second copulations were more than two hours shorter for pairs where the male was exposed to playback compared to pairs where the female was exposed (Table 1b, Figure 2a). They were not affected by male and female body mass (Table 1b). The second copulation lasted roughly two hours longer for pairs where the male was exposed to playback than for pairs where the female was exposed (Table 1b, Figure 2b), resulting in similar sums of mating latency and copulation duration of second mating across treatment levels (Table 1b, Figure 2c). Male and female body mass did not affect the duration of second copulations but the sum of mating latency and copulation duration tended to be shorter when males were mated with larger females (Table 1b).

Figure 2

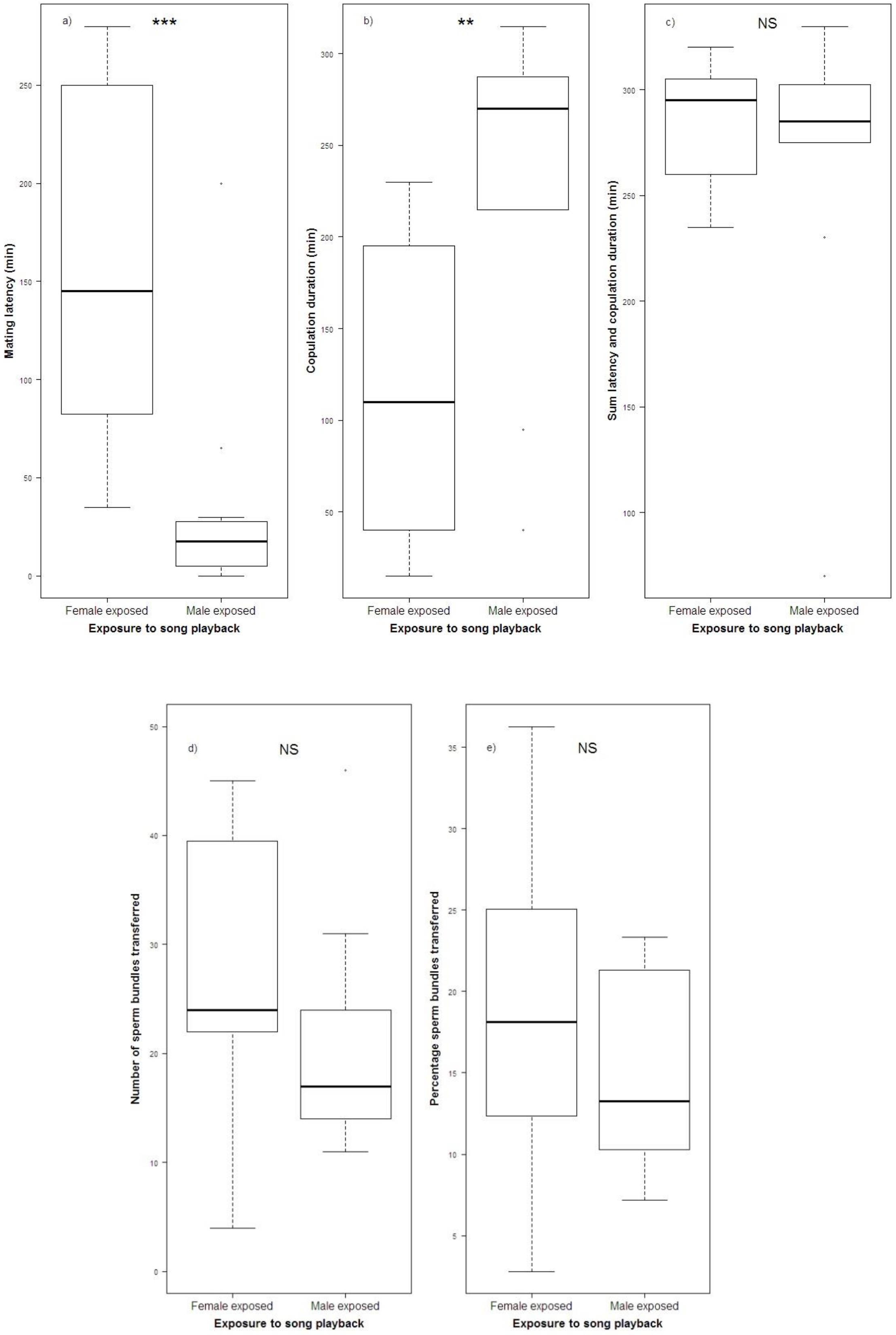

a)
***
Mating latency (min)
0
50
100
150
200
250
Female exposed
Male exposed
Exposure to song playback
b)
**
Copulation duration (min)
50
100
150
200
250
300
Female exposed
Male exposed
Exposure to song playback
c)
NS
Sum latency and copulation duration (min)
100
150
200
250
300
Female exposed
Male exposed
Exposure to song playback
d)
NS
Number of sperm bundles transferred
0
10
20
30
40
50
Female exposed
Male exposed
Exposure to song playback
e)
NS
Percentage sperm bundles transferred
5
10
15
20
25
30
35
Female exposed
Male exposed
Exposure to song playback

Figure 2. Mating behaviour and sperm expenditure of lesser wax moths, *A. grisella,* in relation to perceived male competition in experiment B, where males or females were exposed to playback of courtship song prior to male second mating. *Mating behaviour*: male exposed N = 12; or female exposed to courtship song playback prior to male second mating control N = 11). a) Latencies to forming male second copula, b) time in male second copula and c) sum of latency to and time in male second copula. *Sperm expenditure*: male exposed N = 9; or female exposed N = 11. d) Number of sperm bundles transferred and e) percentage of sperm bundles transferred during second matings. Plots show medians, interquartile ranges (box) and data within 1.5 times the interquartile ranges (whiskers) and circles data outside this range. ** $P < 0.01$; *** $P < 0.001$. NS = not significant.

Sperm expenditure

For eleven control and nine treatment level replicates, eupyrene sperm bundles were successfully prepared from the females of both matings and from the male.

Across treatment levels, the number of bundles as well as the proportion of available bundles transferred decreased from first to second matings within individual males (paired $t = -6.75$, $df = 19$, $p < 0.001$ and $t = -4.13$, $df = 19$, $p < 0.001$, see Figure S4 a,b in OSM). Furthermore, the number as well as the proportion of sperm bundles transferred was positively correlated within individual males (Pearson $r = 0.65$, $t = 3.67$, $df = 18$, $p = 0.002$ and $r = 0.61$, $t = 3.24$, $df = 18$, $p = 0.005$, see Figures S4 c,d in OSM).

Similarly, the number of eupyrene sperm bundles available to males in second matings was not affected by treatment level or by female body mass (Table 1b, Figure 2d). However, heavier males had significantly more sperm bundles available (Table 1b). Neither the number of bundles (Table 1b, Figure 2d) nor the proportion of available sperm bundles transferred (Table 1b, Figure 2e) during second copulations differed between treatment levels or according to female body weight. Furthermore, the within-male difference between first and second matings in the proportion of sperm bundles transferred was also statistically indistinguishable between treatment levels (Table 1b, Figure S2b in OSM). Heavier males, however, transferred a significantly smaller proportion of available sperm bundles and showed a significantly higher difference in the proportion of sperm bundles transferred between first and second matings (Table 1b).

There was no correlation between mating latency or copulation duration of second matings and the number or proportion of sperm bundles transferred during second copulations (all $p > 0.6$).

Comparison of effect sizes between experiment A and B

Controlling for the effects of male and female mass (Table 2), effect sizes for treatment effects on mating behaviour in experiment A and B were statistically indistinguishable for all dependent variables as indicated by non-significant experiment-by-treatment interaction terms (Table 2). Furthermore, the 95% confidence intervals of Cohen's *d* as a measure of standardised effect sizes overlapped widely for these variables (Figure 3). Both tests thus suggest that the effects of playback observed in experiment A are caused by a response of the pair's male and not the pair's female partner.

Controlling for the effects of male and female mass (Table 2), effect sizes for treatment effects on sperm expenditure in experiment A and B were statistically indistinguishable for the major dependent variables as indicated by non-significant experiment-by-treatment interaction terms (Table 2, see also Figures S3 and S4 in OSM). Furthermore, the 95% confidence intervals of Cohen's *d* as a measure of standardised effect sizes overlapped widely for these dependent variables (Figure S3 and S4 in OSM). Both tests thus suggest that the lack of effects of playback observed in experiment A are caused by a lack of response of the pair's male and not the pair's female partner.

Figure 3

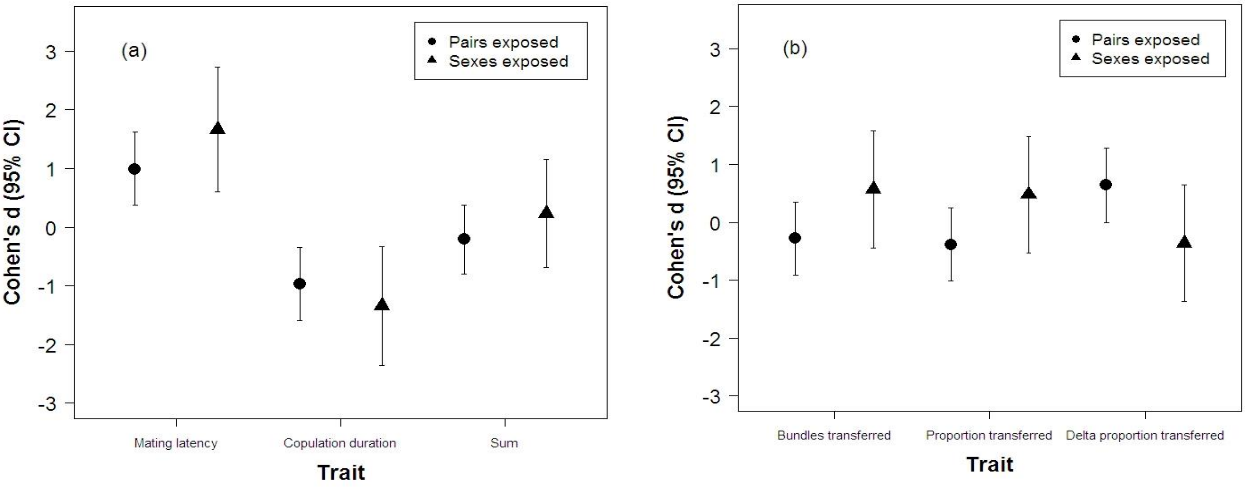


Figure 3. Comparison of standardised effect sizes of treatment effects between experiment A, where experimental pairs were exposed to courtship song playback prior to and during male second mating, and experiment B, where either a pair's male or female partner was exposed to playback prior to male second mating. Plot shows Cohen's *d* with 95% confidence intervals (CI) for a) mating behaviour and b) sperm expenditure.

Table 2

Male second mating	**Estimate**	**SE**	**t**	**df**	**p**
Mating latency (min)					
Intercept	100.42	15.65	–	–	–
Treatment	-73.37	21.64	-3.39	1	**0.001**
Experiment	62.32	27.08	2.30	1	**0.02**
Male mass	1.43	2.83	0.51	1	0.61
Female mass	-0.99	1.14	-0.87	1	0.39
Treatment x experiment	-52.66	37.72	-1.40	1	0.17
Copulation duration (min)					
Intercept	175.36	17.57	–	–	–
Treatment	85.08	24.30	3.50	1	**<0.001**
Experiment	-54.54	30.40	-1.79	1	0.08
Male mass	-0.84	3.18	-0.27	1	0.79
Female mass	1.72	1.28	1.34	1	0.18
Treatment x experiment	25.84	42.35	0.61	1	0.54
Sum latency and duration (min)					
Intercept	275.78	9.46	–	–	–
Treatment	11.71	13.09	0.90	1	0.37
Experiment	7.78	16.37	0.48	1	0.64
Male mass	0.59	1.71	0.35	1	0.73
Female mass	0.73	0.69	1.06	1	0.29
Treatment x experiment	-26.82	22.81	-1.18	1	0.24
Number of bundles transferred					
Intercept	22.58	2.64	–	–	–
Treatment	3.03	3.58	0.85	1	0.40
Experiment	6.11	4.48	1.37	1	0.18
Male mass	0.33	0.47	0.72	1	0.48
Female mass	-0.20	0.49	-0.40	1	0.69
Treatment x experiment	-10.05	6.45	1.56	1	0.13
Proportion of bundles transferred					
Intercept	16.48	1.67	–	–	–
Treatment	2.27	2.32	0.98	1	0.33
Experiment	2.25	2.79	0.81	1	0.42
Male mass	-0.73	0.29	-2.51	1	**0.02**
Female mass	-0.10	0.12	-0.8	1	0.43
Treatment x experiment	-5.69	4.08	-1.39	1	0.17
Delta proportion of bundles transferred					
Intercept	8.43	1.43	–	–	–
Treatment	-3.46	1.98	-1.75	1	0.09
Experiment	-2.84	2.41	-1.18	1	0.24
Male mass	0.58	0.25	2.32	1	**0.02**
Female mass	-0.16	0.10	-1.64	1	0.11
Treatment x experiment	5.46	3.49	1.57	1	0.12

Table 2. Results from linear models comparing the effect sizes of treatment effects from experiment A, where pairs were exposed to *A. grisella* courtship song playback prior to and during male second mating, and experiment B, where either a pair's male or female was exposed to playback prior to male second mating. Note that male and female body mass are mean-centred and the intercept estimates represent the predicted effects for males of average mass from the control level/the treatment level where females had been exposed to playback paired to females of average mass. Significant results are highlighted in bold.

DISCUSSION

The evolution of pre-copulatory mate guarding resulting from male reproductive constraints has yet received little interest, and pre-copulatory mate guarding has almost exclusively been studied in species where females are receptive for a short but predictable period of time (Grafen and Ridley 1983, but see Parker and Vahed 2010). In the present study, we investigated pre-copulatory guarding behaviour of recently mated *A. grisella* males in response to intra-sexual competition. In this species, rather than a lack of female receptivity, pre-copulatory mate guarding results from a male refractory period and takes the form of extended copulas which terminate when males are able to transfer a new spermatophore (Greenfield and Coffelt 1983). In the presently studied population (Indre et Loire, France), pairs remained in copula for up to 20 times longer during a male's second copulation than during its first, which is substantially less than previously reported in a US population (Greenfield and Coffelt 1983).

Our experiments clearly demonstrate that the perception of male competition, simulated by the broadcast of a male courtship song playback, strongly affected pre-copulatory guarding behaviour of *A. grisella* males. We found no difference in the frequency of guarding behaviour as the vast majority of males ceased the female in pre-copula in both treatments. However, pairs experiencing male competition engaged in copula earlier and remained in copula for much longer durations than control pairs, while the total duration of the male's second mating (i.e. sum of latency to and duration of copulation) did not significantly differ across treatment levels (Table 1, Figure 1 and 2).

Besides, we found that guarding behaviours did not vary with female body mass, although the latency to mate a second time appeared marginally shorter with larger females in experiment A (Table 1). In numerous insect species, female body mass represents a good proxy for female reproductive quality, as larger females generally carry more eggs (Honek 1983), as observed in *A. grisella* (Goubault, personnal communication, 2015). However, the absence of evident relationship between female size and male guarding behaviour is in line with earlier studies that also found no relationship between female body mass and strategic sperm allocation (Jarrige et al. 2015). Despite the random allocation of females across treatments, those assigned to the second mating were heavier in the control treatment than in the competition treatment (experiment A, Table A1). Assuming that males are in control of the key behavioural traits (see below), if anything, a supposed male preference for heavier females should lead to shorter latencies and maybe longer durations of second copulations in the control treatment. In contrast, we found the opposite pattern suggesting that female size has a reduced influence on male mate guarding behaviour, if any, in our experiments.

The perception of competitors' presence (through the broadcast of male songs) could also inform males about a potential high risk of sperm competition. They could then respond to this risk by transferring larger ejaculate in order to increase their chances of paternity (Parker 1970, Simmons 2001). In such a situation, longer copulation would simply be a by-product of the time required to transfer larger spermatophore. We, nevertheless, found no relationship between the total duration of extended copulations and the number of eupyrene sperm bundles transferred. We also observed no difference in the number and proportion of eupyrene sperm bundles transferred by males exposed and not exposed to a competitive environment (Table 1, Figure 1e and 2e). This may seem in contradiction with recent results showing that virgin males exposed to social cues of sperm competition risk during early adulthood do transfer larger ejaculates compared to controls (Jarrige et al. 2015). However, the increased sperm expenditure was achieved through increased sperm transfer rate rather than copulation duration, and after a much longer exposure to the competitive environment (3 days instead of a few hours to just 30 min in our study; Jarrige et al. 2015). In any case, here, as sperm expenditure did not vary with the exposure to male competition, we can interpret extended copulations to function mainly as a defence against pre-copulatory male competition.

In experiment A, both partners of the mating pairs were exposed to the playback of male courtship song. At this stage, it remained unclear whether the male or the second female was in control of the latency and the duration of the copulation. By exposing individually either the male or the (second) female to courtship song playback in experiment B, we disentangled male *versus* female contribution to the observed extended copulations. The lack of significance of experiment-by-treatment interactions, as well as the widely overlapping 95% confidence intervals of Cohen's *d* (our measure of standardised effect size), demonstrate that treatment effects were statistically indistinguishable across experiments A and B (figure 3). We conclude that males, not females, of the experimental pairs in experiment A were responsible for the observed differences in mating behaviour across treatment levels.

Given that the total duration of a male's second mating (measured as the sum of latency to and duration of the copulation) was very similar across treatment levels, and that the male sperm expenditure appeared unrelated to mating duration and treatment level, the most parsimonious explanation for the increase in duration between first and second matings is that all males need a similar time to prepare a new spermatophore. The different copulation durations between treatment levels are simply the by-product of the differential mating latencies. Together, these findings suggest that the observed shorter latency to copulate a second time represents a male's adaptation to high levels of male competition, which *A. grisella* males may frequently experience in leks. Indeed, in lek-mating species, mate attraction is often costly and mating opportunities, depending on interactions between male competition and female preferences, might be extremely skewed and stochastic (Höglund and Alatalo 1995). Recently mated males may thus lose mating opportunities if encountering a female during their refractory period (i.e. the period required to produce a new spermatophore). Under such circumstances, adjustment in guarding behaviour according to competition risk could allow males to increase their reproductive success by retaining and monopolising a female until they are physiologically able to inseminate her (Greenfield and Coffelt 1983; see also Parker and Vahed 2010). Here, the frequency of mate guarding was extremely high, however male experiencing social cues of competitive environment expressed shorter latency to engage into pre-copula. This can constitute indirect evidence of the costs of guarding for males. Indeed, if guarding was cheap, males under both treatments would have guarded females as soon as possible. Such costs could involve i) an increased energy expenditure when in pre-copula, and ii) a less effective predator avoidance due to the reduced manoeuvrability of the mating pair (Grafen and Ridley 1983, Scharf et al. 2013), what could be tested in the future by exposing males to an elevated predation risk simulated by playback bat calls. Additionally, assuming that males cannot disengage from an ongoing copulation, mate-guarding may lead males to miss potential mating opportunities with females of higher quality. This could have important consequences on the mating dynamics in leks, by affecting the mating rate of certain males. In leks, male mating opportunities depend on strong and directional female preferences, and thus on the presence and attractiveness of rival males (Höglund and Alatalo

1995). Because most attractive males would have more opportunities to mate than less attractive ones, they should be more likely to engage in guarding, generating a rapid turn-over of males available for reproduction when female encounter rate is high. In contrast, when female encounter rate is low, pre-copulatory guarding behaviour would accentuate attractive males' mating potential, thereby magnifying any existing reproductive skew. Thus, understanding the factors affecting mating dynamics in lekking species would be crucial to understand the maintenance of genetic variance under the strong directional female choice occurring in such systems, also known as the lek paradox (Kirkpatrick and Ryan 1991, Kotiaho et al. 2008 a,b).

More generally, our results suggest that pre-copulatory mate guarding might be expected to evolve in situations where the rate of encountering a mate is stochastic, and where one sex have a restricted time window of sexual receptivity. This could be particularly advantageous in species where males have a sexual refractory period between two successive matings, a situation frequently encountered in many Orthopterans (Vahed 2006, Parker and Vahed 2010). High intra-sexual competition would increase the interest of pre-copula mate guarding, because any unguarded females may rapidly mate with a rival male. For these reason, pre-copulatory mate guarding resulting from male reproductive constraints could be more widespread than previously thought, a potentiality that have so far received only marginal interest. Indeed, if expressed in copula, male mate guarding prior to sperm transfer might be difficult to observe, and potentially concealed with physiological constraints over mating. For instance, in a wide range of species, male copulation duration increases with mating history and the depletion of gametic and non gametic mating resources (e.g. Herberstein et al. 2005, Paukku and Kotiaho 2005, Sandra 2009, Engqvist et al. 2014). Under these circumstances, extended copulations durations might not only reflect an increase in the time necessary for the ejaculate's transfer, but also the expression of pre-copulatory guarding behaviour by which males secure future mates. In addition, although it is unlikely to be the case in *A. grisella*, increased copulation durations might also function against post-copulatory sexual selection by allowing males to maximise or optimize their sperm transfer (Simmons 2001). The present study therefore calls to broaden the view of the factors affecting the evolution of pre-copulatory male mate guarding to male reproductive constraints, and for more investigations over the expression and adaptive significance of this behaviour.

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SUPPLEMENTARY RESULTS

Male first copulation in experiment A

Male first copulation durations were similar across treatment levels and we found no effect of male or female body mass on copulation duration (Table S2a). The total number of eupyrene sperm bundles and the number or proportion of bundles transferred were similar across treatment levels and were unaffected by male and female body mass (Table S2a). Heavier males, however, showed a tendency to have a higher total number of available bundles (Table S2a).

Male first copulation in experiment B

The duration of first copulations of pairs where the male partner was subsequently exposed to playback prior to his second mating took slightly longer than first copulations of pairs where the female partner was subsequently exposed to playback (Table S2b). As in experiment A, we found no effect of female body mass on copulation duration (Table S2b), but copulations were slightly shorter for heavier males (Table S2b).

We found no differences in the total number of eupyrene sperm bundles available or in the number or proportion of bundles transferred between treatment levels and no effect of female body mass (Table S2b). Heavier males, however, had a significantly higher total number of sperm bundles and also transferred more bundles, but not a higher proportion of available sperm bundles during first matings (Table S2b).

Table S1

	Mean per treatment level				
a) Experiment A	**Control (N = 22)**	**Pair exposed (N = 27)**	**t**	**df**	**p**
Male mass (mg)	12.89 ± 2.17	12.75 ± 3.11	0.18	46.0	0.85
First female mass (mg)	28.55 ± 2.93	28.12 ± 3.44	0.48	46.90	0.64
Second female mass (mg)	30.79 ± 6.14	25.03 ± 7.73	2.91	47.0	**0.006**
b) Experiment B	**Female exposed (N = 11)**	**Male exposed (N = 12)**	**t**	**df**	**p**
Male mass (mg)	11.35 ± 3.76	12.08 ± 3.73	-0.47	20.80	0.64
First female mass (mg)	27.88 ± 2.41	29.16 ± 1.87	-1.42	18.90	0.17
Second female mass (mg)	27.92 ± 9.27	30.58 ± 8.27	-0.72	20.20	0.48

Table S1. Results from Welch's t-tests comparing *A. grisella* body mass between treatment levels for males, for females allocated to male first matings and females allocated to male second matings for a) experiment A and b) experiment B, respectively. Significant results are highlighted in bold.

Table S2

		(a) Experiment A					(b) Experiment B				
Male first mating		**Estimate**	**SE**	**t**	**df**	**p**	**Estimate**	**SE**	**t**	**df**	**p**
Copulation duration (min)											
	Intercept	9.88	0.31	–	–	–	8.68	0.31	–	–	–
	Treatment	0.40	0.41	0.96	1	0.34	1.12	0.44	2.51	1	**0.02**
	Male mass	0.05	0.08	0.59	1	0.56	-0.13	0.06	-2.25	1	**0.04**
	Female mass	0.09	0.07	1.43	1	0.16	0.00	0.10	0.04	1	0.97
Number of bundles available											
	Intercept	193.15	13.82	–	–	–	217.19	14.81	–	–	–
	Treatment	-9.48	18.76	-0.51	1	0.62	-35.86	22.57	-1.59	1	0.13
	Male mass	5.72	3.25	1.76	1	0.09	12.76	2.92	4.37	1	**<0.001**
	Female mass	2.9	2.84	-1.02	1	0.31	6.54	4.97	1.31	1	0.21
Number of bundles transferred											
	Intercept	47.46	4.21	–	–	–	51.38	5.24	–	–	–
	Treatment	-4.43	5.72	-0.78	1	0.44	-9.06	7.98	-1.14	1	0.27
	Male mass	0.69	0.99	0.7	1	0.49	2.80	1.03	2.72	1	**0.02**
	Female mass	-0.48	0.87	-0.56	1	0.58	0.25	1.76	0.14	1	0.89
Proportion of bundles transferred											
	Intercept	24.88	1.58	–	–	–	24.07	1.83	–	–	–
	Treatment	-1.33	2.15	-0.62	1	0.54	-0.42	2.80	-0.15	1	0.88
	Male mass	-0.05	0.37	-0.14	1	0.89	-0.33	0.36	-0.92	1	0.37
	Female mass	0.14	0.32	0.41	1	0.68	-0.83	0.62	-1.34	1	0.2

Table S2. Results from linear models analysing the effects of *A. grisella* courtship song playback on mating behaviour and sperm expenditure at first mating for (a) experiment A, where experimental pairs were exposed to playback prior to and during male second mating, and (b) experiment B, where either a pair's male or female partner was exposed to playback prior to male second mating. Note that male and female body mass are mean-centred and the intercept estimates represent the predicted effects for males of average mass from the control treatment level (experiment A) or for males of average mass from the treatment level where females had been exposed to playback (experiment B), respectively, paired to females of average mass in both cases. Significant results are highlighted in bold.

Table S3

Male first copulation	Estimate	SE	t	df	p
Copulation duration (min)					
Intercept	9.91	0.28	–	–	–
Treatment	0.38	0.38	0.99	1	0.32
Experiment	-1.18	0.50	-2.36	1	**0.02**
Male mass	-0.03	0.05	-0.63	1	0.53
Female mass	0.08	0.06	1.43	1	0.16
Treatment x experiment	0.57	0.68	0.83	1	0.41

Table S3. Results from linear models comparing the effect sizes of treatment effects from experiment A, where pairs were exposed to *A. grisella* courtship song playback prior to and during male second mating, and experiment B, where either a pair's male or female was exposed to playback prior to male second mating. Note that male and female body mass are mean-centred and the intercept estimates represent the predicted effects for males of average mass from the control level/the treatment level where females had been exposed to playback paired to females of average mass. Significant results are highlighted in bold.

Figure S1

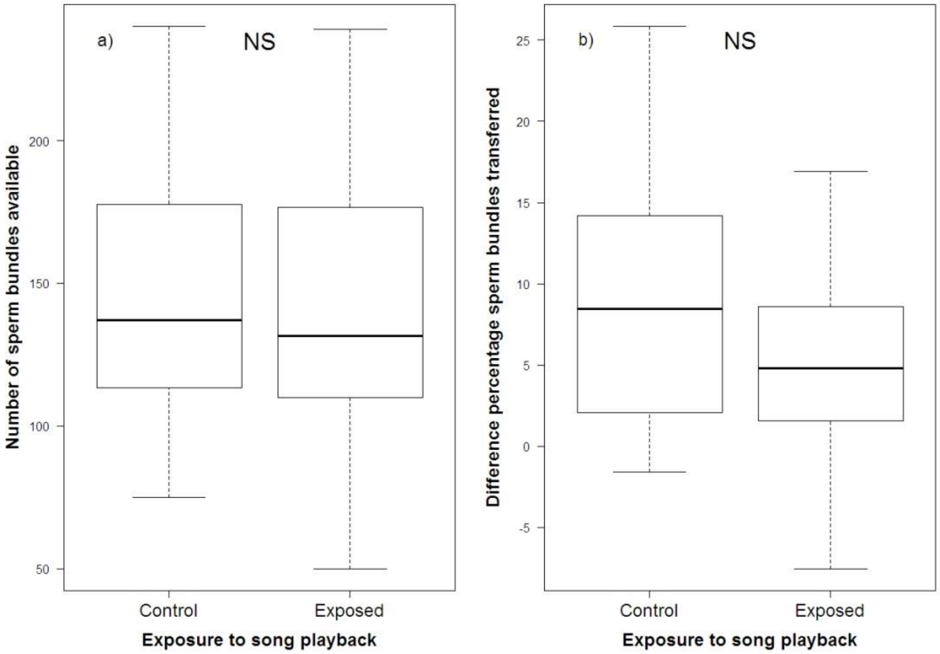

Figure S1. Sperm expenditure of lesser wax moths, *A. grisella,* in relation to perceived male competition in experiment A, where experimental pairs were exposed to playback of courtship song prior to and during male second mating. Pairs exposed: N = 24; or not exposed: N = 20. a) Total number of sperm bundles available at second mating and b) difference between first and second mating in the percentage of sperm bundles transferred. Plots show medians, interquartile ranges (box) and data within 1.5 times the interquartile ranges (whiskers) and circles data outside this range.

Figure S2

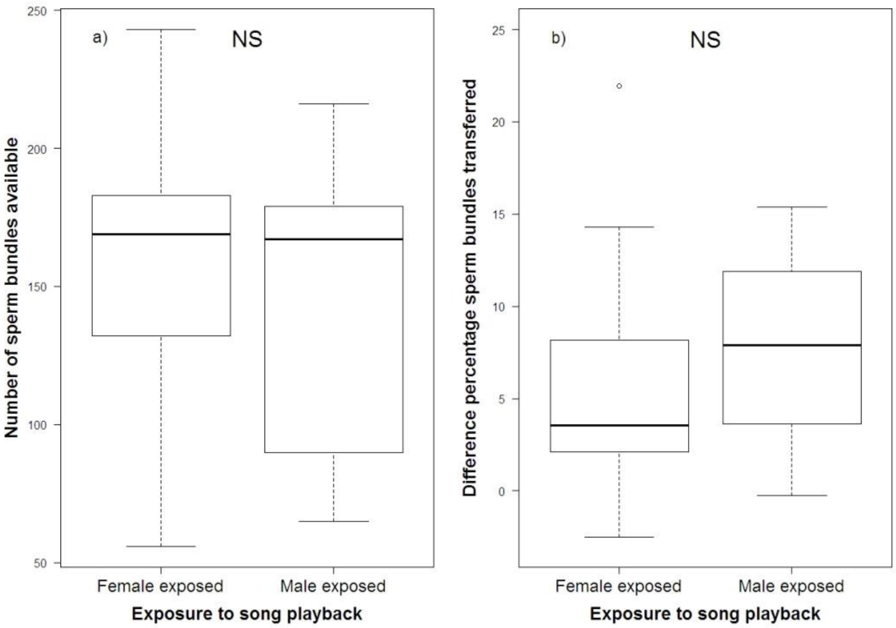

Figure S2. Sperm expenditure of lesser wax moths, *A. grisella,* in relation to perceived male competition in experiment B, where males or females were exposed to playback of courtship song prior to male second mating (males exposed: N = 9; and females exposed: N = 11. a) Total number of sperm bundles available at second mating and b) difference between first and second mating in the percentage of sperm bundles transferred. Plots show medians, interquartile ranges (box) and data within 1.5 times the interquartile ranges (whiskers) and circles data outside this range.

Figure S3

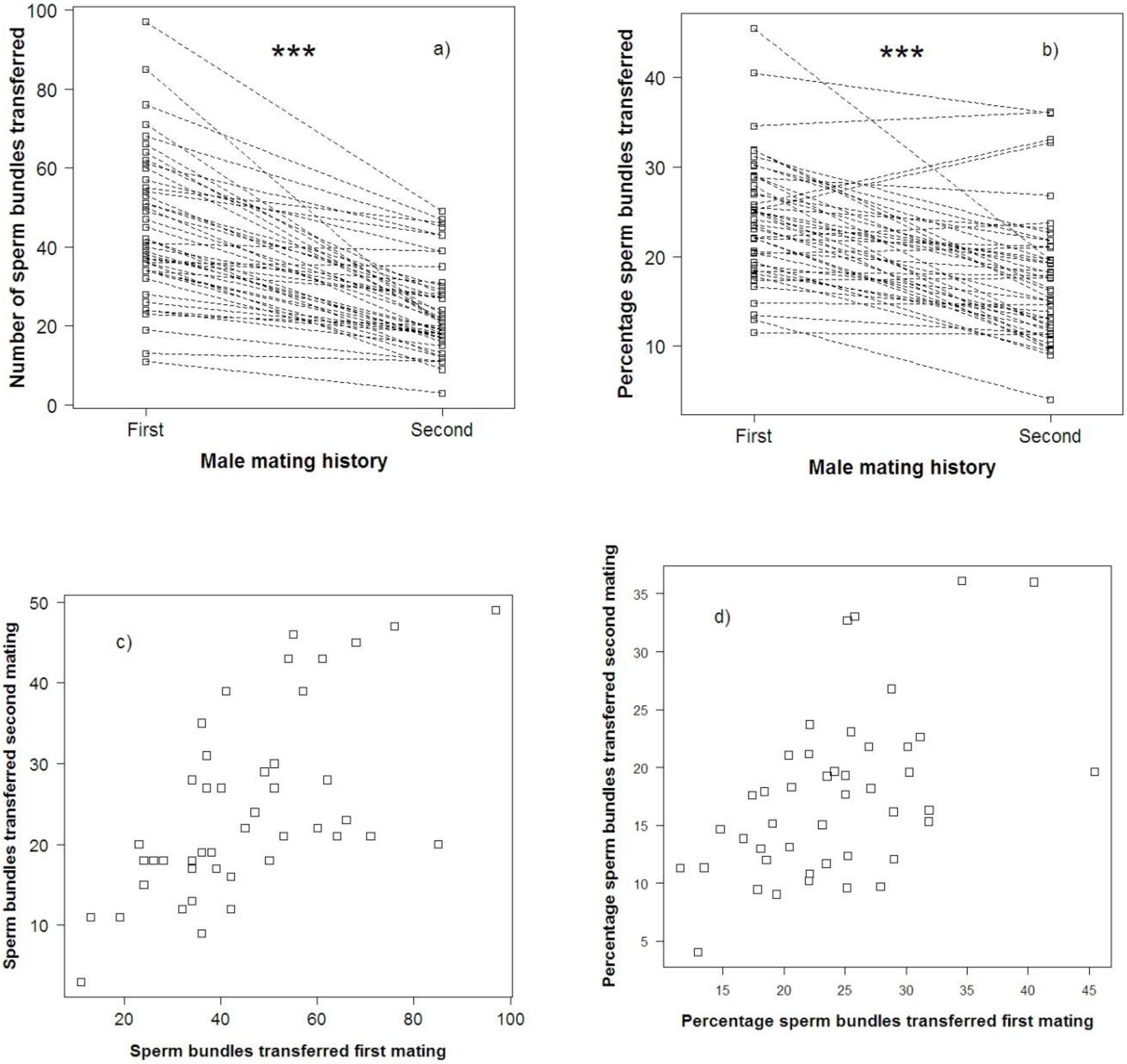
a)
***
Number of sperm bundles transferred
0
20
40
60
80
100
First
Second
Male mating history
b)
***
Percentage sperm bundles transferred
10
20
30
40
First
Second
Male mating history
c)
Sperm bundles transferred second mating
10
20
30
40
50
20
40
60
80
100
Sperm bundles transferred first mating
d)
Percentage sperm bundles transferred second mating
5
10
15
20
25
30
35
15
20
25
30
35
40
45
Percentage sperm bundles transferred first mating

Figure S3. Pairwise comparison of sperm expenditure of the lesser wax moths, *A. grisella,* in relation to perceived male competition in experiment A: experimental pairs exposed to playback of courtship song prior to and during male second mating: N = 24; or not exposed (control): N = 20. a) Number of bundles transferred and b) proportion of bundles transferred. Within male relationship between first and the second mating in c) number of bundles transferred and d) proportion of bundles transferred.

Figure S4

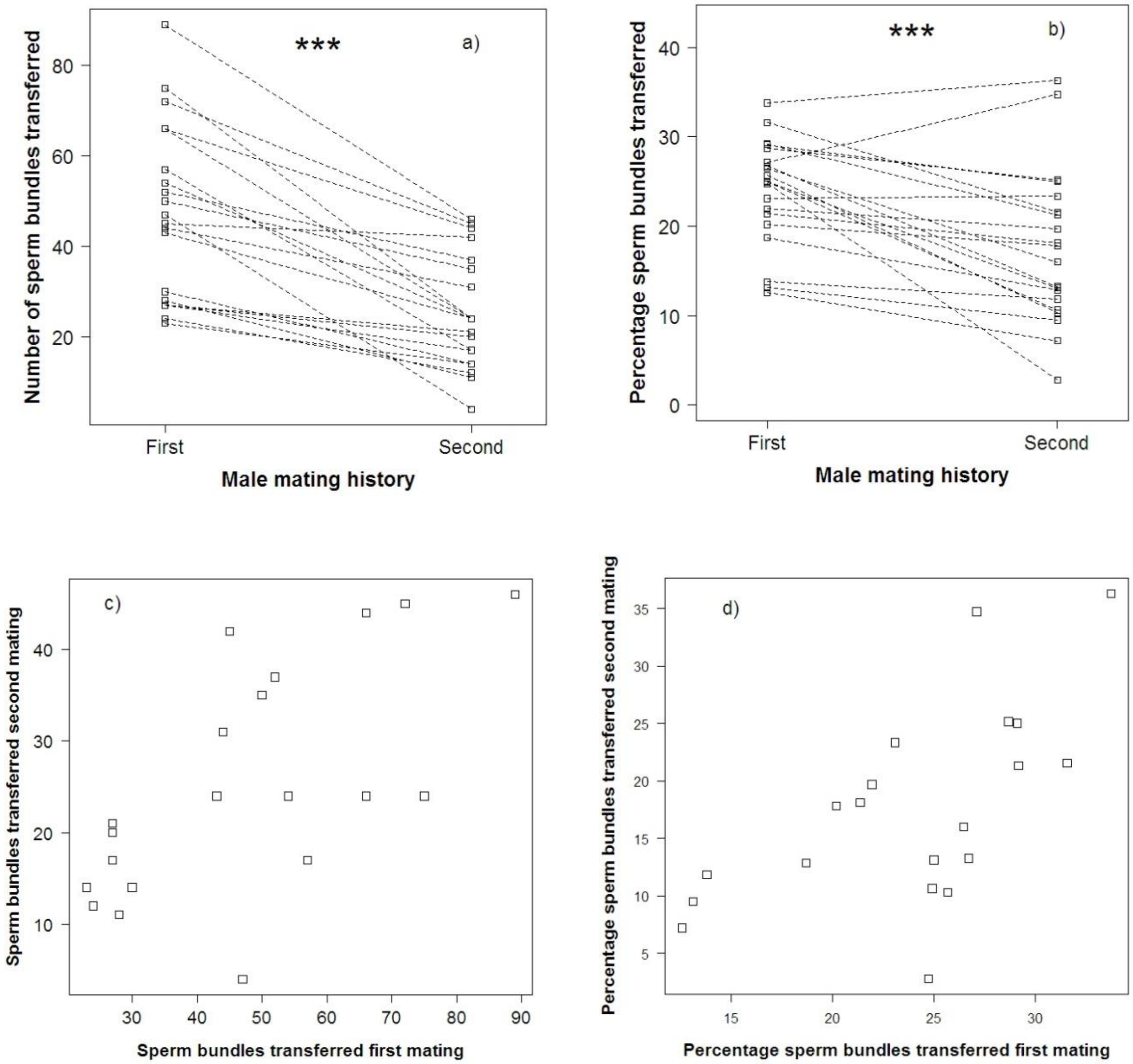
a)
***
Number of sperm bundles transferred
20
40
60
80
First
Second
Male mating history
b)
***
Percentage sperm bundles transferred
0
10
20
30
40
First
Second
Male mating history
c)
Sperm bundles transferred second mating
10
20
30
40
30
40
50
60
70
80
90
Sperm bundles transferred first mating
d)
Percentage sperm bundles transferred second mating
5
10
15
20
25
30
35
15
20
25
30
Percentage sperm bundles transferred first mating

Figure S4. Pairwise comparison of sperm expenditure of the lesser wax moths, *A. grisella,* in relation to perceived male competition in experiment B where males or females were exposed to to playback of courtship song prior to male second mating. Males exposed: N = 9; and females exposed: N = 11. a) Number of bundles transferred and b) proportion of bundles transferred. Within male relationship between first and the second mating in c) number of bundles transferred and d) proportion of bundles transferred.

ARTICLE 3

Sperm depletion and alternative mating phenotypes in the moth *Achroia grisella*

Alicia Jarrige, Mathilde Michon, Virginie Party & Marlène Goubault

En préparation pour Animal Behaviour

ABSTRACT

Although it is certainly true that the production of individual gametes are more costly for females, the transfer of ejaculates comprising thousands of spermatozoa often induce substantial cost for males, that might drastically constrain their reproductive potential. Therefore, in order to maximize their lifetime reproductive success, male should evolve prudent allocation of their reproductive resources according to the likely benefits. In particular, in species in which females are variable in quality, males might sire more offspring by choosing females with the highest reproductive potential. The aim of the present study was to investigate whether and to what extent sperm constituted a limiting reproductive resource, as well as male sperm allocation according to female reproductive quality (body mass), in the lesser wax moth *Achroia grisella*,.

In a first experiment, we quantified the male sperm expenditure of males presented every day of their life with a female of standardized body mass. Our results suggested the existence of two distinct mating types differing in the number of copulations achieved by males and the number of sperm transferred at each copulation. Therefore, we designed a second experiment to determine whether males of both phenotypes exerted differential pre- and/or post-copulatory preferences when given the simultaneous choice between females of different reproductive qualities. This second experiment further confirmed the existence of two mating phenotypes in *A. grisella*, however we found no evidence of the female reproductive quality on the male sperm expenditure. We propose that the observed phenotypes represent alternative mating strategies evolved under post-copulatory sexual selection.

KEYWORDS:

alternative mating tactics; male choice; mating phenotypes; sperm depletion; strategic ejaculation.

INTRODUCTION

Conventional sex roles involve choosy females and competitive males (Andersson 1994). The theoretical explanation behind this view is that female reproductive rate is often lower than that of males because of the maternal higher investment in gamete production and care of offspring (Darwin 1872, Bateman 1948, Trivers 1972, Clutton-Brock and Vincent 1991, Jennions and Petrie 1997). As a consequence, females would gain little, if at all, from each additional mating whereas the reproductive success of males would increase with each successful breeding attempt (Bateman 1948). However, this classical conception has been subjected to both theoretical and empirical revisions (Wedell, Gage et al. 2002, Clutton-Brock 2007, Edward and Chapman 2011). In particular, for males, reproduction often incurs substantial costs in terms of time, energy and risk of injury associated with mate attraction, competition with rival males and ejaculate production, what might strongly constrain the male reproductive potential (Scharf, Peter et al. 2013). Besides, in polyandrous species, the existence of post-copulatory mechanisms (sperm competition and cryptic female choice) can further affect the pay-off of mating and thereby limit the gain of fitness derived from a male successful copulation (Parker 1970, Simmons 2001). Therefore, in order to maximize their lifetime reproductive success, male should evolve prudent allocation of their reproductive resources according to the likely benefits (Simmons 2001, Wedell, Gage et al. 2002, Edward and Chapman 2011). In particular, in species in which females are variable in quality, males might sire more offspring by choosing females with the highest reproductive potential (Bonduriansky 2001, Simmons 2001, Wedell, Gage et al. 2002, Edward and Chapman 2011). An increasing body of work supports this hypothesis by demonstrating that males discriminate females based on cues indicative of her fecundity, such as mating status, age, or body mass (Bonduriansky 2001, Wedell, Gage et al. 2002, Edward and Chapman 2011).

Male mate preferences can be exerted through two distinct and non-mutually exclusive mechanisms: in *pre-copulatory* choice, male can copulate preferentially with higher quality females and reject those of lower quality, while *in-/post-copulation* choice involves variable allocation of reproductive resources (sperm, non-gametic substances, parental care) depending on female quality (Bonduriansky 2001, Edward and Chapman 2011). Male mate choice is expected to evolve whenever the benefits gained by being choosy outweigh the costs. Yet choosiness can induce numerous costs such as the time and energy spent searching for mates (Parker 1983) predation risk (Rowe 1994), and loss of mating opportunities (Martel, Damiens et al. 2008). Besides, the evolution of male mate preferences can depend on complex interactions between selective and competitive mechanisms of sexual selection (Fawcett 2003), such as the presence and strength of female choice, sperm competition and the male's own physiological conditions (e.g. age, mating status; Edward & Chapman, 2011). For example, the degree of sperm depletion is likely to affect the cost of future matings and thus how beneficial it is for a male to be choosy.

The aim of this study was to investigate male sperm depletion in relation to male mating status and male mate choice in the lesser wax moth, *Achroia grisella* (Lepidoptera: Pyralidae), a cosmopolitan symbiont of honeybees, *Apis mellifera* (Hymenoptera: Apidae). Adults have a short lifespan: males typically live 7–14 days and females about 7 days in laboratory. In this species, reproduction and somatic maintenance entirely relies on teneral reserves, as adults possess atrophied mouthparts and can neither feed nor drink (Greenfield and Coffelt 1983). Previous experiments showed that female body mass correlates with fecundity (Goubault, 2015, personal communication). Males exhibit the classical dichotomous spermatogenesis of Lepidoptera (Fernandez-Winckler and da Cruz-Landim 2008), by producing eupyrene (nucleate) spermatozoa aggregated in bundles of 256, and apyrene (anucleate) spermatozoa (Friedlander 1997, Friedländer, Seth et al. 2005). During copulation, both types of sperm are transferred to the female within an endogenous spermatophore. Only eupyrene sperm fertilizes the eggs while the role of apyrene spermatozoa remains to be clarified (Friedländer, Seth et al. 2005, Silberglied, Shepherd et al. 2005). Importantly, spermatogenesis of eupyrene (i.e. fertilizing) sperm stops at pupation and imagoes emerge with a defined number of fertilizing sperm that cannot be replenished (spermatogeny index of 1; Friedlander 1997, Damiens and Boivin 2005). However, the chronology of sperm availability is unusual as eupyrene spermiogenesis (sperm maturation) seems to continue during early adult life (Fernandez-Winckler and da Cruz-Landim 2008). Hence, although the lifetime quantity of fertilizing sperm is fixed, the amount of mature sperm available at a given mating might greatly fluctuate along a male's early life, and therefore require further investigations.

In addition, males are constrained in the rate at which they can transfer a spermatophore, and recently mated males (within an hour) undergo a 4-6 h refractory period during which they cannot transfer a new spermatophore (Jarrige et al, 2015, under review). However, male encountering a female during this interval can secure females through extended copulation durations (precopulatory mate guarding) that is intensified under intrasexual competition (Greenfield and Coffelt , 1983, Jarrige et al., 2015, under review).

The reproductive behavior of *A. grisella* involves intense male competition and female choice, as males gather in leks for six to ten hours every night and display costly ultrasonic advertisement (Reinhold, Greenfield et al. 1998) targeted by strong and directional female choice (Greenfield and Coffelt 1983, Spangler, Greenfield et al. 1984, Jang and Greenfield 1998, Limousin and Greenfield 2009). In addition, recent evidences suggest the existence of post-copulatory mechanisms of sexual selection under the form of sperm competition, and males experiencing a competitive environment during early adulthood increase their sperm expenditure to females (Jarrige, Riemann et al. 2015).

For these reasons, reproduction is likely to be costly for *A. grisella* males and we can expect strategic sperm allocation to have evolved. Due to the possibly complex spermiogenesis occurring in this species, we first investigated whether and to what extent eupyrene (fertilizing) sperm can constitute a limited resource for males. We predicted that males should be more selective as their sperm reserve declines, until a potential terminal investment where males are expected to invest maximally (Clutton-Brock 1984). To do so, we measured the quantity of sperm transferred during the successive copulations of males provided every day with a new virgin female (standardized for age and body mass). Surprisingly, our results suggested two alternative mating phenotypes differing in the number of copulations achieved by males and the number of sperm transferred at each copulation. Therefore, a second experiment was conducted to determine whether males presenting a different phenotype exerted differential pre- and/or post-copulatory preferences when given the simultaneous choice between females of different reproductive qualities (in terms of body mass). In addition, in order to determine whether the phenotype of a male concomitantly affected pre-copulatory courtship song, and could be potentially discriminated by females, we recorded the song of males prior to choice experiments.

MATERIALS AND METHODS

Rearing and maintenance

We studied insects obtained from a lab population derived from several hundred individuals collected in Indre et Loire, France, in October 2007. The population was bred in laboratory using a random breeding protocol which minimizes the loss of genetic variation in the stock population (see Jarrige et al. 2015). Moths were reared in an environmental chamber at 25±1°C, 45% relative humidity under a 12:12 light:dark photoperiod. Larvae were cultured on a standard *ad libitum* diet consisting of wheat, corn and rye flours, water, glycerol, nutritional yeast, honey and beeswax (modified from Dutky et al., 1962). These laboratory conditions closely mimic natural conditions for this species. Under these conditions, generation duration measured from oviposition to oviposition averaged 45 to 55 days. Experimental individuals were isolated during the pupal stage and kept individually in 28-ml plastic cups to ensure virginity and standardized social conditions. Emergences were monitored twice a day, ensuring that individuals identified as new adults were 0 to 16h old.

All experiments took place during the initial 6h of the scotophase (i.e. when mating activities are peaking, Greenfield and Coffelt 1983) in an acoustically insulated chamber that was maintained under environmental conditions identical to those used for the rearing except that diffuse red light (25 W, incandescent) provided illumination. Both males and females were placed in the insulated chamber at least 2 hours prior to experiments for acclimatization.

Experiment 1: Measurement of eupyrene sperm transfer over male successive copulations

To determine if, and to what extent, sperm can constitute a limiting resource for *A. grisella* males, we quantified the number of eupyrene sperm transferred by males to females over successive copulations. Every 24 hours from their emergence (D0) to their death, one hour after the beginning of the scotophase, males of standardized body mass (11-to-15 mg, N = 30) were provided with a virgin female of standardized age (0-1 day old) and body mass (23-31 mg). On the day of the test and prior to the mating trial, males and females were weighed to a precision of 0.1 mg with a Mettler Toledo AX105 DeltaRange balance (Columbus, USA). Females were directly introduced in the male plastic cup for a maximum of 30 minutes, as pilot experiments showed that mating almost never occurs after this delay. The occurrence of mating was monitored and the duration of copulation was timed with a stopwatch. To determine whether mating history affected male survival and mass loss, we compared those measurements to those of control males (11 to 15 mg, N = 30) that were kept in similar conditions but remained unmated from their emergence to their death.

Female dissection and sperm count

Immediately following the natural end of copulation, females were chilled in the freezer during two minutes, then killed by decapitation. The *bursa copulatrix* was removed by ventral opening of the female abdomen. After successful copulation, this organ contains a spermatophore which consists in a seminal sac filled with solitary apyrene spermatozoa and eupyrene spermatozoa aggregated in bundles of 256 (Fernandez-Winckler and da Cruz-Landim 2008). The spermatophore was carefully isolated in a drop of PBS 1X placed on a microscopy slide. The seminal sac was ruptured with a fine needle tip to release the bundles and allow a full dispersion of the sperm mass. After gentle homogenization, eupyrene bundles were counted under dark phase microscopy at x 500 magnification. The total number of sperm bundles transferred by males during their life was calculated by summing the number of bundles transferred to each female they mated. The mean number of bundles transferred per mating was obtained by dividing the total number of bundles by the number of successful copulations achieved by males.

Experiment 2: Male mating phenotypes and mate preferences

The previous experiment outlined the existence of two alternative mating phenotypes in *A. grisella*: a proportion of males, referred as *occasional* males, rejected females more frequently than *regular* males who mated with almost every presented female (see Results & Discussion). A second experiment was then conducted: after determining the mating phenotype of the tested males (*occasional* vs. *regular*), we investigated whether they exhibited different pre- and post-copulation preferences according to their phenotype when given the simultaneous choice between two females of different qualities (in terms of body mass, see below *Male mate preferences*). We also determined whether the phenotype of males could affect their attractiveness to females by measuring their song parameters (see below *Measurement of male signal traits*).

Determining male mating phenotype

Contrary to regular males, occasional males were found to refuse at least one mating opportunity during the first 3 days of their life, a behavior that was never observed in regular males (see Results). Therefore, we used this criterion to determine the mating phenotype of a male. Males standardized for body mass (11-to-15 mg, N = 33) were provided daily (at D0, D1 and D2) with a standard virgin female (24-31 mg, 0-1 day old). If mating took place, females were dissected and the number of eupyrene bundles was counted as previously described. Males refusing at least one (or more) mating during this period of time were considered as occasional, while males that mated every day during this period were categorized as regular (see Results & Discussion).

Male mate preferences

On D3, following song recording (see below *Measurement of male signal traits*), males were allowed to choose between one large (body mass $\geq$ 31 mg) and one small female (body mass $\leq$ 24 mg). All females were 0-1 day old. For each trial, females were kept individually under plastic cover at equal distance of the male (25 cm). Once the male was singing, plastic covers were removed to release both females simultaneously. The latency to the female arrival in the male vicinity, the order of female arrival and the female the male eventually copulated with (large or small) were recorded. Trials where only one female reached the male were excluded. After mating, both males and females were dissected to count the number of bundles transferred to the female as well as the number of mature bundles remaining in the male genital tract.

Male and female dissection and sperm count

We dissected the females and counted the number of eupyrene sperm bundles as described in *Experience 1*. Males were chilled during two minutes in the freezer, decapitated, and the whole reproductive tract was removed after ventral opening (see Jarrige et al. 2015 for a detailed description of its structure). Subsequently, tracts were placed in a drop of PBS 1X on a slide, gently unfolded with two needle tips in order to isolate the seminal vesicles and the testis. Seminal vesicles and testis were then transferred in a new drop of PBS, ruptured, and their contents were gently stirred with a needle tip to ensure homogenization. Eupyrene bundles were then counted under dark phase microscopy at x 500 magnification. The number of bundles available at D3 was calculated by summing the number of bundles transferred to the female at D3 with the number of bundles remaining in the male genital tract. The proportion of eupyrene sperm bundles transferred at D3 was then obtained by dividing the number of bundles transferred to the female at D3 by the number of bundles available at that time.

Measurement of the male signal traits

At D0 and D3, prior to female introduction in the male's cage, we recorded the courtship song of males. Males were recorded directly within their screen cages, as previous studies confirmed that males sing normally in these cages and that the screen does not modify the acoustic parameters of the song (Jang and Greenfield 1998). For clear recordings of a focal male without the influence of neighbors, a barrier of acoustic insulation was placed between neighboring males. In order to determine a potential effect of the treatment on the song displayed by males, we also recorded the song of control (unmated) males under similar conditions.

Song recording was done using a condenser ultrasound microphone (model CM16/CMPA; Avisoft Bioacoustics; Berlin, Germany; frequency response: ± 3 dB, 20-150 kHz), positioned 25 cm from the male and oriented toward him. The microphone output was digitized with an analogue: digital converter (model UltraSoundGate 416-200; Avisoft Bioacoustics) at 16 bits and 250,000 samples/s. A minimum of 30-sec recording was saved for each male on a personal computer using signal processing software (BatSound Pro 4.0; Petterson Elektronik AB; Uppsala, Sweden). From the recording of each sampled male, we selected a 1-s segment in the middle of the recording for analysis of acoustic parameters. Our only criterion was that the 1-s segment did not include brief silent gaps that reflected missing pulse pairs in an otherwise continuous train. We determined the repetition rate of pulse pairs (PR) for the 1-s segment and then randomly selected 20 consecutive pulse-pair (PP). For each, we measured PP periods, from the onset of the first pulse of a PP to the onset of the first pulse of the following PP. We also measured the asynchrony intervals (AI), measured from the onset of the first pulse to the onset of the second pulse of a pair (Limousin and Greenfield 2009). Because the repetition of pulse pairs includes an alternating succession of longer and shorter PP periods as well as an alternating succession of longer and shorter AIs, we measured the average ratio of the longer to shorter PP periods. We also measured the average of the longer and shorter AIs (Figure 1).

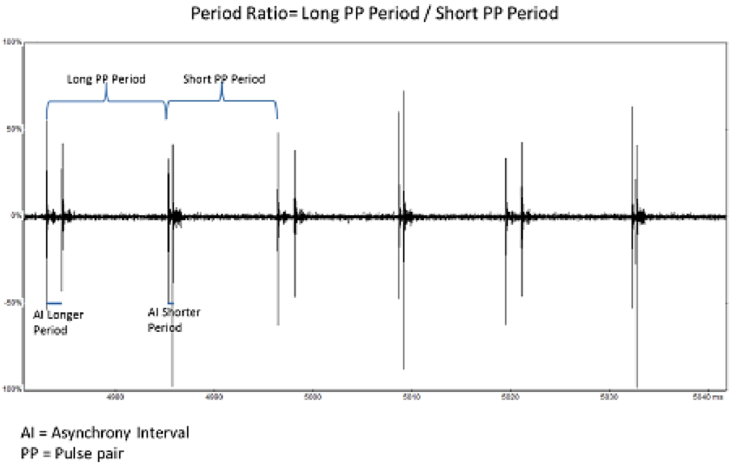

Figure 1. Oscillogram of an acoustic song in *A. grisella* and details of acoustic parameters investigated.

Statistical analysis

All statistical analyses were performed using the computing environment R (R Development Core Team, 2012), with R Studio integrated development environment for R and the package *lme4* and *survival* (Bates, Maechler, Bolker, & Walker, 2014). Additional packages for graphic and optimization of R use included *RVAideMemoire* and *car*.

Experiment 1: Measurement of eupyrene sperm transfer over male successive copulations

Survival analyses were realized using a cox model (function *coxph*). Instantaneous rate of death (constant or not) was determined by plotting the survival curve of individuals. We tested the influence of female body mass on the occurrence of mating using generalized linear mixed models (GLMM function *glmer*) with a binomial distribution of errors, and including male identity as a random effect. We used GLMM considering a *Poisson* distribution of errors to investigate 1) the effect of male age and phenotype on the number of bundles transferred to each female over males' successive copulations, 2) the effect of mating number on the number of bundles transferred to females, and 3) the influence of female mass on the number of sperm bundles received. The identity of individual males was included as a random effect.

We investigated the effect of male body mass at emergence on their phenotype using generalized linear models (GLM, function *glm*) with a *Binomial* family. We used GLM with a *quasipoisson* family to investigate the effect of male phenotype and male body mass on the lifetime number of bundles transferred to females, and on the lifetime number of matings.

Experiment 2: Male mating phenotypes and mate preferences

The effect of 1) mass category (large or small) of the female presented at D3 and 2) male phenotype on the order of female arrival were analyzed by randomly choosing one of the females as focal and using GLM with a *binomial* distribution of errors. A similar approach was used to investigate whether male mated with a particular female according to 1) female order (first or second), 2) female mass category, 3) size difference between females (mass of the larger female minus that of the smaller female) and 4) male phenotype. Note that occasional and regular males had a different mating history at the day of the test (2 or 3 matings for occasional males and 4 matings for regular males). As the number of bundles transferred to females was strongly affected by the number of matings (see results), we also investigated if and how male phenotype and female mass category affected sperm expenditure expressed as the proportion of sperm bundles transferred. The influences of male phenotype and female mass category on the number of bundles transferred were analyzed using GLM considering a *quasibinomial* distribution. The effect of male phenotype and female mass category on the proportion of bundles transferred was tested using GLM with a *quasipoisson* family. When relevant, interactions between variables were investigated. Model simplification was carried out following a stepwise top-down approach. Finally, we investigated the song parameters at D0 and D3 using multivariate analysis of variance (MANOVA, function *manova*). Significance of individual dependent variables was evaluated with a Pillai’s trace test. All statistical tests were two-tailed and we rejected the null hypothesis at $p < 0.05$.

RESULTS

Experiment 1: Measurement of eupyrene sperm transfer over male successive copulations

General mating traits: Overall, males mated on average 9 times during their lifetime (range 4-13, Q25 = 7.25, Q75 = 11, n = 30), and transferred a total of 203 ± 11.22 (mean ± *sem;* range 63-368) sperm bundles. Considering all males over their life, the non-occurrence of mating concerned on average one third of the mating opportunities (32.40 ± 3.53 %). Female body mass had no influence on the occurrence of mating ($\chi_1 = 0.73$, $p = 0.\ 40$). The number of bundles transferred to females decreased with previous mating experience, i.e. the number of previous copulations achieved by a male ($\chi_{29} = 1462.6$, $p<0.0001$, Figure 2) and with male age ($\chi_{29} = 1150.8$, $p<0.0001$). Body mass of the receiving female did not affect the number of bundles transferred ($\chi_{29} = 3.38$, $p<0.076$).

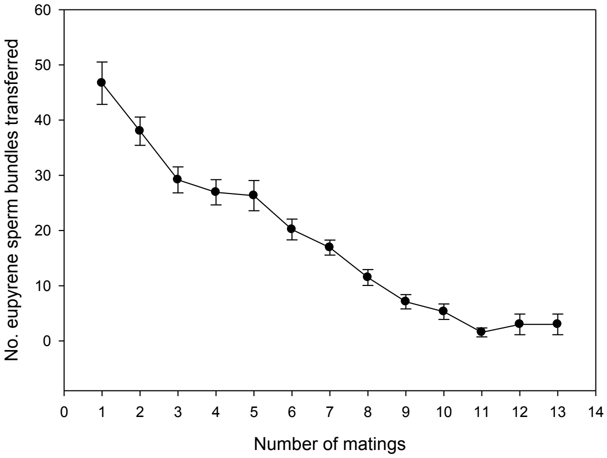

Figure 1. Number of eupyrene sperm transferred over successive matings. N = 30 at first mating. Note that the number of experimental males decrease over time due to natural mortality. Error bars = *sem.*

Mating and survival: Mated males lived significantly shorter than control males ($\chi_1 = 8.25$, $p = 0.004$, Figure 3). Male body mass at emergence influenced male survival with smaller males having shorter life expectancies ($\chi_1 =5.05$, $p= 0.025$). However, neither the number of matings achieved by experimental males nor the total number of bundles transferred during their life had an effect on male survival (respectively $\chi_1 = 1.24$, $p = 0.24$, and $\chi_1 = 3.14$, $p = 0.076$)

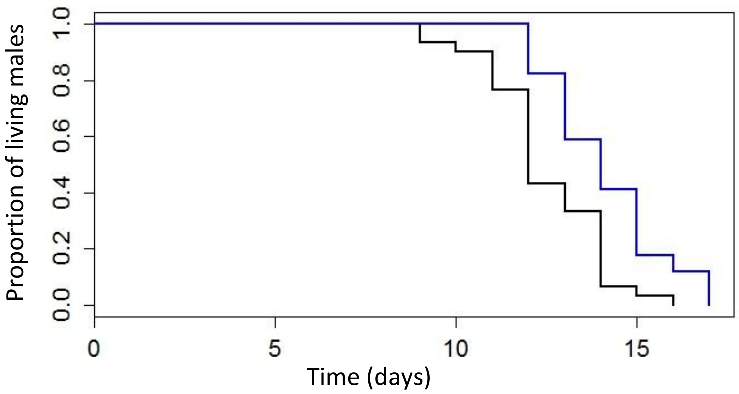

Figure 3. Proportion of living males as a function of time. Black line: mated males, blue line: unmated (control) males.

Male mating phenotype: The total number of copulations achieved by males followed a bimodal distribution (Figure 4). Subsequent graphical analysis of this differential mating pattern revealed that males located on the left hand side of the bimodal distribution had mated less frequently than males located on the right hand side of the bimodal distribution. Importantly, the rate of non-occurrence of matings was noticeable within the first fourth day of the male's life and remained constant within the male's reproductive life (Figure 5). These two distinctive patterns in mating frequency led us to define two mating types, based on the occurrence of copulations within the first days of the male's life: males were defined as *occasional* if they did not mate with at least one (or more) female that was presented to them before the fourth day of their life (that is at D0, D1, D2 or D3), and *regular* if they mated all females in this interval (see Figure 4).

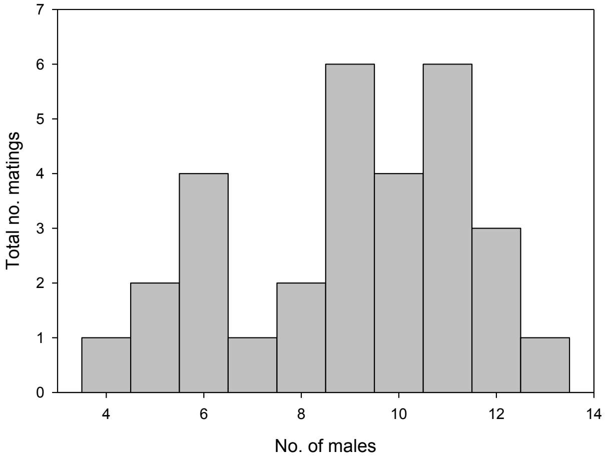

Figure 4. Distribution of the lifetime number of matings achieved by experimental males. N = 30

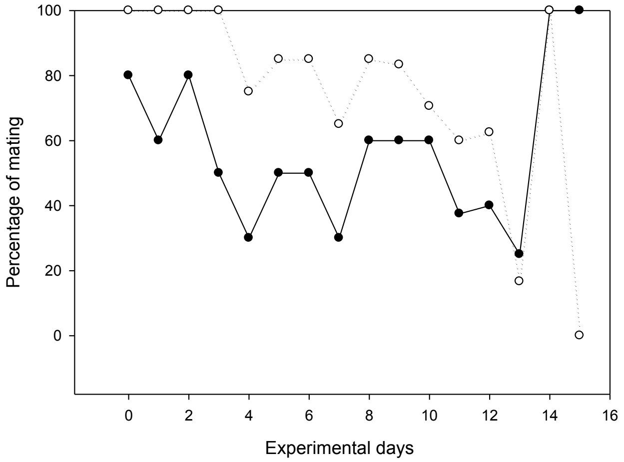

Figure 5. Mating rate according to male phenotype. Solid lines: occasional males, N = 10; dashed lines: regular males, N = 20 (note that the number of experimental males decreases over time due to natural mortality).

Experiment 2: Male mating phenotypes and mate preferences

By definition, male mating type (regular or occasional) affected the lifetime number of copulations achieved by males ($F_{1,29}$ = 11.73, p = 0.004, Figure 6a). Occasional males mated on average 6.8 times ± 0.68 (mean ± sem), range: 4 to 10 times), while regular males mated 10.1 ± 0.41 times (range 6-13 times). Regular males mated significantly more often than regular males (respectively 49.12 ± 4.35 % and 76.84 % ± 3.26 of mating occurrence).

Male body mass at emergence had no influence on male mating phenotype (occasional or regular, G_1 = 38.06, p = 0.73) nor on the total amount of bundles transferred to females ($F_{1,26}$ = 0.020, p = 0.89, Figure 6b). Besides, male survival was not significantly different between males of both phenotypes (χ_1 = 0.19, p = 0.67), and male phenotype did not affect the total lifetime number of eupyrene bundles transferred to females ($F_{1,26}$ = 0.33, p = 0.57). However, we found significant differences in the number of bundles transferred per mating according to male phenotype: occasional males transferred significantly more sperm bundles per mating (32 ± 2.6 bundles) than did regular males (20.8 ± 1.1 bundles), ($F_{1,29}$ = 7.91, p = 0.009, Figure 6c). Furthermore, the decrease in the number of eupyrene bundles appeared stiffer in occasional than regular males, as the interaction between the mating number and male phenotype was highly significant (r = -0.65, $p < 0.0001$, Figure 7). In addition, we found a significant interaction between male age and mating phenotype on the number of bundles transferred ($F_{1,29}$ = 95.21, $p<0.001$).

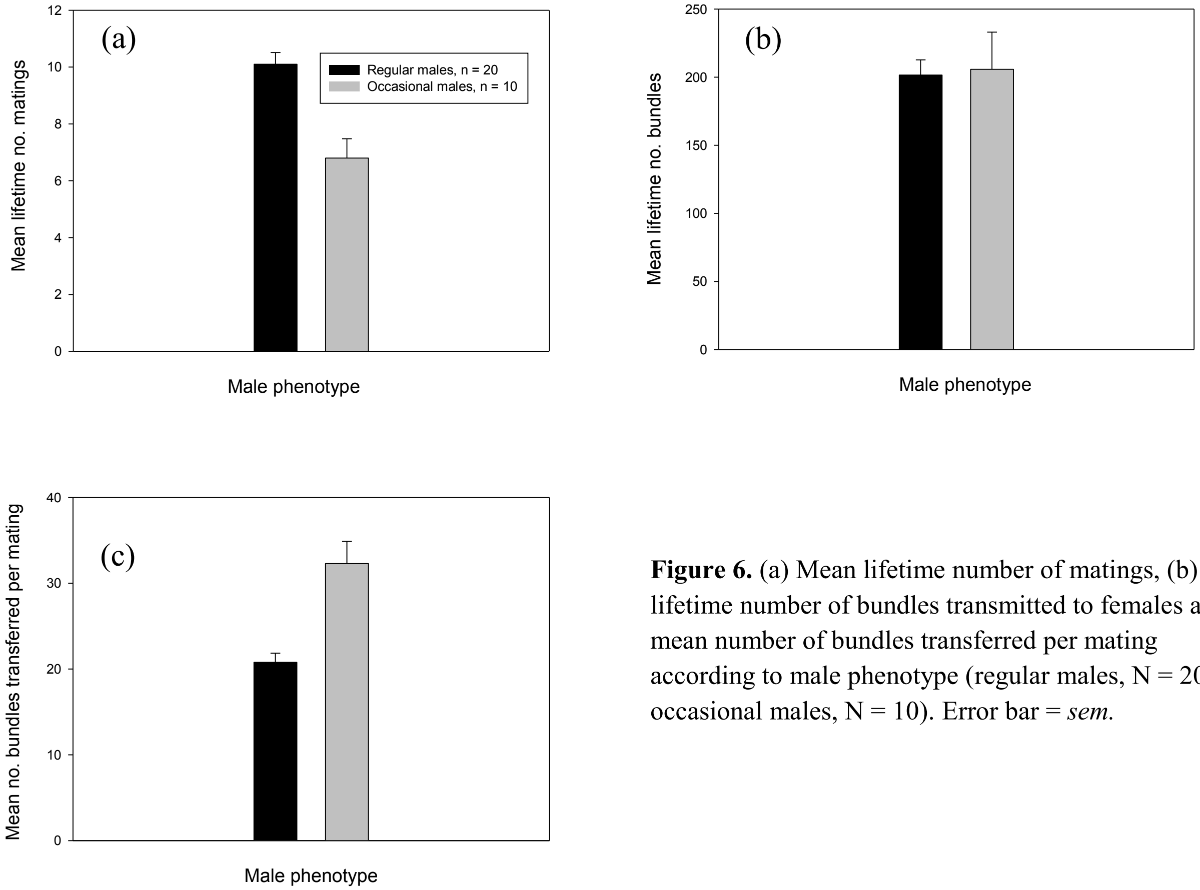

Figure 6. (a) Mean lifetime number of matings, (b) mean lifetime number of bundles transmitted to females and (c) mean number of bundles transferred per mating according to male phenotype (regular males, N = 20; occasional males, N = 10). Error bar = *sem.*

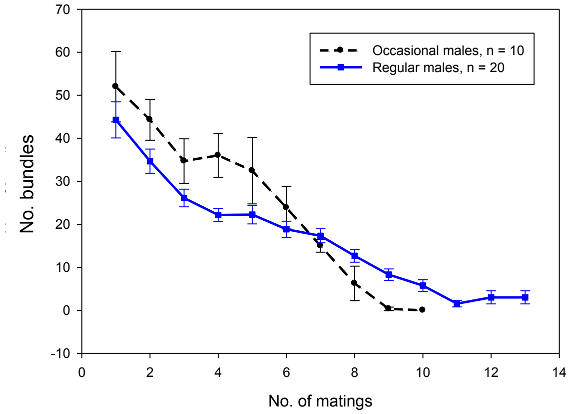

Figure 7. Mean number of bundles transferred by *occasional* and *regular* males over successive copulations. Error bars = *sem*.

Experiment 2: Male mate preferences and mating phenotypes

The order of arrival of the focal female was not influenced by her mass category, although small females tended to reach males prior to large females (G_1 = 48.67, p = 0.06, Figure 8a). Small females were more likely to arrive first in trials involving occasional males than in trial involving regular males (G_1 = 41.25, p = 0.006, Figure 8a). Overall, males tended to mate with the female arrived first, although the effect was not significant, (G_1 = 47.79, p = 0.07). The probability that males mated with a female was not influenced by the female mass category nor the size difference between the two females (respectively G_1 = 52.68, p = 0.96; G_1 = 52.45 p = 0.64; Figure 8b). Finally, males did not mate a particular type of female (large or small) according to their phenotype, although regular males tended to mate preferentially with small females (G_1 = 49.70, p = 0.08, Figure 8).

The number of bundles transferred to females did not differ in relation to the female mass category (G_1 = 161.40, p = 0.28, Figure 8c). However, consistent with the results found for experience 1, male phenotype strongly influenced the number of bundles transferred to females as occasional males transferred significantly more bundles per mating than occasional males (G_1 = 119.67, p = 0.0001, Figure 8c). Finally, we found no influence of male mating phenotype and female mass category on the proportion of bundles transferred to females (respectively G_1 = 3.03, p = 0.80; G_1 = 2.83, p = 0.09).

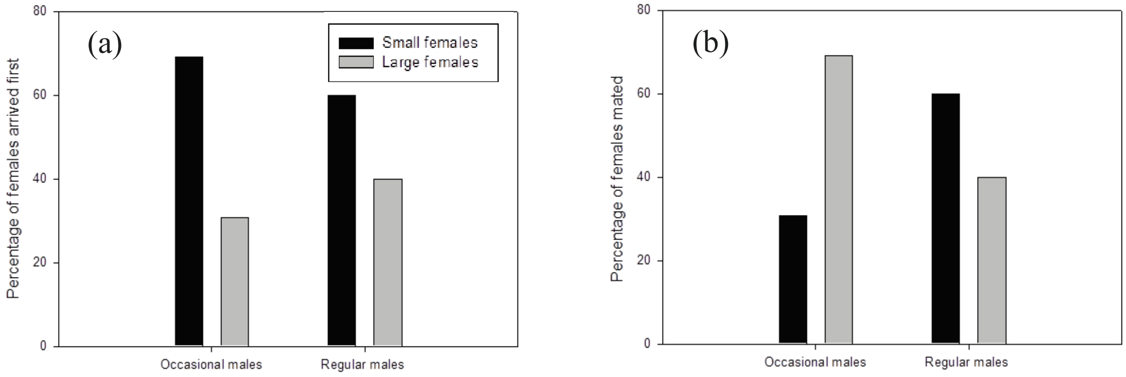

Figure 8. (a) Percentage of females arriving first depending on their mass category and male mating phenotype and (b) percentage of mated female depending on their mass category and male mating phenotype.

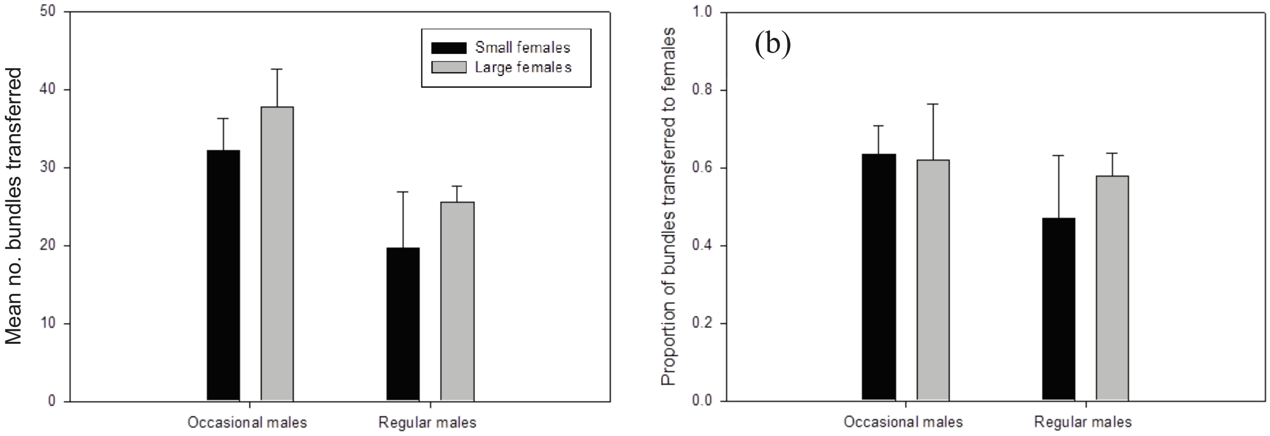

Figure 9. (a) Average number of bundles transferred to females depending on their mass category and male mating phenotype and (b) proportion of bundles transferred to females depending on their mass category and male mating phenotype. Error bars = *sem.*

Male song traits

We found no significant differences between the different parameters of courtship song in regular and occasional males at D0 and D3. However, our results revealed a significant difference in the asynchrony interval of the shorter period between regular and control males (see table 1 below).

Song parameter	Df	F value	P value
Pulse pair rate (PR)	2	0.10	0.90
Pulse pair ratio	2	0.93	0.40
Long pulse pair period	2	0.81	0.45
Short pulse pair period	2	0.77	0.47
Asynchrony interval longer period	2	4.16	**0.02**
Asynchrony interval shorter period	2	0.77	0.47
Pulse pair rate (PR)	2	0.13	0.87
Pulse pair ratio	2	0.03	0.97
Long pulse pair period	2	0.04	0.96
Short pulse pair period	2	0.29	0.75
Asynchrony interval longer period	2	3.42	**0.04**
Asynchrony interval shorter period	2	2.38	0.10

Table 1. Results from Manova on male courtship song parameters at D0 and D3. Significant results are in bold.

DISCUSSION

In the present study, our first purpose was to investigate the dynamic of sperm allocation of males provided daily with a new female, in order to determine whether and to what extent eupyrene sperm constitute a limited resource in *A. grisella*. Our results showed a sharp decrease in the number of eupyrene sperm bundles transferred by males to females over successive copulations, indicating a rapid and irrevocable depletion of eupyrene spermatozoa (Figure 3). Therefore, although sperm maturation (spermiogenesis) still occurs in young adult males and results in an increase of the number of eupyrene sperm bundles available during early reproductive life, eupyrene sperm is likely to constitute a limiting resource for male reproductive success. Such a constraint has been previously documented in a wide range of taxa (Scharf, Peter et al. 2013), a condition under which males are expected to strategically allocate their limited resources in order to maximize the number of offspring they sire (Bonduriansky 2001, Wedell, Gage et al. 2002, Edward and Chapman 2011). Besides, mated males lived significantly shorter than virgin males (Figure 3), suggesting a potential trade-off between survival and reproduction. These results further confirm that reproduction induces substantial costs for males in this species.

In addition, the first experiment revealed that the lifetime number of copulations achieved by males followed a bimodal distribution (Figure 4), with some males mating with most of the females they encountered (regular males), while others mated significantly less frequently (occasional males). Regular males were predominating, representing two third of the experimental individuals. Further investigation of this dichotomous pattern revealed that regular and occasional phenotypes could be predicted within the first 4 days of the male reproductive life as regular males mated all females before their fifth mating opportunity (Figure 5). Furthermore, occasional and regular mating phenotypes differed in the sperm expenditure to females with regular males mating more frequently and transferring fewer sperm bundles per mating, and occasional males achieving significantly fewer matings but transferring more sperm at each mating event (Figure 6a,b). However, although the non-mating occurrence was significantly greater in occasional males, the total lifetime number of sperm transferred to females did not differ between mating phenotypes (Figure 6c).

Two hypotheses could account for this dichotomous mating rate. First, occasional and regular males could achieve differential success under pre-copulatory female preferences. Because *A. grisella* female mate preferences have been demonstrated to rely essentially on male courtship song traits, we compared song traits of both phenotypes of males, in particular those preferred by females including call rate and asynchronous intervals (Jang and Greenfield 1996, Jang and Greenfield 1998, Limousin and Greenfield 2009).We found no evidence that any of the male song traits studied differed between both phenotypes, indicating that females cannot discriminate between male phenotypes on the basis of their song. Although we cannot exclude that females might use other types of cues or signals emanating from males that would remain to be identified, it suggests that the contrasting mating rate between phenotypes would most likely result from differential male mate rejection frequency than from female preferences for certain males.

In such a situation, occasional and regular types could result from different physiological constraints on mating: occasional males might have a longer refractory period leading them to reject any female encountered during this period. However, this hypothesis is unlikely because the delay of 24h given to males in experiment 1 should be more than sufficient for males to recover from previous mating events as the refractory period does never exceed 6h in this population (Jarrige et al, 2015, under review), and this duration mimics realistic natural conditions for female encounter rate as mating activities peaks every night at the beginning of the scotophase (Greenfield and Coffelt, 1983). Furthermore, the observed mating phenotypes are doubtfully the consequence of differential spermiogenesis, first because occasional males transfer more sperm bundles per mating, and second because the higher rate of mate rejection of occasional males would, in that case, be expected to occur only within the male early reproductive life and until the end of sperm maturation (i.e. 3 to 5 days after their emergence, Jarrige, 2013, personal observation). Besides, *A. grisella* males recently mated can display a form of pre-copulatory mate guarding in which males cease the female and extend the copulation duration until being able to transfer a spermatophore again (Greenfield and Coffelt, 1983, Jarrige et al. 2015, under review). In the present study, this behavior was never observed, suggesting that the differential rate of mating between occasional and regular males likely reflects two distinct phenotypes regarding the allocation of copulations.

It is surprising, however, that occasional males missed one or more mating opportunities during the first days of their life, because future reproduction remains unpredictable and uncertain compared to present reproduction (Williams 1966). Thus, males should cease any given chance to mate whenever they arise. However, strategic allocation of copulations could be adaptive if it allows males to invest their limited gametic resources only to females associated with greater fertilization returns, such as females of higher reproductive quality or in which the risk of sperm competition is lower (Wedell, Gage et al. 2002, Barry and Kokko 2010).

In the first experiment, the females were standardized for body mass, and presented sequentially every 24h. Under these conditions, male mating preferences might be difficult to observe (Barry and Kokko 2010). We therefore conducted a second experiment to specifically investigate whether males of both mating phenotypes exerted differential mate choice by mating preferentially or adjusting their sperm expenditure according to the reproductive quality of females presented simultaneously. As common in insects, the quality of *A. grisella* females varies with body size as larger females are more fecund (Honek 1993, Goubault, personal communication 2015). We thus predicted that occasional males would be more selective than regular ones, and compensate the loss of mating opportunities by exerting preferences for females of higher reproductive potential. However, in contrast with our predictions, we found no clear evidence supporting the existence of pre- and post-mating selectivity associated with mating phenotype when males where simultaneously given the choice between a small and a large female. This confirms the results obtained in the first experiment that mate rejection occurs independently of the female reproductive quality (Figure 8, 9). These results are also in line with earlier studies in this species that also found no relationship between female body mass and strategic sperm allocation (Jarrige et al. 2015).

One possible inference about the maintenance of occasional phenotype would be that occasional males are advantaged in sperm competition. In *A. grisella*, 10 to 20 % of the females have been observed to remate, which corresponds to an overall sperm competition risk of roughly 30% (Engqvist, Cordes et al. 2014). Previous experiments demonstrated that males increased their eupyrene sperm expenditure when experiencing social cues of elevated sperm competition risk during early adulthood (Jarrige et al., 2015). Therefore, because they transferred more sperm in average, the occasional phenotype could be advantageous as a male's share of paternity would ultimately increase with male ejaculate size (Simmons 2001, Parker and Pizzari 2010). In particular, the risk of sperm competition in *A. grisella* might have been underestimated under natural conditions, as the number of bundles transferred to females drop drastically after a few matings (Figure 3). Thus, females mating with sperm depleted males might be more prone to seek for additional mates, thereby creating further opportunities for sperm competition.

Here, we propose that occasional males might enhance their average sperm expenditure at mating by increasing the latency between two successive copulations, resulting in the dichotomous mating pattern observed in this study. Indeed, although adults emerge with a strictly fixed number of eupyrene sperm bundles, the number of bundles available for transfer might vary with the time elapsed since last mating, according to the time necessary for the descent of spermatozoa from the testis and/or seminal vesicles to the male cuticular duplex, from where they can subsequently be transferred to the female.

The mating phenotypes observed here are reminiscent of bet-hedging strategies, by which males optimize their reproductive success under fluctuant and unpredictable environments (Fox and Rauter 2003). Notably, individuals could maximize their lifetime fitness, measured as the geometric mean of the fitness gain at each copulation, by using different tactics regarding their reproductive investment. In the present case, while some males might achieve a greater reproductive success in situations of high sperm competition and/or low female encounter rate (occasional phenotype), the regular phenotype could lead to about the same fitness success by lowering the arithmetic mean at each mating, thereby consisting in a low risk strategy. Indeed, as typical in lek mating systems, the mating rate of a male primarily depends on the outcome of competitive interactions with rivals and the direction and intensity of female preferences (Höglund and Alatalo 1995). Thus, for males, female encounter rate as well as the chances of fertilization associated with female quality (fecundity, risk of sperm competition) could be highly stochastic and males could achieve equal reproductive success through two alternative mating behaviors. This could eventually select for disruptive selection and result in the evolution and maintenance of alternative mating strategies, such as the "*resident-satellite*" behaviors adopted by males ruff *Philomachus pugnax* (Hill 1991, Plaistow, Johnstone et al. 2004).

Finally, the reproductive success of *A. grisella* males relies on complex interactions between pre- and post-copulatory selective episodes of sexual selection (Jang and Greenfield 1996, Jang and Greenfield 1998, Limousin and Greenfield 2009, Jarrige, Riemann et al. 2015), the latter depending on the former. Therefore, resource allocation strategies would be of central importance for male overall reproductive success, especially considering that adults emerge with a defined amount of energetic resources that will have to be partitioned between pre- and post-copulatory traits as well as somatic maintenance. Although eupyrene spermatogenesis ends at pupation, the male ability to transfer a spermatophore could be constraint by the production of non-gametic material, such as apyrene sperm or materials involved in packaging the sperm. Under these conditions, male phenotypes could reflect male body condition and subsequent limitations in their reproductive potential. Yet, here, male body mass at emergence did not affect the phenotype of males, and we found no significant difference in the survival of occasional and regular males. However, it is important to point out that the present study was conducted using males of average body mass, and it remains plausible that variation in the total amount of energetic resources acquired during larval development and subsequent variations in body mass might affect the future mating phenotype of a male. Supporting this assumption, a recent study demonstrated that song and sperm production were affected by larval diet in *A. grisella* (Cordes, Albrecht et al. 2015). The role of developmental conditions on mating phenotypes has been demonstrated in various species, such as *Onthophagus* beatles, in which underfed larvae develop into small males that sneak matings, while well-fed larvae develop into large horned fighting males (Eberhard and Gutierrez 1991).

To conclude, our results clearly demonstrate that eupyrene sperm constitute a limiting resource for *A. grisella* males' reproductive success. More surprisingly, our results revealed a discontinuous distribution in male mating behaviors, suggesting the strategic allocation of copulations and sperm resources. Such a variation in reproductive phenotypes requires further investigations, in particular to understand whether and to what extent such behaviors are adaptive and result from male physiological constraints.

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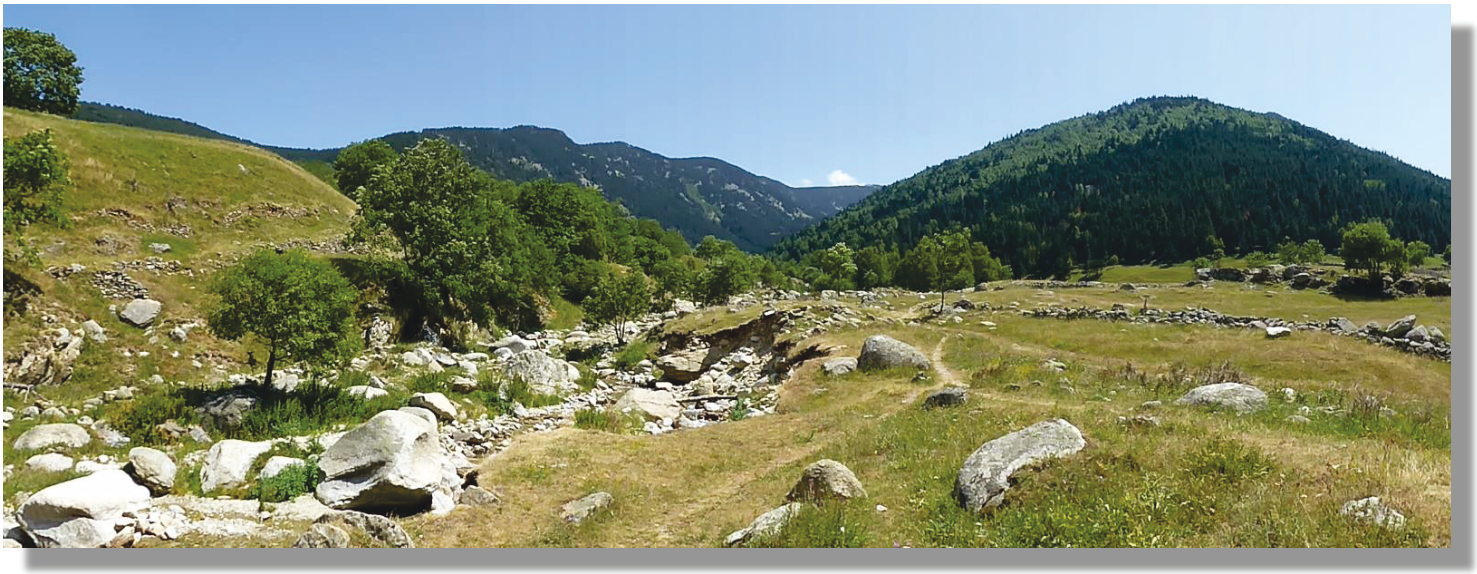
CHAPITRE 3 : Allocation des ressources non gamétiques chez *Ephippiger diurnus*



Le Col de Mantet, habitat naturel de *E. diurnus.* Crédit photo : A. Jarrige

CHAPITRE 3. Allocation des ressources non gamétiques chez *E. diurnus*

Lors de l'accouplement, les mâles transfèrent souvent bien plus que leurs spermatozoïdes aux femelles. Tout d'abord, les éjaculat se résument rarement aux gamètes, et consistent généralement en un cocktail de substances parmi lesquelles on trouve, entre autres, des protéines, des carbohydrates, des lipides et de l'eau (Poani, 2006). Ces substances jouent souvent un rôle fondamental dans la concrétisation des chances de paternité des mâles, notamment en affectant la physiologie de la femelle, son immunité ou son comportement reproducteur (Perry et al. 2013, South & Lewis 2011). La quantité de ces substances para-spermatiques peut parfois être extrême, et aboutir à des transferts de substances non gamétiques pouvant atteindre entre 30 et 40% du poids du mâle (Gwynne 2001). Aussi, tout comme le sperme, ces substances peuvent être couteuses à produire ou à collecter et fortement limiter le potentiel reproducteur des mâles (Boggs 1995 ; voir Chapitre 1, Partie 4.2), et sélectionner pour l'évolution d'allocations adaptatives de leur composition et de leur transfert aux femelles (Chapitre 1, Partie 4.2).

Le record de taille en ce qui concerne le poids des de ces transferts relativement au poids du mâle revient à la sauterelle *Ephippiger diurnus* (Orthoptera, Tettigoniidae) ou le spermatophore transféré aux femelles à l'issue de l'accouplement peut atteindre jusqu'à 40 % du poids du mâle (voir Chapitre 1, Partie 4.2). Ce spermatophore se compose d'une ampulla, contenant les spermatozoïdes, et d'un spermatophylax gélatineux que les femelles consomment à l'issue de l'accouplement. La production de ces donations s'avère extrêmement couteuse pour les mâles, et l'existence d'une période réfractaire de 48h à 72h suivant le transfert d'un spermatophore limite fortement la fréquence à laquelle les mâles peuvent s'accoupler (Voir Chapitre 1, Partie 4.2, et Encadré 2). Sous ces conditions, il est prédit que les mâles octroient prudemment leur spermatophylax à l'égard des bénéfices potentiels : en particulier, si les femelles varient dans leur qualité reproductive, il est attendu que les mâles transfèrent préférentiellement, ou ajustent la taille/qualité de leurs donations aux femelles de meilleure qualité.

Le troisième chapitre de ce manuscrit est donc consacré à l'étude des allocations aux allocations de ressources non gamétiques chez *E. diurnus.*

Le présent chapitre s'articule en deux parties. Dans une première partie, afin de réaliser des prédictions réalistes concernant les contraintes auxquelles sont soumis les mâles en conditions naturelles, et leurs conséquences sur les variations d'allocations de ressources non gamétiques, nous avons tout d'abord réalisé une étude de terrain au cours de laquelle nous avons mesuré la variation de qualité des femelles et du sexe ratio opérationnel au cours de la saison de reproduction. Dans un second temps, toujours en conditions naturelles, nous avons également étudié les préférences pré et post-éjaculat des mâles au regard de la qualité de la femelle (poids, Article 4).

Toutefois, lorsque l'éjaculat est complexe, les variations des différents éléments qui le composent peuvent avoir des effets contrastés sur la réponse sexuelle des femelles et le succès reproducteur des mâles (Gillot 2003). Aussi, dans un second temps, nous avons analysé la composition biochimique du spermatophylax (eau, acides aminés libres et protéiques), et considéré les variations de ces éléments au regard de la qualité des femelles (poids et âge, Article 5).

ARTICLE 4

Amino acid composition of the bushcricket spermatophore and the function of courtship feeding: variable composition suggests a dynamic role of the nuptial gift

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ABSTRACT

Nuptial gifts are packages of non-gametic material transferred by males to females at mating. These gifts are common in bushcrickets, where males produce a complex spermatophore consisting in a sperm-containing ampulla and an edible sperm-free spermatophylax. Two non-mutually exclusive hypotheses have been suggested to explain the function of the spermatophylax: the *paternal investment* hypothesis proposes that it represents a male nutritional investment in offspring; the *mating effort* hypothesis proposes that the spermatophylax maximises the male's sperm transfer. Because gift production may represent significant energy expenditure, males are expected to adjust their investment relative to the perceived quality of the female. In this study, we first examined the free and protein-bound amino acid composition of the nuptial gift in the bushcricket, *Ephippiger diurnus* (Orthoptera: Tettigoniidae). Second, we investigated whether this composition was altered according to female age and body weight. Our study represents the first investigation of both free and protein-bound amino acid fractions of a bushcricket spermatophylax. We found that composition of the nuptial gift varied both qualitatively and quantitatively with respect to traits of the receiving female: older females received larger amounts of protein-bound amino acids (both essential and non-essential), less water and less free glycine. This result suggests that gift composition is highly labile in *E. diurnus,* and we propose that gift allocation might represent a form of cryptic male mate choice, allowing males to maximize their chances of paternity according to to the risk of sperm competition that is associated with mate quality.

KEYWORDS: *Ephippiger diurnus*; free amino acids; male mate choice; protein bound amino acids; strategic allocation

INTRODUCTION

Nuptial feeding, *i.e* material donations transferred to the opposite sex at mating, is widespread among insects and encompasses a wide variety of forms, including prey, body parts and glandular secretions (Vahed 1998, Lewis and South 2012). One of the most intensively studied examples of courtship feeding can be found in bushcrickets (Orthoptera: Tettigoniidae), where males transfer the product of their accessory glands as an edible sperm-free spermatophylax attached to a sperm-containing ampulla, together forming the spermatophore (Gwynne 2001). Following copulation, the female consumes the spermatophylax while the sperm migrate to her genital tract (Gwynne 2001).

The function of nuptial feeding has been the focus of considerable debate among evolutionary biologists for several decades and two main, non-mutually exclusive, hypotheses have been proposed (Vahed 1998, Vahed 2007a, Lewis and South 2012). According to the *paternal investment hypothesis*, donations would consist of substances, potentially nutritive, that enhance female ‘condition’ (e.g. higher egg load or survival, larger eggs), and ultimately increase the number or quality of the male’s offspring. Alternatively, the *mating effort hypothesis* proposes that donations ‘protect’ the donor’s sperm by prolonging the female remating interval, hence reducing the risk of sperm competition. Current studies show that spermatophore function probably differs among bushcricket species (Wedell 1994a, Vahed 1998, Gwynne 2008).

Bushcricket spermatophylaxes mainly consist of water, protein (4-27 % of the wet mass), and a small amount of lipids (Heller et al. 1998, 2000). Female condition is thought to be predominantly determined by the amount of free and protein-bound amino acids obtained via the spermatophylax. In line with the parental effort hypothesis, protein-bound amino acids, especially essential ones, may strongly affect female fecundity, because vitellogenesis is protein-limited, (Wedell 1994ab, Wheeler 1996, Klowden 2013). In support of this hypothesis, ingested protein-bound amino acids have been found to be incorporated into female soma and eggs in bushcrickets (Wedell 1996, Rooney and Lewis 1999, Wedell and Karlsson 2003), and experimental evidence showed that gift consumption positively affects female longevity and reproductive output (Vahed 1998, Gwynne 2001, 2008). In contrast, free amino acids are phagostimulants in many insects and may instead improve the gift's gustatory appeal and/or texture (Simpson and Simpson 1992, Sakaluk 2000, Calatayud et al. 2002, Warwick et al. 2009, Gershman et al. 2012). Beside, the low concentration of free amino acids in the spermatophylax implies that these substances are unlikely to represent a significant contribution to the female's diet. Consequently, this fraction may be more indicative of the male's mating effort than his parental effort.

Despite the considerable variation in spermatophylax size between species (from 2 to 40 % of male body mass), gift-giving behaviors are costly for males and strongly limit their reproductive rate (Gwynne 2001, Lewis and South 2012). Consistent with this premise, males from various bushcricket species strategically allocate their resources by selectively mating with particular females, or by adjusting the size of their donation, and thereby protein content, according to i) female quality (Wedell 1996, Jarrige et al. 2013), ii) the risk of sperm competition (Simmons and Kvarnemo 1997) and iii) their own physiological condition (Wedell and Ritchie 2004, Jarrige et al. 2013). In contrast to other types of nuptial gifts transferred prior to mating (Vahed 1998), bushcricket spermatophylaxes are manufactured by male reproductive glands during copulation (Gwynne 2001). This opens the opportunity for cryptic male mate choice through the manipulation of the biochemical composition of the gift according to female quality.

However, to our knowledge, no study has yet jointly investigated the amino-acid composition of nuptial gifts and their variation with regard to female traits. In this study we focused on determining the free and protein-bound amino acid composition of spermatophylaxes produced by males in Ephippiger diurnus (Orthoptera: Tettigoniidae) and measuring the extent to which males modify spermatophylax composition with respect to traits (age and body weight) of the receiving female.

In this species, males produce an unusually large spermatophore (20-30 % of their body mass) that appears to be particularly costly: its transfer results in a 4-5 days refractory period (Wedell and Ritchie 2004).

Moreover, the nitrogen content of the spermatophylax decreases with successive matings (Wedell and Ritchie 2004, Jarrige et al. 2013), suggesting that at least part of these resources cannot be replenished via feeding. Consequently, males allocate their scarce resources strategically by modifying the size, and thereby the total protein content, of their donation according to mate quality (Jarrige et al. 2013).

The present study provides a detailed analysis of the amino acids content in the *E. diurnus* spermatophylax. Our results revealed quantitative, as well as qualitative, variations in the gift's amino acid composition according to female body mass and age. Together, these results indicate that gift production represents a plastic process by which males adjust their reproductive expenditure according to the potential fitness returns associated with mate quality.

METHODS

Rearing and maintenance

Ephippiger diurnus (Dufour, 1841) (Orthoptera: Tettigoniidae) used in experiments were offspring of individuals collected in the field at Col de Mantet (42°29'N, 2°3'E, Pyrénées Orientales, France) in July 2008. Eggs were cultured following the standard methods for this species (Hartley and Dean 1974) which consisted of two diapauses of 60 days at 4 ± 2°C, separated by an interval of 120 days during which eggs were kept at 20 ± 2°C. Eggs were placed on cotton covered by filter paper in Petri dishes and regularly sprayed with a 1% methyl-4-hydroxybenzoate solution to prevent desiccation and mold development. Nymphs were reared individually in 5 cm diameter x 8 cm height plastic containers and fed *ad libitum* with cabbage bee pollen, and flaked goldfish food. After the final molt, adults were individually transferred to larger plastic cages (10 cm diameter x 15 cm height) and provided with the same diet as nymphs. Rearing and experiments took place in environmental chambers maintained at 25 ± 2°C on a L:D 16h:8h cycle.

Mating and spermatophores

Mating: To obtain spermatophores, 28 males aged from 25 to 35 days after final ecdysis were randomly paired with virgin females ranging from 16 to 63 days old; 24 females were 24-to-49 days old, 1 female was 16 days old and 3 females were between 56 and 63 days old. *E. diurnus*, have a long lifespan, and females within this age range are able to reproduce and produce viable eggs. Mating took place between 8am and 15pm, the peak period for singing and mating in *E. diurnus* (Busnel 1955). Males and females were weighed on a microbalance (± 1 mg; Mettler-Toledo, Greifensee, Switzerland) prior to mating sessions, and female age was recorded.

Spermatophore collection and analysis: Immediately after mating, spermatophores were carefully removed from the females' genitalia. Fresh ampullae and spermatophylaxes (SPFx) were weighed separately on a microbalance (± 1 mg; Mettler-Toledo, Greifensee, Switzerland). We estimated the water content of a spermatophylax by comparing its fresh and dry weight. Desiccation was achieved by freeze-drying (primary drying: 1 h at -10°C, 25 mbar, secondary drying: -76°C, 0.001 mbar overnight; Bioblock Scientific Alpha1-4LDplus lyophilizator). The dried subsamples were ground to powder with a mortar and stored at -80°C until subsequent analysis.

Free and protein-bound amino acid analysis

From a subset of 5 mg of powdered spermatophylax, free amino acids were extracted with 1.2 mL acetonitrile 25 % in HCl 0.01 N (1:3, v:v). From another subset of 5 mg of powdered spermatophylax, proteins were hydrolyzed into their protein-bound amino acids in a sealed glass tube at 150°C for 2 h with 500 µL of 4 M methanesulfonic acid after flushing out air with a gentle stream of nitrogen gas. Following hydrolysis, the hydrolysates were partially neutralized with 1 mL sodium carbonate 1 M. Prior to analysis, samples were transferred to a 1.5 mL Eppendorf tube, and pH was checked to confirm that it was between 1.5 and 5.0. Free and hydrolyzed protein-bound amino acids were extracted and derivatized as described in the EZ:faast amino acid analysis kit (Phenomenex Ltd, Aschaffenburg, Germany). Subsequent samples were then concentrated under a stream of nitrogen gas and immediately injected into the GC-MS system composed of an AutoSystem XL gas chromatograph (ZB-AAA column (10 m x 0.25 mm), Phenomenex Ltd) coupled to a TurboMass mass spectrometer (Perkin-Elmer, Courtabœuf, France). Helium served as the carrier gas and its flow was held constant at 1.1 mL/min. The oven temperature program was a 30°C/min ramp from 110°C to 320°C, with the temperature of the injection port maintained at 250°C. The MS ion source (electronic impact) and inlet line temperatures were 200°C and 310°C, respectively. The scan range was 3.5 scans/s and atomic masses between 45-450 daltons were detected. Under these conditions, a 2 μL sample was injected in splitless mode during 30 sec. We used Norvaline at 200 nmol·mL^{-1} as an internal standard. Calibration curves for each of the standard physiological amino acids were produced using an original concentration of 200 nmol·mL^{-1}. Chromatogram analyses were completed using the TurboMass™ Software (version 5.4.2; Perkin-Elmer, Courtabœuf, France). The total free and protein bound amino-acid content of spermatophylaxes were calculated by multiplying their content in 1 mg of dry spermatophylax by the spermatophylax total dry weight.

Statistical analysis

Relationships between amino acid content and female body mass, female age, and male body mass were investigated using Spearman correlation tests, as most of the data were non-normally distributed. To account for the number of comparisons being performed and avoid false positives, the alpha value was adjusted following the Benjamini-Hochberg procedure with a false discovery rate of 0.10 (Benjamini and Hochberg 1995, Reiner et al. 2003).

All data were analysed using SigmaStat 3.5 Software.

RESULTS

Spermatophore composition:

Data are presented as mean ± SEM in table 1, and mean ± SEM (median, Q1, Q3) in the text, except for percentages. Spermatophores weighed on average 823.00 ± 24.33 (Md: 791.98, Q1: 637.39, Q3: 892.13) mg and contained a spermatophylax of 678.44 ± 20.24 (Md: 659.56, Q1: 612.65, Q3: 733.36) mg. Spermatophylax and ampulla weights were positively correlated (r_s = 0.49, P = 0.007, N = 28). Water represented 85.12 ± 0.31 (Md: 85.34, Q1: 80.83, Q3: 86.05) % of the spermatophylax fresh mass. In total, spermatophylaxes contained 75.81 ± 7.16 (Md: 55.68, Q1: 35.04, Q3: 95.64) mg of amino acids, representing 11.15 ± 1.03 % of their fresh mass and 74.20 ± 6.46 % of their dry mass. Free amino acids represented only 4.78 ± 0.34 % of this amount, while protein-bound amino acids represented the remaining 95.22 ± 0.34 %. Amino acid composition is detailed in Table 1. Two pairs of amino acids, serine and threonine, and cysteine and phenylalanine, were co-eluted, and our data therefore show the combined amounts of each pair (Table 1).

Free amino acids: In total, the spermatophylaxes contained on average 3.12 ± 0.17 (Md: 3.26, Q1: 0.97, Q3: 3.66) mg of free amino acids. Most amino acids (2.50 ± 0.14, Md: 2.40, Q1: 6.31, Q3: 2.81 mg) were non-essential, with glycine predominating (70.46 ± 2.5 % of the total free amino acid content). Essential free amino acids, including serine and cysteine, represented only 0.62 ± 0.07 (Md: 0.52, Q1: 0.15, Q3: 0.73) mg (Table 1).

Protein-bound amino acids: Spermatophylaxes contained a total of 72.70 ± 7.11 (Md: 52.76, Q1: 32.49, Q3: 92.15) mg of protein-bound amino acids. In this fraction, leucine, lysine and histidine, all essential amino acids, dominated, accounting for respectively 20.94 ± 1.06 %, 18.06 ± 1.09 % and 11.54 ± 1.17 % of the total. Essential amino acids formed the major part of this fraction with 54.74 ± 5.89 (Md: 40.31, Q1: 23.75, Q3: 68.73); 70.56 ± 1.29 %) mg (Table 1).

Male body mass:

The amount of non-essential amino acids of both types, free and protein-bound, was negatively correlated with male body mass (Table 2). In contrast, spermatophylax water content increased with male body mass (Table 2).

Female body mass and age:

No significant relationship was found between female body mass and the general features or amino acid content of the spermatophylax they received (i.e. mass, water content and ampulla weight), although larger females tended to receive more essential free amino acids than smaller females (Table 2). In contrast, older females received larger spermatophylaxes (Table 2 & Fig.1a), containing more protein-bound amino acids, and less water and free glycine (Table 2 & Fig. 1b,d,e,f). They also received a heavier ampulla (Table 2 & Fig. 1c). No significant relationship between female age and the total amount of free amino acids (without glycine) contained in the spermatophylax was found (Table 2). Female age and body weight were not correlated (r_s= 0.09, p = 0.65, N = 28).

Table 1

	Free amino acids (mg)	Protein-bound amino acids (mg)	Free amino acid (% total)	Protein bound amino acids (% total)
Ala	0.04 ± 0.004	0.82 ± 0.09	1.51 ± 0.22	1.17 ± 0.05
Gly	2.20 ± 0.14	3.96 ± 0.30	70.46 ± 2.5	5.85 ± 0.34
Val *	0.09 ± 0.01	5.70 ± 0.57	3.09 ± 0.27	8.08 ± 0.37
Leu *	0.09 ± 0.09	15.13 ± 1.72	2.82 ± 0.21	20.94 ± 1.06
Ile *	0.01 ± 0.002	3.20 ± 0.41	0.42 ± 0.07	4.33 ± 0.26
Thr* + Ser	0.05 ± 0.01	0.72 ± 0.07	1.74 ± 0.19	1.10 ± 0.10
Pro	0.07 ± 0.01	3.18 ± 0.24	2.52 ± 0.23	4.72 ± 0.29
Asn	0.04 ± 0.01	0.14 ± 0.02	1.27 ± 0.21	0.19 ± 0.02
Arg+ Asp	0.03 ± 0.004	1.024 ± 0.20	1.08 ± 0.30	1.43 ± 0.21
Met *	0.003 ± 0.001	0.42 ± 0.08	0.10 ± 0.01	0.67 ± 0.12
Glu	0.53 ± 0.02	4.19 ± 0.60	1.60 ± 0.41	6.21 ± 0.72
Phe * + Cys	0.010 ± 0.02	6.71 ± 0.87	3.36 ± 0.53	9.07 ± 0.65
Gln	0.01 ± 0.001	0.06 ± 0.02	0.15 ± 0.03	0.10 ± 0.04
Lys*	0.004 ± 0.001	13.95 ± 1.92	0.14 ± 0.02	18.06 ± 1.09
His *	0.25 ± 0.054	8.71 ± 1.21	7.59 ± 1.40	11.54 ± 1.17
Tyr	0.06 ± 0.012	4.59 ± 0.61	1.79 ± 0.31	6.28 ± 0.51
Trp *	0.011 ± 0.003	0.20 ±0.04	0.35 ± 0.07	0.27 ± 0.05
Essential	0.62 ± 0.07	54.74 ± 5.89	0.97 ± 0.15	70.55 ± 1.29
Non-essential	2.50 ± 0.14	17.96 ± 1.62	3.82 ± 0.26	24.67 ± 1.15
Total	**3.12 ± 0.17**	**72.70 ± 7.11**	**4.78 ± 0.34**	**95.22 ± 0.34**

Table 1. Composition (mean ± sem) of *E. diurnus* spermatophylaxes in free and protein-bound amino acids. N = 28.

* indicates essential amino acids (Dadd, 1985).

Table 2

	Male body mass (mg)	**Female body mass (mg)**	**Female age (days)**
Spermatophylax features			
Mass (mg)	r_s = 0.01, p = 0.95, α = 0.1	r_s = - 0.14, p = 0.48, α = 0.06	**r_s = 0.49, p = 0.008, α = 0.04**
Water content (% of fresh mass)	**r_s = 0.57, p = 0.002, α = 0.01**	r_s = - 0.16, p = 0.42, α = 0.02	**r_s = - 0.59, p < 0.0001, α = 0.01**
Ampulla weight (mg)	r_s = - 0.15, p = 0.44, α = 0.07	r_s = - 0.14, p = 0.48, α = 0.07	**r_s = 0.60, p < 0.0001, α = 0.02**
Free amino acids (mg)			
Essential	r_s = - 0.27, p = 0.17, α = 0.05	r_s = 0.43, p = 0.02, α = 0.01	r_s = 0.15, p = 0.46, α = 0.1
Non-essential (without glycine)	**r_s = - 0.39, p = 0.04, α = 0.04**	r_s = 0.05, p = 0.78, α = 0.09	r_s = 0.30, p = 0.12, α = 0.08
Glycine	rs = - 0.13, p = 0.52, α = 0.08	rs = - 0.04, p = 0.84, α = 0.1	**rs = - 0.39, p = 0.04, α = 0.07**
Total	rs = - 0.40, p = 0.04, α = 0.03	rs = 0.16, p = 0.43, α = 0.04	rs = 0.23, p = 0.23, α = 0.09
Protein-bound amino acids (mg)			
Essential	rs = - 0.12, p = 0.54, α = 0.09	rs = 0.16, p = 0.42, α = 0.03	**rs = 0.51, p = 0.005, α = 0.03**
Non-essential	**r_s = - 0.48, p = 0.01, α = 0.02**	r_s = 0.10, p = 0.61, α = 0.08	**r_s = 0.41, p = 0.03, α = 0.06**
Total	rs = - 0.24, p = 0.22, α = 0.06	rs = 0.15, p = 0.43, α = 0.05	**rs = 0.50, p = 0.008, α = 0.05**

Table 2. Relationship between male and female general features and amino acid content of spermatophylaxes. Spearman rank correlation, α-value are given following Benjamini-Hochberg procedure and for a false discovery rate of 0.10. N = 28. Significant results are in bold.

Figure 1

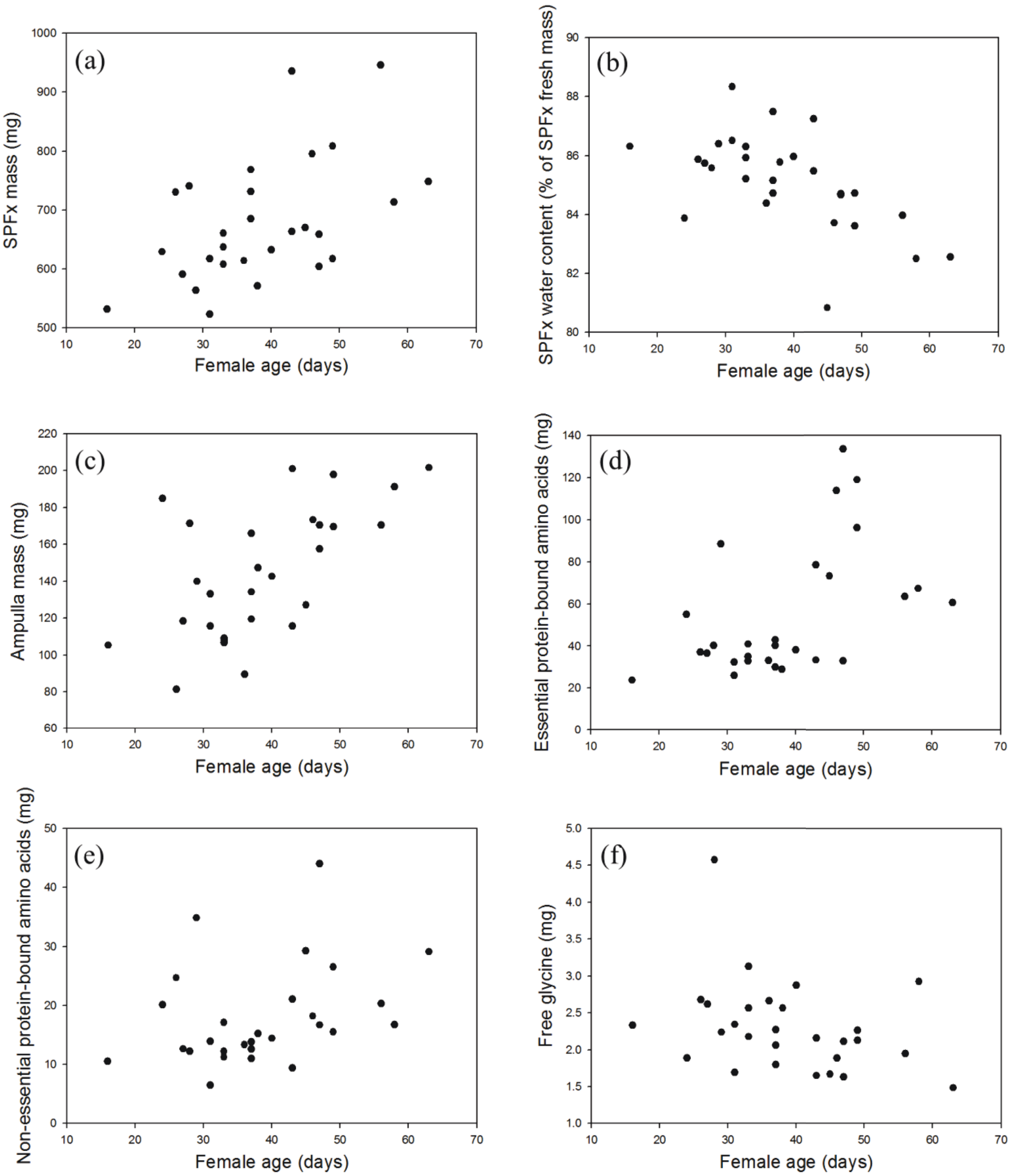

Fig. 1 Relationship between female age and (a) spermatophylax mass; (b) spermatophylax water content, (c) ampulla mass; (d) essential protein-bound amino acids; (e) non-essential protein-bound amino acids and (f) free glycine.

DISCUSSION

This study is the first to simultaneously investigate the free and the protein-bound fractions of bushcricket nuptial gifts. Consistent with previous findings in *E. diurnus* (Wedell 1994b, Jarrige et al. 2013), spermatophylaxes consisted of approximately 85 % of water and 11 % of amino acids. More detailed measurements revealed that the amino acid fraction consisted of 5 % of free amino acids and 95 % of protein-bound amino acids. Among free amino acids, glycine was largely predominant. However, glycine was not abundant in the protein-bound fraction, most of which were essential amino acids (Table 1). Larger males produced spermatophylaxes containing more water and a lower amount of non-essential amino acids (both free and protein-bound) (Table 2). These adjustments could reflect a trade-off between somatic maintenance and gift production, as somatic costs may increase with body mass. However, our results revealed that the most significant variations in gift composition were related to female traits. Indeed, males transferred a larger amount of protein-bound amino acids (both essential and non-essential), less water and less free glycine to older mates (Table 2). These relationships suggest that *E. diurnus* males adjust the amino-acid composition of their nuptial gifts, and that these adjustments may represent an adaptation for maximizing reproductive success when mating with females of variable 'quality'.

In nature, sexually receptive mates can be rare and of variable quality (Jarrige et al. 2015, in prep). Rejecting partners would not be advantageous. In contrast, by adjusting the composition of nuptial gifts to female quality, males could invest the adequate resources, thereby moderating their loss in reproductive potential (Vahed 2007) without dismissing any mating opportunity. Because physical contact during pre-mating interactions, as observed in *E. diurnus* (Busnel

1955), could provide males with reliable cues about female quality (Edward and Chapman 2011), we propose that the observed modifications in amino acid composition reflect a form of cryptic male mate choice.

In *E. diurnus,* recent field observations suggested that female body mass is a poor predictor of fecundity, compared to age (Jarrige et al., 2015 in prep.). Indeed, for a similar mass, young females carry few mature eggs but plenty of body fat, while older females carry more mature eggs but less fat (Jarrige et al. 2015, in prep.). Due to their age, older females are also less likely to mate again. These factors, combined with the last male sperm precedence effect observed in *E. diurnus* (Hockham et al. 2004), reduce the risk of sperm competition in such females. It would therefore be advantageous for males to provide older females with more nutritious donations in order to extend their survival, fecundity, and ultimately the number of offspring they would sire. Conversely, males mating with younger females would benefit from increasing an investment that prolongs their mate's refractory period. Thus, males would lower the high risk of sperm competition expected in this situation.

Our detailed analysis of gift composition in amino acids allow us to infer possible functions of the spermatophore in *E. diurnus*. In insects, protein content of a nuptial gifts is often considered as a measure of its nutritive quality because female vitellogenesis is protein-limited (Wheeler 1996, Klowden 2013). Hence, females might use protein-bound amino acids in the spermatophylax to sustain their metabolic and/or reproductive activities. In contrast, free amino acids would only marginally contribute to a female's diet, because they are present at extremely low amounts in the spermatophylax (Table 1). Their presence, however, might strongly affect the male fertilizing success by acting as a phagostimulant: it would lengthen the duration of female gift consumption (Calatayud et al. 2002, Warwick et al. 2009, Gershman et al. 2012), hence potentially favoring sperm transfer into the female genital tract. High levels of free glycine may enhance this effect by increasing the gummy consistency of the spermatophylax, which prolongs its handling time by females (Heller et al. 1998, Warwick et al. 2009). This small amino acid has also been found to increase female's refractory period (Heinrich et al. 1998, Calatayud et al. 2002, Warwick al. 2009, Gordon al. 2012), thus reducing her remating rate. Finally, because water is not limited in the natural habitat of *E. diurnus*, its presence in the spermatophore is unlikely to represent a critical contribution to female diet.

In summary, older females received significantly more essential and non-essential protein-bound amino acids, less water and free glycine and, although not reaching significance after correction for multiple analyses, larger females tended to receive a larger amount of essential free amino acids. This pattern suggests that *E. diurnus* males provide older females with more nutritious gifts containing less manipulative or deceiving substances, while larger females tend to receive more substances likely to exploit their sensory responses. Thus, the spermatophylax may function primarily as mating effort in younger females in which sperm competition can be high, whereas it would increase paternal investment in older, potentially more fecund females, in which sperm competition is reduced but survival limited (Hockham et al. 2004, Jarrige et al. 2013). Moreover, the positive relationship between spermatophylax and ampulla size suggests that the gift may simultaneously serve as a sperm protection device (mating effort) (Table 2).

The notion that nuptial feeding can concomitantly functions as parental and mating effort within a species is not novel, and has been documented in numerous taxa (Reinhold and Heller

1993, Vahed 1998, Vahed 2007b, Gwynne 2008, Lewis and South 2012). However, our study provides evidence that males are not only able to adjust the size of their donation, but also to finely tailor its amino acid content in regard to female traits. Such a sophisticated manipulation of proteins has already been observed in the ejaculate of *Drosophila melanogaster* (Sirot et al. 2011). Together with the present work, it suggests that strategic allocation of amino-acid and proteins might be widespread, especially in species where mating involves a costly, complex product of male metabolism.

Importantly, the potentiality that males might strategically tailor spermatophylax composition according to mate quality put into question the classical attempt to sort nuptial gift into the dichotomous *mating* or *parental* effort function, because the confounding effect of cryptic male mate choice would render gift function highly labile within the successive mating episodes of the same individual. More theoretical and empirical studies are therefore required to investigate the extent to which these variations affect the fitness of males and females and their evolutionary consequences on both sexes.

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ARTICLE 5

Pre and post-copulatory male mate choice under natural conditions in a bushcricket

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En préparation pour Animal Behaviour

ABSTRACT

Contrasting with a long assumed idea, reproduction also incurs substantial costs to males. In particular, ejaculate (spermatozoa and other non-gametic materials) can be costly to produce, and strongly constraint male's lifetime reproductive potential. Therefore, males, like females, might use complex strategies to optimize the pay-off of each reproductive event. For example, in species where females vary in quality, males might obtain a greater fitness return by strategically allocating their reproductive resources to females that show the highest reproductive potential. However, if male preferences for mate quality have been well documented under laboratory controlled conditions, the few field studies investigating male preferences suggest that the expression of choice by males is scare under natural conditions. Thus, the importance of male mate choice and the factors affecting its evolution in the wild is not yet clear, surging the need for further investigations.

In the present study, we investigated male mate choice under natural conditions in *Ephippiger diurnus*. During mating, males provide females with one of the largest spermatophores described in bushcrickets. For males, the production of this donation represents a significant energy expenditure, which strongly limits the rate at which they can transfer a spermatophore. Therefore, we predicted that males would adjust their investment relative to the perceived quality of the female. Here, we investigated *E. diurnus* male pre and post-copulatory selectivity for female quality (body mass) in the field during two successive periods of the reproductive season. Besides, in order to better understand the natural conditions that may influence the selective constraints and pressures encountered by males, we also measured the sex ratio, operational sex ratio and variance in female quality (female body mass, mating status and number of mature and immature eggs in their ovaries), over the two investigated periods. During both study period, OSR was strongly biased toward males, and males attempted to cease any mating opportunity that was presented to them. Beside, males transferred larger gift toward larger females, and increase their gift expenditure over the reproductive season.

KEYWORDS: *Ephippiger diurnus*, male mate choice, spermatophore, female quality, operational sex ratio

INTRODUCTION

The Darwin-Bateman paradigm constitutes the bedrock of the modern conceptual view of sexual selection: while females' fitness is predominantly constrained by a lack of resources, males' chances of paternity are mainly limited by their access to females' ova, resulting in the evolution of distinct sex roles with, in most cases, selective females and competitive males (Bateman, 1948; Darwin, 1872; Dewsbury, 2005). However, accumulated evidences from different taxa show that sex roles are much more diverse than predicted under this classical view of sexual selection theory (Bonduriansky, 2001; Gowaty, Steinichen, & Anderson, 2003; Rillich, Buhl, Schildberger, & Stevenson, 2009; Rosvall, 2008). In particular, it is increasingly clear that reproduction also incurs substantial costs to males, which stem from ejaculate production (spermatozoa and other non-gametic materials), mate attraction and/or competition with rival males to access to females (Scharf, Peter, & Martin, 2013). In addition, mate attraction and intra-sexual competition can be major causes of increased injury or predation risk (Scharf et al., 2013). Therefore, males, like females, might use complex strategies to optimize the pay-off of each reproductive event. For example, in species where females vary in quality, males might obtain a greater fitness return by strategically allocating their reproductive resources to females that show the highest reproductive potential (Edward & Chapman, 2011; Hoefler, 2007; Wedell, Gage, & Parker, 2002). As for female choice, male preferences can be exerted either pre-copulatory by mating preferentially with particular females over others, or post-copulatory through strategic allocations of the ejaculate (sperm or other non gametic materials) according to the female reproductive quality (Edward & Chapman, 2011).

However, despite an increasing amount of studies investigating male mate choice, only a very few have been carried out under natural conditions (but see MacLeod & Andrade, 2014). Although laboratory studies can probe the ability of males to discriminate among female phenotypes, they cannot establish the frequency or consequence of male mate choice in nature (e.g. Jennions & Petrie, 1997). In particular, ecological factors, both biotic and abiotic, can play a crucial role in determining the dynamic of mating interactions and mate choice. For example, the relative abundance of sexually receptive females and rival males can exert strong influence on the evolution of male mate choice, because this will determine the costs and benefits associated with choosiness (Edward and Chapman, 2011). Such costs would involve time and/or energy losses when prospecting for mates, increased predation risk, and/or loss of mating opportunities (Scharf et al. 2013). Thus, the frequency and importance of male mate choice under natural condition is not yet clear (Edward & Chapman, 2011) and field studies are urgently needed (Barry & Kokko, 2010).

In the present study, we aimed to investigate male mate choice under natural conditions in *Ephippiger diurnus*, (Orthoptera: Tettigoniidae), a flightless bradyporine bushcricket commonly found in the Mediterranean area of southern Europe (Duijm, 1983; Oudman, Duijm, & Landman, 1990). During mating, *E. diurnus* males provide females with one of the largest spermatophores described in bushcrickets, consisting in a large sperm free spermatophylax (up to 30 % of the male body mass) attached to a sperm containing ampulla (Wedell & Ritchie, 2004). Once the copulation ends, the female consumes the gelatinous spermatophylax while the sperm contained in the ampulla enters her genital tract. Following this transfer, the male enters a 48-72-h sexual refractory period during which he neither sings nor courts females (Busnel, 1955). Therefore, a male's lifetime capacity to transfer spermatophores is strongly limited, a condition under which it would be advantageous for them to choose which female to mate with and/or to adjust its donation in relation to the reproductive value of the female that they met. Along these lines, previous laboratory studies suggested that males adjusted their allocation according to the female traits (age and body size; Jarrige, Greenfield, & Goubault, 2013; Jarrige, Riemann, Goubault, & Schmoll, 2015).

Here, we investigated *E. diurnus* male pre and post-copulatory selectivity for female quality (body mass) in the field during two successive periods of the reproductive season. Besides, in order to better understand the natural conditions that may influence the selective constraints and pressures encountered by males, we also measured the sex ratio, operational sex ratio and variance in female quality (female body mass, mating status and number of mature and immature eggs in their ovaries), over the two investigated periods.

MATERIALS AND METHODS

We studied a field population found at 1700 m in Col de Mantet, Pyrénées Orientales, France (42, 28°'N, longitude 2, 18°'E), during two periods in summer 2014 (12th - 23rd August and 12th - 24th September 2013). Under natural condition, the reproductive season is relatively brief, as the first adults typically emerge in July and the reproductive season peaks between mid-August and mid-September. Although adults can live up to 4 months under laboratory conditions, the life expectancy of adults is markedly shorter in the field, as individuals die with the first frosts, generally occurring early October at this altitude. Only eggs overwinter in the soil, resulting in non-overlapping generations.

Mate attraction and copulation: As typically observed in Tettigoniids, *E. diurnus* males group and display intense acoustic calls to attract sexually receptive females from the vicinity (Busnel, 1967; Duijm, 1983). Calling and mating activities usually take place from sunrise to noon, under dry and sunny weather (Busnel, 1955). The courtship song is produced by the friction of a toothed structure located at the basis of the vestigial forewings and consists in stridulations of 4-5 syllables in this population. Previous work on this population showed that females prefer males whose songs include a number of syllables higher than average or with higher amplitude (Ritchie, 1992; Ritchie, 1996).

The mating sequence of this species has been described (Busnel, 1955; Duijm, 1983; Stiedl & Kalmring, 1989): upon a female's approach (60 cm or closer), a male ceases calling and both individuals may *tremulate* (i.e. drums rapidly their abdomen on the substrate; (Busnel, 1955; Stiedl & Kalmring, 1989). After antennal contact, the male presents his abdomen to the female's head. The female mounts on the male's back and the pair joints their genitalia and proceeds to copulation. At each step, any individual can interrupt the mating sequence by leaving and prospecting for another mate.

Measure of sex ratio:

To measure the natural sex ratio, we delineated squared quadrats of 5.20 m side length, and thoroughly collected all individuals present within. The location of quadrats was chosen randomly and on average two quadrats were explored every other day.

Age, sex and sexual maturity (juvenile or adult) of individuals were noted and collected individuals were placed individually in 15 x 9 cm (height x diameter) plastic containers with mesh covers. Males and females were provided *ad libitum* with flowers constituting the natural diet of *E. diurnus*, completed with 4 pollen pellets and a pinch of dehydrated fish flakes. Individuals were then brought to our field facilities and housed under natural photoperiod and temperature. Males and females were kept in separate chambers until their sexual receptivity was tested (see below) on the following day. For each period (August and September), the sex ratio was obtained by dividing the total number of collected males by the total number of collected females.

Operational sex ratio

The operational sex ratio (OSR) at a given time was defined by the quotient of the number of males ready to mate on the number of sexually receptive females. Therefore, we measured the OSR by testing the sexual receptivity (see below) of individuals collected within quadrats. At a given period (August or September), the operational sex ratio was obtained by dividing the total number of sexually receptive males by the total number of sexually receptive females.

Male sexual receptivity: In *E. diurnus*, sexually mature males only sing when they are physiologically able to mate and transfer a spermatophore (Busnel, 1955; Jarrige et al., 2013; Wedell & Ritchie, 2004). We thus investigated male sexual receptivity by observing male singing behaviour on the morning following their capture. Observations took place from 9 am to 12 am, and males observed calling at least once during this period were considered as sexually receptive while males remaining silent were categorized sexually unreceptive.

Female sexual receptivity: We determined the sexual receptivity of females by measuring their phonotactic response to a standard male call broadcasted with a loudspeaker, following a method successfully tested in the laboratory in July 2012. The experimental setting consisted in a 3.4m mesh platform suspended at 1.20m from the floor and at the extremity of which was placed a loudspeaker. Females were released at the opposite end of the platform, at 3m from the loudspeaker. To avoid the confounding effect of stress in the observed behaviour, females were released by opening their plastic container without direct manipulation. The broadcast of a male synthetic song (see below) was initiated upon female's release. Each female was tested twice during 2 trials separated by at least 40 minutes. Females were considered sexually receptive when they reached and actively investigated a zone of 20 cm around the loudspeaker in both trials. After both trials, females were weighed with a field scale (± 0.1 mg, model Kern CM 150-1N -S3, Kern and Sohn, Balingen, Germany).

Courtship song playbacks: To construct the synthetic courtship call, we recorded the songs of males with an electret measurement microphone (model CM16/CMPA; Avisoft Bioacoustics; Berlin, Germany; frequency response: ± 3 dB, 20-150 kHz) whose output was amplified (preamplifier adaptor; Avisoft Bioacoustics; Berlin, Germany) and sent to a digital tape recorder (model HD-P2; Tascam; Tokyo, Japan). The analogue digital converter in the tape recorder sampled the song at 192 kHz and 16 bits. From this song, we constructed a 2 minutes synthetic call by selecting high syllable chirps (5 and 6 syllables) and editing them at the rate of 25 chirps per minute, corresponding to the third quartile for this trait in the studied population (Jarrige et al. 2013). The subsequent file was played using signal processing software on a notebook computer (Avisoft-SASLab Pro, Avisoft Bioacoustics; Berlin, Germany) and sent to the broadcasting loudspeaker (model UltraSoundGate Player BL/Pro 35 70115, Avisoft Bioacoustics, Berlin, Germany).

Variance in female quality

Subsequently to female receptivity trials, we assessed the variance in female reproductive quality by investigating their mating status (virgin or non-virgin) as well as the number and maturity of their eggs present in their ovaries. To do so, a randomly chosen fraction of the collected females were weighed on a field scale (± 0.1 mg, model Kern CM 150-1N -S3, Kern and Sohn, Balingen, Germany), then frozen for 10 minutes at -20 °C and killed in 70 % ethanol. After dorsal opening, ovaries and spermatheca were removed and placed in a small dissection pool. In *E. diurnus*, the maturity of eggs can be determined based on their coloration: immature eggs appear small and yellow, while mature eggs are larger and brown (see Figure 1a). Similarly, the mating status (virgin or non-virgin) was easily assessed by the visual observation of the spermatheca: this organ appears totally flat and transparent in virgin females which contrasts with the round, opaque and orange appearance found in previously mated females (see Figure 1b).

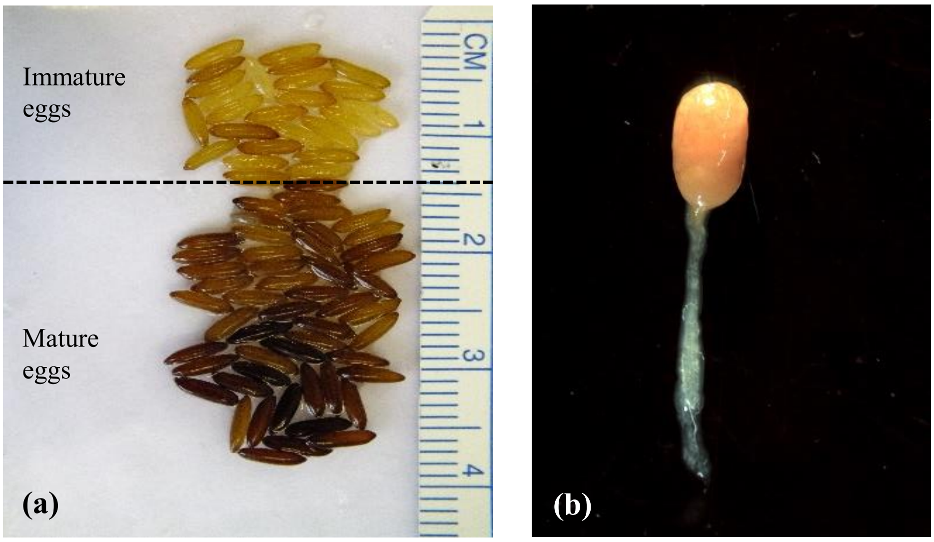


Figure 1: (a) Coloration criteria used for mature and immature egg characterisation. (b) Spermatheca of a mated female, appearing round shaped and orange.

Male mate preferences

Experiment 1: In this experiment, we investigated male and female precopulatory behaviours as well as the occurrence of mating according to female quality (body mass). Sexually receptive males were weighed on a Kern scale (± 0.1 mg, model CM 150-1N -S3, Kern and Sohn, Balingen, Germany), then transferred in 25 x 16 x 14.5cm (height x width x depth) plastic container containing mesh and wooden sticks. Subsequently, either a small (2.57 to 2.70 mg) or a large female (3.04 to 4.42 mg) was introduced directly into the male container. Females used in this experiment were collected from the field outside of the quadrats and kept isolated in plastic containers under the conditions described above (see *Measure of sex ratio*). To ensure that they were receptive, we then tested their sexual receptivity (see *Female sexual receptivity*) daily until they became receptive again.

After 40 minutes, if the pair did not mate, the female was gently removed. After a delay of 20 minutes, the male was provided with another female of a different body size. The absolute value of size difference between the two females ranged from 0.31 to 1.87 mg. The order of assignment of the smaller and larger females was random. For each mating trial, we monitored the occurrence of behaviours indicative of male and female motivation to mate with their current partner (see Table 1 for details). The observed behaviours were subsequently grouped in 2 main categories according to whether or not they reflected the individual's motivation to mate, by summing the number of occurrence of each behaviour within a category (Table 1). In males, strong predisposition to mate is manifested by tremulation behaviours, and/or by the presentation of its back to the female. In females, eagerness to copulate can be exerted through tremulation and palping behaviours as well as by attempting to mount on the male's back. Although male calling song reflects with certainty the male sexual receptivity, this behaviour was analysed separately because it was difficult to determine whether the song was displayed toward the presented female (motivation to mate) or, on the contrary, to attract another further female (mate rejection). In the present experiment, we only retained trials were both individuals undoubtedly perceived each other though body contact (e.g. antennal contact).

Male behaviours	Female behaviours
Motivation to mate	**Motivation to mate**
Approaching the female	Approaching the male
Tremulation	Tremulation
Presenting back to the female	Palping the male's back
	Mounting the male's back
Mate rejection or disinterest	**Mate rejection or disinterest**
Self-grooming	Self-grooming
Exploring the container	Exploring the container
Moving away from female	Moving away from male
Attacking the female	Attacking the male
Others	**Others**
Antennal contact	Antennal contact
Calling	

Table 1. Details of male and female behaviours monitored in *Experiment 1*. Tremulation: drumming of the body on the substrate. Exploration: individuals walk around in the container. Palping: the female palps the male back or abdomen with her pedipalps. Mouting: the female mount on the male's back.

To conduct meaningful investigation of the number of occurrence of male and female behaviours, we needed to retain only individuals that had a similar time duration to display behaviours and with two distinct females. Therefore, all the males that mated during one of the trials were excluded from subsequent analysis. Investigation of mating occurrence and spermatophore transfer was realized in experience 2 (see below).

Experience 2: In this experiment, we investigated male post-copulatory mating behaviours by analyzing the number of mating occurrence and the size of the spermatophore transferred according to the female quality. Prior to mating trials, receptive males were weighed on a Kern scale and individually transferred in 15 x 9 cm (height x diameter) cm plastic container containing mesh and wooden sticks. Subsequently, males were provided with a randomly chosen receptive female (see *Female sexual receptivity*) of known body mass. Females were left for a maximum of 2 hours and 30 minutes. If mating took place, the spermatophore was immediately removed from the female genitalia and weighed. If males did not mate with the presented female, they were transferred back in their plastic container and tested again the following day with another randomly chosen female.

Statistical analysis

All statistical analyses were performed using the computing environment R (R Development Core Team, 2012), with R Studio integrated development environment for R and the package *lme4* (Bates, Maechler, Bolker, & Walker, 2014).

Sex ratio, operational sex ratio and variation in female quality

We investigated the influence of the study period (August or September) on the sex ratio by pooling the observations from the different quadrats and comparing the proportion of males and females for each period using χ^2 comparison tests. A similar approach was used to compare i) the proportion of sexually receptive males and females (OSR) and ii) the proportion of virgin *versus* mated females between both periods. We tested for differences in

i) the number of juveniles ii) the number of mature and immature eggs carried by females between the two periods using generalized mixed models (GLMM, function *glm)* with a Poisson distribution of errors. The influence of female body mass on the number of mature and immature eggs carried by female was tested with GLM with a Poisson distribution of errors. The effect of the study period on female body mass was investigated with linear models (LM, function *lm*). Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality (Crawley, 2007).

Experiment 1

We used generalized linear mixed models (GLMM, function *glmer)* with a *Poisson* family to investigate the number of occurrence of male i) motivation, ii) rejection and iii) song behaviours. As explanatory fixed effects, we included male body mass, female body mass and the order of presentation of female (first or second). Furthermore, we included male and female identity as random effects to account for the fact that each male was sequentially presented with two females, and that some females were used more than once over the course of the experiment. We investigated the effect of male body mass, female body mass and female order on the occurrence of mating (yes/no) using generalized linear mixed models with a binomial family and including male and female identity as random effects.

The influence of female body mass, study period, male motivation behaviours and calling behaviours on the female motivation behaviours were tested using GLMM with a *Poisson* family and with male and female identity as random effects.

Experience 2

We investigated mating occurrence (yes/no) using GLMM with a *binomial* family. As fixed effects, we entered male body mass, female body mass, and study period, and included male and female identity as a random effect. We tested the influence of male body mass, female body mass and the study period on the mass of the spermatophore and the spermatophore mass as percentage of male mass using linear mixed effect analysis (LME, function lme), including male and female identity as a random effect. Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality

(Crawley, 2007). Significance of fixed effects was determined by removing the focal terms from a maximum likelihood fit of the model. P-values in the context of LME analyses always refer to the increase in model deviance when a term is removed from a model compared against a χ^2 distribution using a likelihood ratio test. All statistical tests were two-tailed and we rejected the null hypothesis at $p < 0.05$.

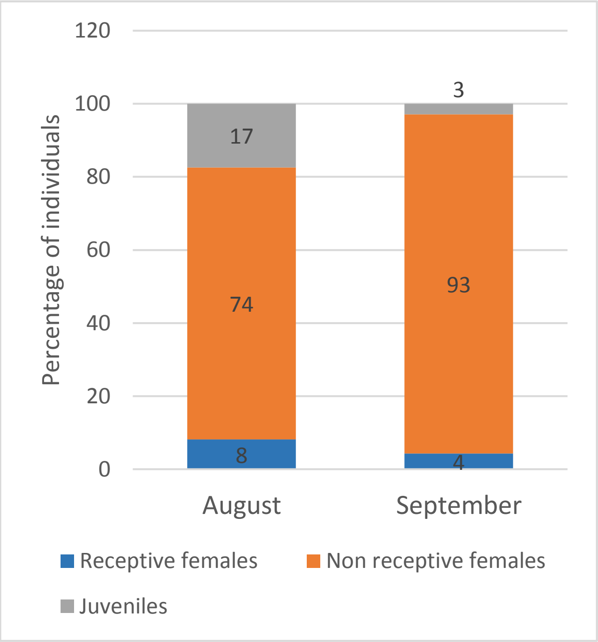
RESULTS

Measure of sex ratio

During this study, we investigated 33 quadrats (15 in August and 18 in September) within which 311 individuals were collected: 172 in August and 140 in September.

During both periods, the sex ratio was biased toward males with 1.28 males for one female in August and 1.07 in September. We found no difference in the proportion of males and females between both periods ($\chi^2 = 0.71$, df = 1, p = 0.40). Similarly, we found no difference in the number of juveniles collected between August and September (August: 15 juveniles, September, 2 juveniles; Fisher test, p = 0.53, Figure 2).

Operational Sex Ratio: overall 178 males and 139 females were tested for sexual receptivity (August: 86 females and 104 males; September: 70 females and 75 males). The OSR was highly biased toward males with 10.2 receptive males for one receptive female. We found no difference in the proportion of receptive and non-receptive individuals of both sex between the two periods (August: 62 receptive males, 42 non-receptive males, 7 receptive females, 64 non receptive females, OSR = 8.86; September: 40 receptive males, 35 non receptive males, 3 receptive females, 65 non-receptive females, OSR = 13.33; $\chi^2 = 4.73$, df = 3, p-value = 0.19; Figures 2 and 3).



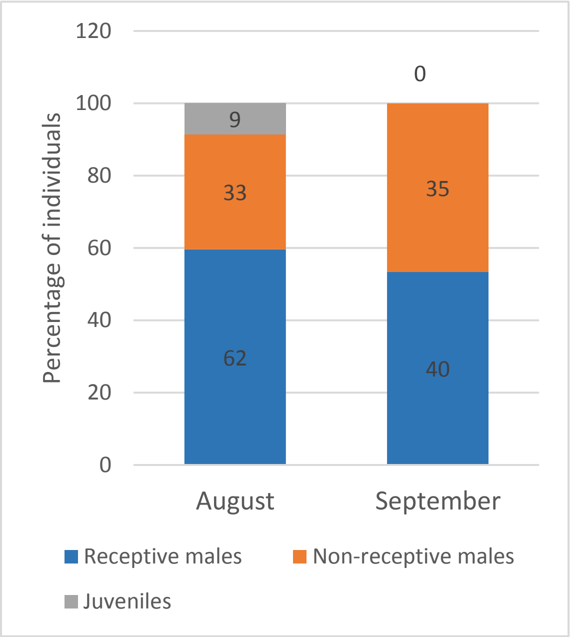


Figure 2. Percentage of sexually receptive, non-receptive and juvenile females according to the study period (August or September 2015). Figures indicate the number of individuals of each category.

Figure 3. Percentage of sexually receptive, non-receptive and juvenile males according to the study period (August or September 2015). Figures indicate the number of individuals of each category.

Variance in female quality

In total, 70 females were dissected in August and 68 in September. We found an highly significant difference in the propotion of sexually mature females beween August and September ($\chi^2 = 25.04$, df = 1, $p < 0.0001$, Figure 4): the majority of females were virgin in August while most of them were mated in September. In addition, females were significantly larger in September ($G_1 = 6.34$, $p < 0.0001$). We found a significant relationship between female body mass and the number of eggs, both mature and immature, present in their ovaries (respectively $z = 31.22$, $p < 0.0001$; $z = 30.44$, $p < 0.0001$). Similarly, the number of eggs carried by females strongly differed between both periods: in September females carried a greater total number of eggs ($z = 28.91$, $p < 0.0001$), both matures and immatures (respectively $z = 30.45$, $p < 0.0001$; $z = 12.34$, $p < 0.0001$; Figure 5).

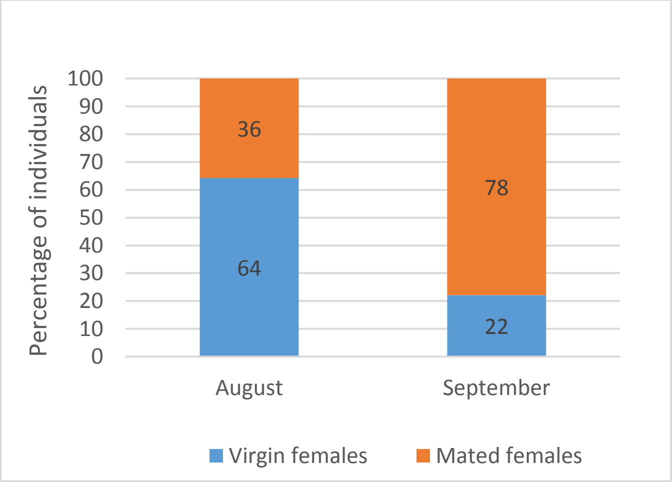


Figure 4. Percentage of virgin and mated females according to the study period. Figures indicate the number of individuals within each category.

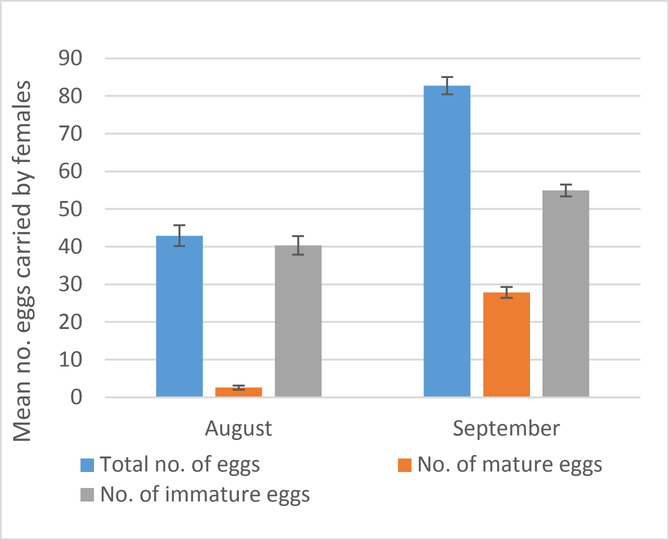


Figure 5. Mean number of eggs carried by females according to the study period. Labels indicates the number of individuals within each category. Error bar = *sem*.

Male mate preferences

Experience 1

Male mating behaviour prior to spermatophore transfer

In experiment 1, males were sequentially provided with one small and one large female in a random order. For the present analysis, we retained 44 mating trials in August and 36 trials in September where the male did not mate (see Material and Methods). Behaviours indicative of male motivation to mate were not affected by the period although males tended to call more frequently in September (motivation behaviours: $F_{(1,75)} = 2.53$, $p = 0.12$; rejection behaviours: $F_{(1,75)} = 0.06$, $p = 0.81$; calling: $F_{(1,75)} = 3.22$, $p = 0.07$). Males displayed significantly more motivation behaviours and calling song toward the second female that was presented to them (respectively $F_{(1,75)} = 9.52$, $p = 0.02$; $F_{(1,75)} = 293.84$, $p < 0.0001$), but rejection behaviours were not affected by female order ($F_{(1,75)} = 0.91$, $p = 0.34$).

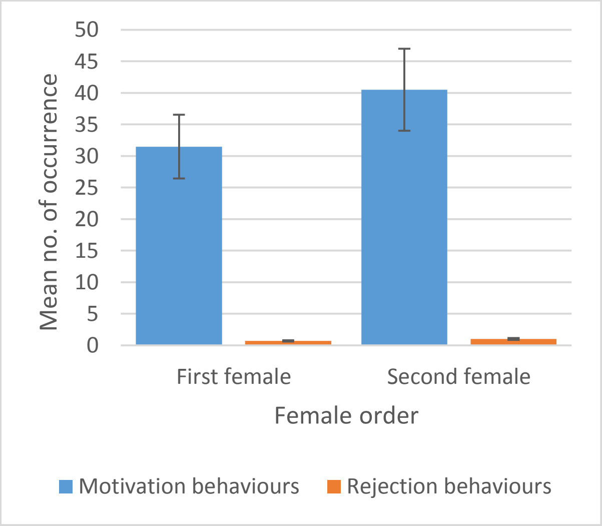


Figure 6. Mean number of occurrence of motivation and rejection behaviours according to female order. Error bars =*sem*.

Male body mass did not affect the occurrence of mating behaviours (motivation behaviour: $F_{(1,75)} = 0.14$, $p = 0.91$, rejection behaviours: $F_{(1,75)} = 5.33$, $p = 0.91$, calling song: $F_{(1,75)} = 0.10$, $p = 0.96$). However, male mating behaviour was influenced by female body mass as males displayed significantly more motivation behaviours, more calling songs and less rejection behaviours toward females of higher body mass (respectively $F_{(1,75)} = 20.27$, $p < 0.0001$; $F_{(1,75)} = 130.90$, $p < 0.0001$; $F_{(1,75)} = 4.55$, $p = 0.03$; Figure 7)

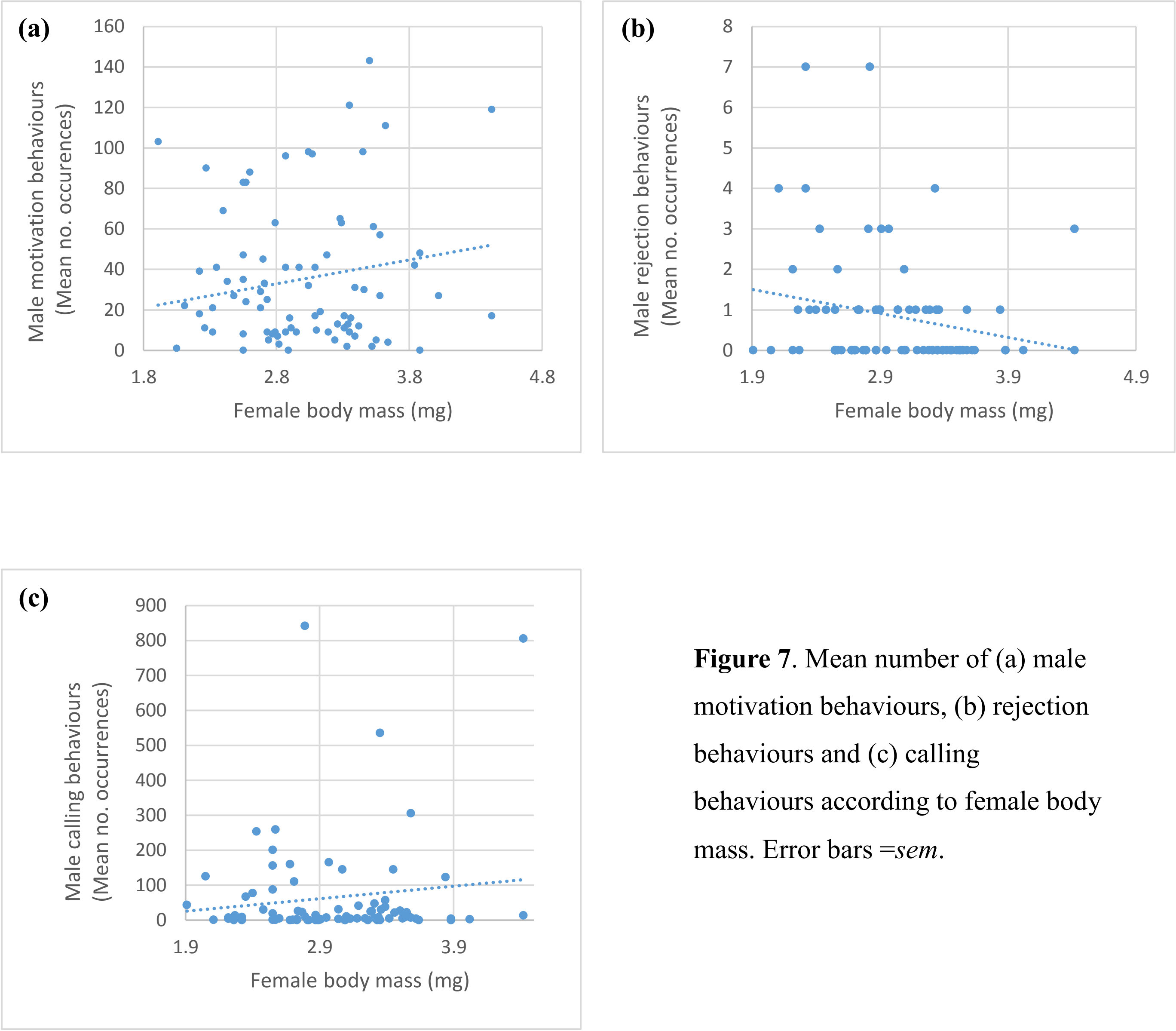


Figure 7. Mean number of (a) male motivation behaviours, (b) rejection behaviours and (c) calling behaviours according to female body mass. Error bars =*sem*.

Female mating behaviour

Behaviours indicating female motivation to mate were less frequent in August than September ($F_{(1,75)} = 0.01$, $p = 0.001$), and did not vary with male motivation or calls $F_{(1,75)} = 0.35$, $p = 0.19$; $F_{(1,75)} = 0.07$, $p = 0.61$).

Experience 2

Mating occurrence and spermatophore transfer

In the second experiment, males were presented with one female of random body mass. Overall, we tested 63 males on 138 mating trials. The occurrence of mating did not vary with male body mass ($F_1 = 0.98$, $p = 0.33$) however larger males transferred significantly larger spermatophore ($\chi^2_{(1)} = 49.20$, $p = 2.32$).

The period affected both the frequency of copulations and the spermatophore transfer as males mated less frequently in September ($F_1 = 2.80$, $p = 0.005$; Figure 8a), but provided females with a larger spermatophore ($\chi^2_1 = 22.94$, $p < 0.0001$; Figure 8b) that represented a larger percentage of their body mass ($\chi^2_{(1)} = 18.41$, $p < 0.0001$; Figure 8c).

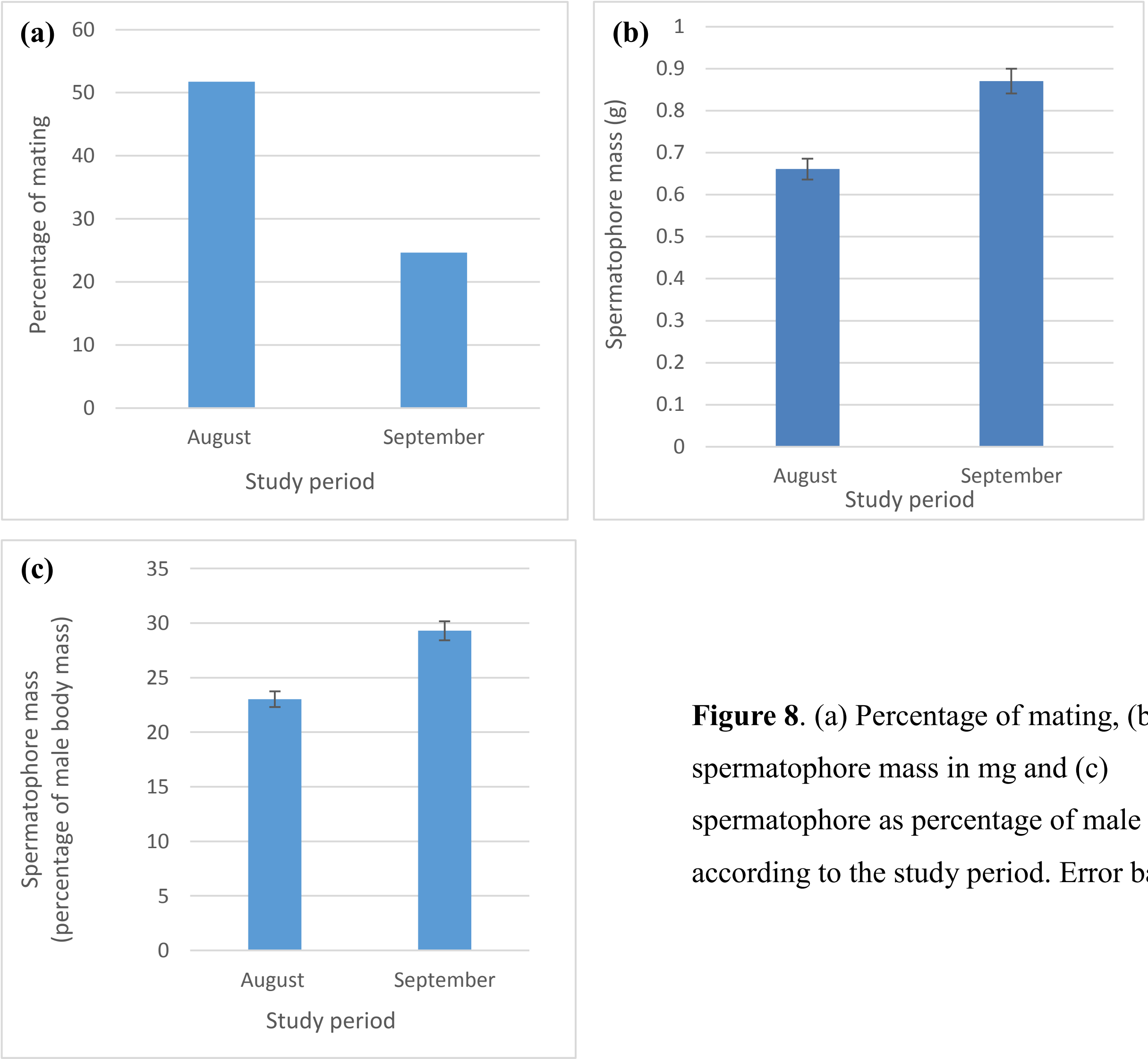


Figure 8. (a) Percentage of mating, (b) spermatophore mass in mg and (c) spermatophore as percentage of male body mass according to the study period. Error bars = *sem*.

However, the body mass of the females used in this experiment did not vary with the study period ($G_{(1)}$ = -0.55, p = 0.58) and the spermatophore size did not vary with female body mass (frequency of copulation: F_1 = 0.028, p = 0.98; spermatophore mass: $\chi^2_{(1)}$ = 1.77, p = 0.18; percentage of spermatophore mass according to male body mass: $\chi^2_{(1)}$ = 0.34, p = 0.56).

DISCUSSION

Male mate choice has been rarely observed and quantified under natural environment. The aim of the present study was, therefore, to investigate pre- and post-copulatory male preference for female quality in the bushcricket *E. diurnus* under natural conditions during two periods of the reproductive season. Beside, in order to better understand the factors that might affect male reproductive success, we also measured the OSR and variance in female quality during these two study periods.

When mating is costly, for example when the reproductive resources are constrained or limited, males are expected to strategically allocate their reproductive resources to optimize the pay-off of each reproductive event and thereby maximize their lifetime reproductive success (Edward & Chapman, 2011; Wedell et al., 2002). In particular, when females are variable in their reproductive quality, males are expected to mate preferentially with, or invest maximally toward females of higher reproductive quality (Bonduriansky, 2001; Edward & Chapman 2011). However, male mate choice is not expected to evolve when the OSR is biased toward males, as the rate at which males are likely to encounter sexually receptive females would be very low (Bonduriansky, 2001; Edward & Chapman 2011). Thus, when mating opportunities are rare, males are expected to invest maximally regardless of female quality, and are therefore not expected to reject any potential mate.

In the present study, the OSR was strongly biased toward males during the two investigated periods, suggesting that males' potential mating opportunities were scarce. In line with the general predictions, our results showed rare mate rejection behaviours in males (Table 1, Figure 6). Nonetheless, our results revealed that males adjust their precopulatory behaviours according to the female quality, as males displayed more motivation behaviours and calling songs toward larger females (Figures 7 a,b). In insects, the preference for larger mate is widespread, as the fecundity of females often correlates with their size (Honek, 1993; Ridley, 1983). In the present study, this relation also prevails in *E. diurnus*, as larger females carried significantly more mature and immature eggs. Besides, females were significantly larger and carried more mature and immature eggs later in the reproductive season. These results contrast with previous investigations of the relationship size-fecundity in this species, lead in 2012 in the same population, and were such a pattern was non-existent (Jarrige, 2012, unpublished data).

Furthermore, we showed that male motivation behaviours and calls were more intense toward the second female presented to them, irrespectively of their quality. This difference in behaviour could result from the male past experience: here, males would readjust their investment in mate attraction following an unsuccessful previous mating opportunity.

However, the study of the size of the spermatophore transferred by males to females did not reveal any variation in the size of the gift (both in absolute mass and percentage of male body mass) in relation to female quality. Here again, these results contrast with previous laboratory experiments showing that males *E. diurnus* provided larger females with larger donations (Jarrige et al., 2013).

However, we must note that the observed differences between laboratory and field conducted experiments might simply be the by-product of the differential conditions experienced by individuals under both environments. Notably, lab-reared individuals have access to *ad libitum* food resources, while individuals under natural conditions might be more constrained by food availability (Ritchie, Sunter, & Hockham, 1998). This could have affected both affected male capacity in gift production as well as the variance in female fecundity.

In the present study, mating occurrence within mating trials was significantly lower later in the reproductive season, despite that females displayed more behaviours indicative of their willingness to mate. However, it is delicate from the present to determine with certainties whether the observed differences in mating pattern results from male and/or female behaviours, as the non-occurrence of mating could result from a complex mixture between male and female pre-copulatory mate preferences.

Besides, males transferred larger gifts that represented a higher percentage of their body mass later in the reproductive season. We propose three non-mutually exclusive hypotheses to account for the differences in gift expenditure between the two study periods.

As the analysis of female quality variance reveals an important increase in female body mass and fecundity along the reproductive season, the increase in male donation could reflect male strategic allocation associated with female fecundity, by increasing the number and/or quality of eggs and offspring sired by the gift giving male (paternal investment hypothesis; Gwynne, 2001; Vahed, 1998, 2007). Such differential allocation of nuptial gifts in relation to female quality has been found in other Orthopteran species (e.g. Kvarnemo & Simmons, 1998; Simmons & Kvarnemo, 1997; Wedell, 1992). Although the body mass of the females used in the second experiment was not significantly different between the two study periods, males could nonetheless have been selected to increase their gift expenditure in relation to the phenology of the reproductive season, if the increase in female body mass and fecundity over the reproductive season is a general rule in this species.

Furthermore, the observed increase in gift expenditure could reflect male terminal investment, as males would have fewer opportunities for future reproduction in September both due to higher risk of mortality associated with age and changes in climatic conditions. Thus, the trade-off between current and future reproduction should be resolved in favour of high reproductive effort into current reproduction (Williams 1966; Clutton-Brock 1991; Forslund & Part 1995).

Second, the observed variations in spermatophore size could also reflect male's response to sperm competition risk. In *E. diurnus*, previous studies demonstrated a pronounced advantage for the last sperm (last sperm precedence; Hockham, Graves, & Ritchie, 2004). Thus, as the reproductive season advances, greater are the chances for a male to be the last one to inseminate a female. Therefore, the gift might serve as a protection for the ejaculate by increasing the female's refractory period following a mating: females cannot remate before the full consumption of the spermatophore. Larger gifts thereby reduce the risk of sperm competition (mating effort hypothesis; Vahed, 1998, 2007). Under both of these hypotheses, males would therefore achieve greater fitness gain from transferring larger spermatophores to females in September. These results are consistent with previous work on this species in which males were found to transfer larger gifts to older females (Jarrige et al., 2013).

Finally, because *E. diurnus* males are limited in the rate at which they can manufacture a new spermatophore, the differences in gift allocation between the two periods could account for the lower mating rate observed in September. Indeed, the transfer of larger spermatophores could result in a longer refractory period between two successive copulations, as commonly documented in bushcrickets (Gwynne, 2001), thereby reducing the frequency at which males could mate.

Therefore, we showed that despite an OSR highly biased toward males, *E. diurnus* males adjusted both their pre- and post-copulatory sexual behaviours in response to complex reproductive constraints. Although it is currently assumed that sex roles (competitive versus choosy) mostly depend on the OSR (Emlen & Oring, 1977; Gwynne, 1991), our results provide interesting empirical evidence that male mate choice can evolve under male biased OSR, and call for further investigation of male strategic allocation of resources under the complex and dynamic network of factors affecting the male fitness under natural conditions.

CHAPITRE 4 : DISCUSSION GENERALE

Dans le cadre de ces travaux de thèse, je me suis intéressée aux **allocations stratégiques** de ressources reproductives sous la forme de transferts **gamétiques** et **non gamétiques** chez les mâles au sein de deux espèces d'insectes, à l'égard de facteurs impliquant la **variance de la qualité de la femelle** (Articles 2 à 5), **l'intensité de la compétition intrasexuelle pré-copulatoire** (Article 2) et du **risque de compétition spermatique** (Article 1). Mes résultats montrent que les mâles allouent stratégiquement leurs ressources reproductives selon des modalités variées au travers i) de variations de comportements de parade ou de gardiennage des femelles avant le transfert de sperme (Article 2 et 5), ii) de la variation de la qualité et/ou de la quantité des transferts gamétiques et non-gamétiques faits aux femelles (Article 1 et 4), ou encore iii) de du partitionnement des ressources gamétiques au travers phénotypes distincts (Article 5).

D'une manière générale, ces résultats soulignent l'importance des mécanismes post-éjaculat dans les variations de la réponse sexuelle des mâles (Article 1 et 4), tandis que la qualité des femelles semble avoir un effet plus nuancé sur les allocations de ressources gamétiques et non gamétiques (Article 2, 3, 4 et 5). Ce résultat est surprenant, dans la mesure où les mâles de ces deux espèces subissent d'importantes contraintes liées à la reproduction (Chapitre 1, Partie 4), et que les femelles présentent des variations notables au niveau de leur qualité reproductive, deux conditions sous lesquelles l'évolution de préférences est théoriquement attendue (Bonduriansky 2001, Edward & Chapman 2011). Aussi, ces résultats m'ont amenée à m'interroger sur les mécanismes sous-jacents à l'évolution des allocations stratégiques, et notamment l'évolution de préférences chez les mâles des deux espèces étudiées et plus largement dans le règne animal.

Comme cela a maintenant été énoncé maintes fois au cours de ce manuscrit, la reproduction est une activité souvent contrainte et couteuse pour les mâles, en conséquence de quoi leur potentiel reproducteur s'avère souvent limité (Chapitre 1, Partie 1). Les mâles devraient donc, tout comme les femelles, allouer prudemment leurs ressources de sorte à maximiser leurs chances de paternité. Toutefois, la condition nécessaire à l'évolution d'une allocation stratégique donnée est qu'elle génère un **bénéfice net en termes de fitness**, c'est-à-dire qu'elle induise plus d'avantages qu'elle n'engendre de coûts. Cependant, les épisodes sélectifs auxquels sont soumis les mâles sont souvent plus complexes que ceux existants chez les femelles (Chapitre 1, Partie 2, et ci-dessous), puisque les mâles doivent à la fois être en mesure de concrétiser l'acte d'accouplement, et d'avoir de bonnes chances de fertiliser les gamètes de la femelle. **Aussi, le succès reproducteur des mâles n'est mesurable qu'à l'issue de l'ensemble des étapes de sélection auxquelles ils sont soumis, et les allocations stratégiques attendues sont donc celles qui permettent aux mâles de maximiser leurs chances de paternité à l'issue de ces deux étapes sélectives successives**. Pour mieux comprendre l'évolution des allocations stratégiques chez les mâles, et plus particulièrement l'évolution de préférences, j'ai tout d'abord résumé le succès reproducteur des mâles sous la forme d'un modèle prenant en compte l'ensemble des épisodes sélectifs et des facteurs qui l'affectent (Chapitre 1, Partie 2, et Figure 12 ci-dessous). Les résultats de mes travaux de thèse sont ensuite discutés au regard de ce modèle et de la théorie générale de la sélection sexuelle et des rôles sexuels.

Modèle du succès reproducteur des mâles et allocations stratégiques des ressources reproductives

Ce modèle résume les épisodes sélectifs et facteurs connus pour affecter le succès reproducteurs des mâles (détaillés dans le chapitre 1 de ce manuscrit). Ce modèle, applicable pour les espèces à fertilisation interne et chez lesquelles il n'existe pas de soins parentaux, s'articule comme suit (Figure 12):

$$\boxed{SR = S_\alpha \,.\, (\beta \,.\, \Phi)}$$

S_α: somme du nombre d'accouplements = somme du nombre d'épisodes reproducteurs ou le mâle transfère avec succès son sperme à la femelle.

β: qualité de la femelle = 1 pour une femelle dont la qualité reproductive (fécondité) est égale à la moyenne pour la population, entre 0 et ≤ 1 pour les femelles de qualité inférieure à la moyenne et ≥ 1 pour les femelles de qualité supérieure à la moyenne de la population.

Φ: chances de fertilisation = 1 lorsque les mâles ont 100 % de chances de fertiliser la totalité des œufs de la femelle, 0 lorsque les chances de fertilisation sont nulles.

Le succès reproducteur des mâles (SR), exprimé en nombre de gamètes fertilisés, dépend i) du nombre de femelles avec lesquelles ils parviennent à s'accoupler avec succès (α), ii) de la qualité reproductive des femelles accouplées (β), et iii) des probabilités qu'ont leurs spermatozoïdes de féconder les gamètes de la femelle (ϕ). Si l'un des termes de cette équation est nul, le succès reproducteur des mâles est équivalent à 0 et les mâles ne produisent pas de descendants.

Le premier terme de cette équation concerne logiquement le nombre d'accouplements réussis, c'est-à-dire le nombre de femelles auxquelles les mâles parviennent à transférer leur sperme avec succès. Ensuite, l'expression de choix pour la qualité reproductive de la femelle revient pour les mâles à maximiser leurs investissements (en termes d'acception des femelles et/ou de tout autre compartiment de l'effort reproducteur des mâles) en faveur des femelles les plus fécondes. Aussi, dans le présent modèle, les femelles de qualité moyenne (c'est-à-dire dont le nombre d'ovules fécondables est égal à la moyenne de la population) ont une valeur de 1. La variance de qualité des femelles est exprimée sous la forme d'une plus-value ou d'une moins-value à cette valeur moyenne (e.g. une femelle de bonne qualité aura une valeur supérieure à 1, une femelle de qualité inférieure à la moyenne aura une valeur comprise entre 0 et 1). Aussi, toutes choses étant égales par ailleurs, les mâles peuvent atteindre un succès reproducteur équivalent en s'accouplant peu, mais avec des femelles de meilleure qualité, ou en augmentant la fréquence des accouplements avec des femelles de qualité moindre. Par exemple, si les males s'accouplent avec 3 femelles de qualité moyenne, leur succès reproducteur SR sera égal à $SR = S_3.(1.1) = 3$. Le même succès reproducteur peut donc théoriquement être atteint en s'accouplant avec 2 femelles de qualité reproductive supérieure si les bénéfices retirés compensent la perte d'opportunités d'accouplement: $S_2.(1.5).1) = 3$ (ici, une opportunité d'accouplement est perdue et la compensation en termes de qualité doit donc atteindre 0.5, c'est-à-dire que le nombre d'œufs fécondables chez les femelles préférées excèdent de moitié celui des femelles non préférées).

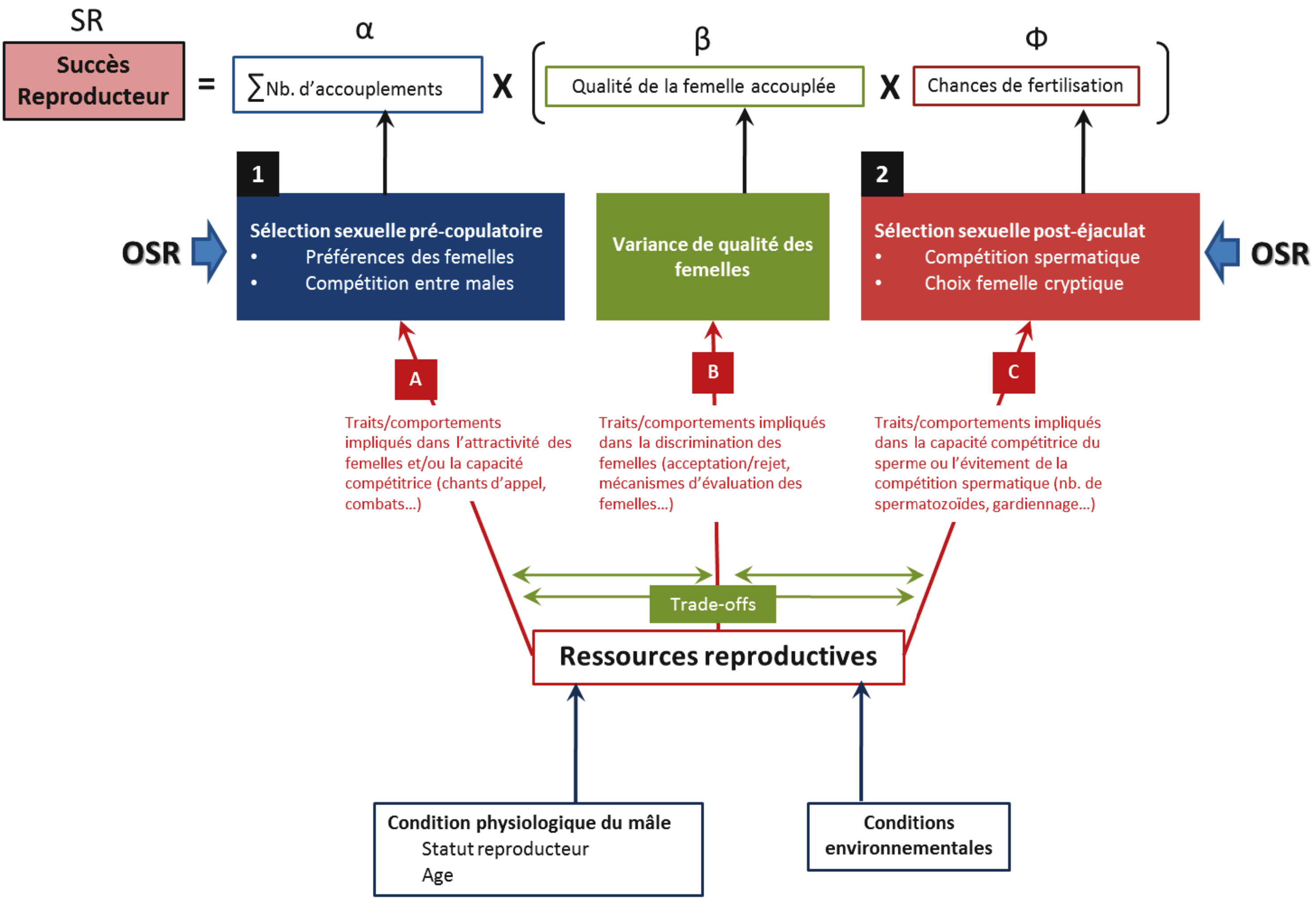


Figure 12 : modèle résumant les épisodes sélectifs et les facteurs influençant le succès reproducteur des mâles (voir texte).

Enfin, ultimement, le succès reproducteur des mâles dépend du nombre de spermatozoïdes effectivement impliqués dans la fécondation des ovules des femelles accouplées. Dans le modèle, les chances de fertilisation des mâles sont représentées par une valeur comprise entre 0 et 1, où 0 correspond à un taux de fertilisation nulle, et 1 représente la fertilisation par le mâle de la totalité des gamètes de la femelle. A ce titre, l'existence de sélection sexuelle post-éjaculat peut fortement affecter les chances de fertilisation des mâles, (Chapitre 1, Partie 2.2.1, Encadré 1). Notamment, s'il existe de la compétition spermatique, **la maximisation de ϕ dépendra de la quantité et de la qualité des transferts gamétiques et/ou non gamétiques (e.g. substances accessoires réduisant la probabilité de réaccouplement de la femelle) et/ou de l'intensité des comportements de gardiennage post-**éjaculat (Chapitre 1, Partie 3.4). Aussi, les mâles peuvent être avantagés s'ils réduisent leur effort pour accéder aux femelles mais maximisent leurs chances de fertilisation. Prenons un exemple : si les mâles s'accouplent avec 4 femelles de qualité moyenne et ont une chance de fertiliser à chaque fois 50 % des œufs de la femelle, alors le succès reproducteur $S_4.(1.(0.5)) = 2$. Le même succès reproducteur peut donc être obtenu en s'accouplant deux fois moins, mais en fécondant 100% des gamètes de la femelle $S_2.(1.1) = 2$.

Aussi, la maximisation du succès reproducteur des mâles peut potentiellement se faire au travers de la maximisation de chacun des termes de cette équation, et par le biais de variations de réponses sexuelles variées visant à maximiser i) l'accès des mâles aux femelles (comportement de cours, intensité des combats etc., flèche A, Figure 12), ii) la qualité des femelles accouplées (acceptation ou rejet des femelles, ajustement de la quantité de gamètes au nombres d'œufs fécondables, etc. ; Chapitre 1 ; Partie 3.1) au regard de la qualité de la femelle etc., flèche B, Figure 12) ou encore iii) la maximisation des chances de fertilisation des mâles, notamment s'il existe de la compétition spermatique (ajustement des transferts gamétiques/non gamétiques aux femelles, comportements de gardiennage etc. (flèche C, Figure 12).

La situation devient particulièrement complexe dès lors que les pressions de sélection auxquelles sont soumis les mâles sont composites **et** que les femelles sont de qualité variable, une situation fréquente au sein du règne animal (Andersson 1994, Parker 1970, Simmons 2001). En effet, la maximisation de SR peut alors théoriquement impliquer des variations d'investissements conjointes visant à maximiser α, β et Φ. L'évolution d'une stratégie d'allocation de ressource va donc dépendre de :

a) L’existence potentielle de trade-offs entre les investissements dans les traits favorisant le succès reproducteur pré-copulatoire (attraction des femelles, préférence pour la qualité de la femelle, etc.) et les traits favorisant le succès reproducteur post-éjaculat des mâles (Figure 12, flèches vertes). Dans ce cas, il existe des corrélations inverses entre certains des termes du modèle (α, β et Φ). Par exemple, investir de façon maximale dans l’accès aux femelles peut permettre aux males d’augmenter le nombre d’accouplements qu’ils réalisent, mais parallèlement réduire leurs chances de succès post-éjaculat, notamment en diminuant les ressources disponibles pour la production de sperme (Scharf et al. 2014).

b) La variation de paramètres intrinsèques et extrinsèques aux mâles, tels que leur condition physiologique, l’OSR ou les conditions environnementales. En effet, la variation de ces paramètres peut fortement conditionner la quantité de ressources reproductive dont disposent les mâles, ainsi que l’intensité de la compétition inter-males et/ou la disponibilité des femelles sexuellement réceptives (flèches bleues, Figure 12), et ainsi affecter la balance des coûts/bénéfices associés à une stratégie donnée (Chapitre 1, Partie 3).

On comprend alors aisément que la maximisation du succès reproducteur des mâles peut être extrêmement complexe en impliquant des variations de réponse sexuelle composites. Ce véritable casse-tête évolutif rend particulièrement ardu l’établissement de prédictions sur les allocations stratégiques et les rôles sexuels attendus.

En effet, si les variations d’investissements des mâles dans les traits et comportements impliqués dans l’accès aux femelles ou à leurs gamètes sont créditées d’un flot de confirmations empiriques, l’évolution de préférences des mâles constitue encore aujourd’hui une question largement non résolue. En particulier, le modèle classique de la sélection sexuelle (Chapitre 1) prédit l’évolution de préférence au sein d’un sexe dès lors que l’OSR est biaisé en faveur de l’autre sexe. Aussi, lorsque les deux sexes ont un OSR ou un PR équivalent et qu’il existe des différences dans la qualité des individus, un certain degré de préférence devrait théoriquement être exprimé au sein des deux sexes (McNamara & Collins 1990 ; Crowley et al. 1991). Cela suppose que le choix mutuel du partenaire devrait être observé chez un nombre non négligeable d’espèces, ce que semble contredire la majorité des études empiriques réalisées à ce jour (Johnstone et al. 1996 ; Kokko & Johnstone 2002).

A ce titre, mes résultats et le présent modèle apportent une lumière nouvelle en ce qui concerne l’évolution des choix mâles sous pressions de sélection composites. Tout d’abord, il est important de noter le caractère essentiel de la sélection sexuelle chez les mâles : les épisodes sélectifs pré et post- éjaculat sont séquentiels et dépendants (1 et 2 Figure 12), puisque le succès post-éjaculat des mâles dépend au préalable de leur capacité à s'accoupler. Pour cette raison, **les investissements des mâles dans les traits permettant d'accéder aux femelles sont une condition *sine qua none* à la concrétisation de leur paternité**. Aussi, les variations des réponses sexuelles des mâles à l'égard des facteurs qui influencent la capacité des mâles à s'accoupler, c'est-à-dire les variations de réponses sexuelles des mâles au regard des pressions de sélections sexuelles pré-copulatoires, sont primordiales dans la maximisation de succès reproducteur des mâles (A et 1, Figure 12).

Aussi, si l'accès des mâles aux femelles est conditionné une sélection pré-copulatoire forte, les mâles ne peuvent réduire leurs investissements pour l'accès aux femelles en dessous d'un certain seuil, au risque de ne pas s'accoupler. S'il existe par ailleurs de la sélection post-éjaculat, les mâles doivent alors investir dans les traits favorisant leurs chances de fertilisation. Dans le cas contraire, les chances de paternité des mâles restent faibles, ce qui peut représenter une perte nette pour les mâles des ressources préalablement investies pour l'accès aux femelles. Aussi, **il est attendu que les mâles maximisent également leurs investissements dans les traits/comportements favorisant leurs chances de fertilisation**, et minimisent ainsi le risque de voir leur capital énergie/temps diminué de l'ensemble des ressources investies lors des étapes sélectives ultérieures (e.g. investissement dans les chants d'appels, les combats etc.).

Cela peut en particulier freiner, voire contre sélectionner, l'évolution de préférences pour la qualité reproductive de la femelle. En effet, comme mentionné précédemment, si les ressources reproductives des mâles sont limitées, il est attendu que les mâles les allouent en priorité en vue d'accéder aux femelles et à leurs gamètes. De plus, l'expression de préférences peut s'avérer couteuse au travers i) du temps et/ou de l'énergie dépensé à chercher les femelles de meilleure qualité, ii) de l'investissement dans des mécanismes permettant l'évaluation efficace de la qualité de la femelle rencontrée, et le risque d'erreur qui y est associé et iii) du risque de perte d'opportunités d'accouplement, notamment dans le cas où les préférences se manifestent par le rejet de certaines partenaires (Edward & Chapman 2011, Chapitre 1, Partie 3.1). L'expression de préférences pourrait alors aboutir à une diminution des ressources disponibles pour les autres compartiments affectant la fitness des mâles (termes α et ϕ du modèle, Figure 12), et ainsi diminuer le succès reproducteur global des mâles. Renforçant cet effet, les pressions de sélection pré et post-éjaculat peuvent être corrélées à la qualité de la femelle : les femelles les plus fécondes peuvent être plus sélectives, être l'objet de compétition inter-male plus marquée, ou encore être associées à des risques de compétition spermatique plus importants (Edward & Chapman 2011, Wedell et al. 2002). Dans ce cas, seuls les mâles en meilleure condition physiologique, c'est-à-dire ceux dont les ressources reproductives, l'attractivité ou les capacités compétitrices sont les plus élevées pourraient retirer des bénéfices de l'expression d'un choix (Chapitre 1, Partie 3.3 et 3.6).

En d'autres termes, lorsque les **pressions de sélection s'exerçant sur les capacités d'acquisition des partenaires et/ou de leurs gamètes sont fortes, les traits qui y sont associés devraient en premier lieu favorisés. Aussi l'évolution de choix pour la qualité reproductive de la femelle ne peut être attendue que si l'expression de préférences ne grève pas par ailleurs le succès lors des épisodes sélectifs pré et post-éjaculat.**

Chez les deux espèces étudiées dans le cadre de cette thèse, les mâles subissent d'intenses pressions de sélection pré-copulatoire intra et intersexuelle, puisque les femelles discriminent les mâles sur la base de leurs chants d'appels émis lors du regroupement de mâles (Greenfield & Coffelt 1983, Busnel 1955). L'accès aux femelles requiert ainsi d'importants investissements énergétiques dans la production des chants (Chapitre 1, Partie 4). L'intensité de la sélection sexuelle pré-éjaculat est particulièrement drastique chez *A. grisella*, du fait de la structure en lek des groupes de males et de l'incapacité des adultes à renouveler leur capital énergétique (Kunike, 1930). Au sein de ces deux espèces, l'allocation d'une quantité substantielle de ressources dans l'accès aux femelles est donc primordiale au succès reproducteur des mâles, et il a été démontré que les mâles augmentent leur investissement dans les chants d'appels en présence de compétiteurs (Busnel et Dumortier 1956, Jia et al. 2001).

De plus, l'existence de compétition spermatique est avérée chez les deux modèles étudiés (Hockam et al. 2004, Article 1). **Aussi, dès lors qu'il existe de la compétition spermatique, il est attendu que les mâles allouent aussi leurs ressources reproductives au regard du risque et de l'intensité des mécanismes post-éjaculat rencontrés.** Les résultats obtenus dans le cadre de cette thèse vont dans ce sens, puisque les mâles *A. grisella* augmentent leurs transferts gamétiques lorsqu'il existe un risque de compétition spermatique (Article 1), et que les variations de taille et de qualité du spermatophylax des mâles *E. diurnus* semblent en partie être influencées par la probabilité de réaccouplement de la femelle liée à leur âge (Article 4 et 5). La maximisation de l'investissement reproducteur des mâle au regard du risque de compétition spermatique permet par ailleurs aux males de réduire le risque de voir leur capital énergie/temps diminué de l'ensemble des ressources investies dans les chants d'appels et le transfert de substances gamétiques et non gamétiques aux femelles, sans que les chances de retour sur leur paternité en soient pour autant améliorées.

Enfin, nos résultats montrent que les mâles *A. grisella* n'expriment pas de variations de réponses sexuelles au regard de la qualité de la femelle (Articles 1 à 3). Chez cette espèce, il est donc probable que les avantages liés aux préférences soient marginaux en comparaison des avantages résultant des allocations stratégiques pour l'accès aux femelles et la fertilisation de leurs gamètes. En revanche, chez *E. diurnus*, les mâles maximisent leurs comportements pré copulatoire et ajustent leur la taille et la qualité de leurs donations au regard de la taille de l'âge des femelle rencontrée (Articles 4 et 5). Toutefois, les résultats obtenus chez cette espèce suggèrent que la variance de qualité de femelles (fécondité) et le risque de compétition spermatique sont confondus (i.e. les femelles les plus grosses sont les plus fécondes, mais aussi les plus âgées et donc les moins susceptibles de se réaccoupler. Si c'est le cas, β et Φ vont dans le même sens, et les avantages pour les mâles à être sélectifs seraient nets.

De plus amples études restent néanmoins nécessaires au sein de ces deux espèces pour déterminer i) la **valeur adaptative des allocations stratégiques** exprimées par les mâles, c'est-à-dire quantifier leur impact sur les chances de paternité des mâles, ainsi que la **répartition des ressources reproductives entre les différentes allocations stratégique**s exprimées par les mâles (trade-offs), au regard de la variation de facteurs variables (intensité de la compétition intra-sexuelle, de la compétition spermatique etc.).

Conclusion : une explication alternative aux rôles sexuels

Historiquement, la reprise des travaux de Bateman par Trivers marque un tournant dans la théorie de la sélection sexuelle en fournissant une explication théorique aux préférences des femelles pour les mâles : les femelles seraient le sexe sélectif car leur reproduction est souvent plus contrainte que celle des mâles. Cette avancée théorique est rapidement assortie de nombreuses confirmations empiriques illustrant l'importance de la sélectivité des femelles au sein du règne animal. Aussi, si la multiplication du nombre d'accouplements est bénéfique au succès reproducteur des mâles, le succès reproducteur des femelles atteint rapidement un plateau au-delà duquel chaque accouplement n'apportera pas, ou peu, de bénéfices. Pour ces dernières, il ne s'agirait donc pas d'augmenter le nombre d'épisodes reproducteurs réalisés, mais leur qualité, en choisissant les mâles qui leurs apporteront les meilleurs bénéfices directs (nourriture, territoires) et/ou indirects (génétiques). Au contraire, la maximisation des chances de paternité des mâles reposerait principalement sur leurs investissements dans l'acquisition de partenaires.

La découverte des nombreuses contraintes liées à la reproduction des mâles a donc naturellement amené les chercheurs à se poser la question de la sélectivité des mâles pour les femelles (Bonduriansky, 2001, Edward & Chapman, 2011). L'existence de préférences des mâles pour les femelles s'avère alors répandue au sein du règne animal, toutefois, les facteurs sous-jacents à son évolution restent largement incompris et les prédicteurs utilisés classiquement tels que l'OSR, le PRR et le PI (Chapitre 1, Partie 3.2), révèlent rapidement leurs limites (Kokko & Johnstone, 2002). L'une des raisons majeures de l'échec à comprendre l'évolution des choix chez les mâles, et plus largement des rôles sexuels, trouve son origine dans le fait que les préférences chez les mâles ont souvent été abordées avec les mêmes attendus que les choix des femelles, c'est-à-dire en considérant qu'une reproduction couteuse pour les mâles assortie de la variation de qualité des femelles devrait théoriquement aboutir à l'évolution de préférences (Chapitre 1). En effet, selon moi et au vu de mes présents résultats, la validité de ce raisonnement est rendu caduc par la différence fondamentale entre mâles et femelles en ce qui concerne la complexité des épisodes sélectifs auxquels sont soumis individus.

Chez les espèces à fertilisation interne, au-delà de l'anisogamie, la différence majeure entre mâles et femelles réside dans le fait que le transfert de gamètes s'effectue toujours du mâle vers la femelle. Aussi, **il n'existe pas chez les femelles d'équivalent à la sélection intra-sexuelle post-éjaculat, et ces dernières ont toujours la certitude que seuls leurs gamètes seront impliqués dans la production de la descendance.** Par ailleurs, si l'OSR est équilibré, la population de gamètes mâles excède largement la population de gamètes femelles et pour cette raison, la probabilité qu'un ovule soit fécondé surpasse fortement celle d'un spermatozoïde d'être fécondant. Aussi, bien que les gamètes femelles soient individuellement plus couteux à produire, les chances de retour associées à cet investissement sont également plus grandes : une lecture alternative des travaux de Bateman illustre d'ailleurs parfaitement le fait que les femelles atteignent un succès reproducteur maximal après seulement quelques accouplements. Pour cette raison, les femelles peuvent faire l'économie des adaptations souvent couteuses pour se prémunir du risque de compétition intra-sexuelle post-éjaculat, comme la multiplication des gamètes, les comportements de gardiennage etc. ou le partitionnement de leurs ressources entre plusieurs épisodes sélectifs. Par ailleurs, les bénéfices associés à l'expression de préférences sont nets, puisqu'il n'existe pas d'équivoque quant au fait que les gamètes de la femelle seront impliqués à l'issue de l'accouplement, ce qui n'est pas le cas des mâles. Aussi, pour les raisons évoquées ci-dessus et considérant les nombreux coûts liés à chaque étape de sélection auxquelles font face les mâles, il me semble que l'idée générale selon laquelle l'évolution de préférences au sein d'un sexe reflète les contraintes reproductive de ce sexe mérite d'être largement nuancée. En effet, chez les espèces à fertilisation interne ou chez lesquelles il n'existe pas de soins parentaux, une explication alternative aux rôles sexuels « classique » pourrait être fournie en considérant non pas l'asymétrie d'investissement dans la production individuelle de gamètes, mais en considérant sa conséquence probable sur l'asymétrie de complexité entre épisodes sélectifs subis par les individus. A ce titre, le fait pour les femelles de garder leurs gamètes « à domicile » lors de la fécondation les soumet souvent à des épisodes sélectifs plus simples que ceux rencontrés chez les mâles, et leur permet souvent de s'exempter d'adaptations couteuses pour accéder aux mâles et/ou à leurs gamètes. Aussi, les femelles seraient majoritairement sélectives… tout simplement parce qu'elles peuvent « se le permettre », ayant peu d'incertitudes sur le devenir de leurs gamètes. Au contraire, l'évolution de préférences chez les mâles n'est qu'un terme d'une équation plus complexe (Figure 12), et ne peut être attendue que si les avantages issus des choix sont maintenus lors des épisodes sélectifs ultérieurs et ne réduisent pas la capacité des mâles à accéder aux femelles. Aussi, l'expression de préférences peut être considérée comme « un luxe reproductif » dont le coût pour les mâles reste souvent élevé, comme l'illustrent les résultats obtenus dans le cadre de ces travaux de thèse.

Cette situation pourrait être généralisable à un grand nombre d'espèces, et expliquer la rareté du choix mutuel, et plus largement la faible fréquence du choix des mâles relativement aux choix des femelles dans le règne animal. Aussi, il apparait indispensable de considérer les épisodes sélectifs qui affectent le succès reproducteur des males dans leur ensemble pour comprendre l'évolution des allocations stratégiques - préférences ou autres - qui en résultent.

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ANNEXES

Male song as a predictor of the nuptial gift in bushcrickets: On the confounding influence of male choice

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ABSTRACT

In species where males provide females with nuptial gifts, females can benefit from choosing mates offering the larger or higher quality donation. The existence of such preferences assumes that females can reliably assess a male's gift before mating. However, in certain species, e.g. most bushcrickets, prior, direct assessment of the nuptial gift is impossible because females choose males before the gift's transfer. Any female assessment of a male's expected donation could therefore only be made via reliable premating cues or signals. In the bushcricket *Ephippiger diurnus*, males provide females with a large spermatophore at mating, which consists of an ampulla, housing sperm, surrounded by a nutrient-containing spermatophylax that females consume. As female mate choice occurs before spermatophore emission, females may rely on the premating acoustic signals that males emit to attract females. Here, we investigate whether certain parameters of *E. diurnus* male song could reliably inform females about the material benefits to come. We found no evidence that any male song traits studied (call rate, syllable number, peak frequency) could predict spermatophylax nutritional contents. However, our results provide good evidence that male mate choice is likely to occur concomitantly with female choice as the size and quality of the nuptial gift appeared to vary with female traits (age and size). This added presence of male choice would render pre-mating assessment of direct benefits transferred by males effectively impossible for *E. diurnus* females.

KEYWORDS:

Ephippiger diurnus; honest signalling; male mate choice; male song; sexual selection; spermatophore; spermatophylax;

INTRODUCTION

Female mate choice is expected to arise whenever mating with certain males over others can result in differences in fitness (Andersson 1994). These differences are assumed to reflect either indirect genetic benefits or direct material ones that males provide at mating. Indirect benefits are received when certain paternal genes enhance the mating success or viability of progeny in the next generation (Fisher 1930, Kokko et al. 2003). Direct benefits, on the other hand, increase the fitness of the choosing female in the present generation, and may derive from paternal care of offspring, courtship feeding, resources defended on a mating territory, reduced infection by disease agents that can be transferred to the female, and sperm number (Hoelzer 1989, Andersson 1994). Because of their immediate effects on female fitness, it has been argued that direct benefits should be more important to choosy females than indirect ones (Møller & Jennions 2001).

In many insects, males transfer a ‘nuptial gift’ to females at mating (Vahed 1998, Gwynne 2001). These donations can be prey items, male glandular secretions, or even male body parts. In bushcrickets (Orthoptera: Tettigoniidae), males often transfer the product of their accessory glands in the form of a complex spermatophore (McCartney et al. 2008, Vahed & Gilbert 1996, Wedell 1993). This structure consists of a sperm-containing ampulla to which a gelatinous, sperm-free spermatophylax is attached (Boldyrev 1915). Spermatophylax size is highly variable among species, representing from 2-40% of male body mass (Gwynne 2001). Chemical analyses show that the spermatophylax consists mainly of water and proteins, the latter comprising from 4-27 % of the wet mass, but a small amount of lipids may also be present (Gwynne 2001). Following this transfer, the female consumes the spermatophylax while the sperm contained in the ampulla enter her genital tract (reviewed by Gwynne 2001). Two hypotheses, which are not mutually exclusive, have been proposed to explain the evolution and current function of nuptial gifts in bushcrickets. First, the spermatophylax may function as male mating effort by preventing females from removing or prematurely consuming the ampulla before sperm transfer is complete. Thus, the spermatophylax serves to protect the ejaculate or to increase the female’s refractory period following a mating, thereby reducing sperm competition (Vahed 1998, 2007b). Second, the spermatophylax might function as paternal investment if the substances transferred to females increase the number and/or quality of eggs and offspring sired by the male making the donation (Vahed 1998, 2007b, Gwynne 2001).

From the female perspective, experimental evidence suggests that spermatophylax consumption can be associated with direct, nutritious benefits in various species, enhancing female fecundity and/or lifespan (Gwynne 2001, Lehmann 2012) or provide females with nitrogen (Voigt et al. 2006) and/or energy for body homeostasis (Voigt et al. 2008). Hence, within species where males supply females with such nutritious advantages, females are expected to prefer males providing the most or the best benefits (Møller & Jennions 2001). However, to exercise such mate choice, females would have to perceive differences among males that reliably indicate the level of paternal investment to be offered (Kokko et al. 2003). In some insects, nuptial gifts can be assessed by the females prior to mating, as in hanging-flies (Mecoptera: Bittacidae) where females refuse to copulate with males providing small or unpalatable prey items (Thornhill 1976, 1977). In most bushcrickets, however, prior, direct assessment of the nuptial gift is impossible because females choose males before the formation and transfer of the spermatophylax. Consequently, any female assessment of a male's expected paternal investment could only be made via reliable premating cues. Although females might also exert some post-copulatory choice, (Eberhard 1996, Simmons 2001), it would be by the means of sperm selection after several matings and not via the removal of a low quality spermatophore. Once the spermatophore is attached, females are usually unable to detach it from their genitalia before its total consumption or degradation during which the sperm are transferred to the spermatheca (ACJ, Pers. Obs).

Male bushcrickets broadcast loud advertisement songs that play a major role in female mate choice (Gwynne 2001). Several authors (see Gwynne 2001, Gerhardt & Huber 2002) have suggested that advertisement songs may serve as pre-mating cues in female discrimination of male nuptial gifts, as the songs vary among males (Gerhardt & Huber 2002) and are physiologically costly for them to produce (Prestwich 1994). But, for song to serve as a reliable predictor of a forthcoming nuptial gift, certain acoustic parameters of song would have to be 'condition-dependent' (sensu Grafen 1990), and condition would also be expected to influence the size and or quality of the gift. Some indirect evidence supports these criteria. In certain bushcricket species females do exercise a choice of larger males (Gwynne 1982, Wedell &

Sandberg 1995, Lehmann & Lehmann 2008), and in others, females prefer male signal traits such as greater song length (Galliart & Shaw 1991), greater syllable rate (Brunel 2012, Tuckerman et al. 1992) or lower carrier frequency (Wedell & Sandberg 1995, Latimer & Sippel 1987) that may reflect greater available energy or larger body size, respectively. Similarly, male body mass may be correlated with nuptial gift size (reviewed in Gwynne 2001, Lehmann 2012, but see Tuckerman et al. 1992 for an absence of such a correlation). Thus, a discriminating female could potentially determine which males are likely to provide superior direct benefits at mating by paying attention to their long-range advertisement songs. Importantly, she would make this determination without expending time and energy and exposing herself to predation, costs that individual visits to and close inspection of various local males could entail (Searcy & Andersson 1986, Brown & Gwynne 1997).

The above findings showing a relationship between female preference and song traits, female preference and male body size, male body size and nuptial gift, and female fitness and a male's nuptial gift, suggest that female bushcrickets could use song to estimate the direct benefits to come. However, none of these studies have explicitly investigated the direct relationship that may exist between song traits and nuptial gift quality. The measure of this relationship is critical because both song and nuptial gift vary throughout a male's adult life, especially with his age, mating status and the time elapsed since the last mating (Wedell 1994, Lehmann & Lehmann 2000, 2008, 2009, Wedell & Ritchie 2004). The only way to determine whether females obtain greater direct benefits by preferentially mating with males showing particular song traits is by testing the relationship that exists between these two parameters.

We investigated such a relationship between male song traits and spermatophylax quality in the bushcricket *Ephippiger diurnus* (Orthoptera: Tettigoniidae). As in many bushcrickets, pair formation in *E. diurnus* results from the attraction of sexually receptive females by a male advertisement call (Busnel & Dumortier 1955). Because *E. diurnus* males transfer an extremely large spermatophylax to the female during mating (over 30% of male body weight, Busnel & Dumortier 1956, Wedell 1994), this species is most suitable for addressing the question of male signalling as a pre-mating cue for direct, nutritional benefits. In particular, we asked *i)* whether song traits can reliably predict spermatophylax quality in *E. diurnus* and also *ii)* whether male age or male mating status influences this possibility. Because the donation of a large spermatophylax may represent a considerable investment for a male (Vahed 2007a), males may also be expected to display, to some extent, mate discrimination and preferences (Trivers 1972, Bonduriansky 2001). A previous study on *E. diurnus* revealed a reversal of conventional sex roles wherein males became the choosier sex when both sexes were deprived of food (Ritchie et al. 1998). As in other facultative sex-role reversed bushcricket species, male investment is thought to be one of the main causes underlying these shifts in mating behavior (see Gwynne 2001 for a review). We therefore also explored *iii)* whether age and body mass of the receiving female influenced the size or quality of the spermatophylax that a male donated, and *iv)* whether potential male choice might reduce the predictive value of male songs.

MATERIALS AND METHODS

Population studied

Ephippiger diurnus (Orthoptera: Tettigoniidae), is a flightless bradyporine bushcricket common in the Mediterranean area of southern Europe (Duijm 1990, Oudman et al. 1989, 1990). We studied *E. diurnus* that descended from a population found at 1700 m on the Col de Mantet in Département Pyrénées Orientales, France (42°28´N, 2°18´E), previously designated as *E. cunii* Bolivar (Harz 1969, Hartley & Warne 1984, but see Kidd & Ritchie 2000, Spooner & Ritchie 2006 for a genetic based view on the *Ephippiger* complex). Previous work on this population in the field and laboratory showed that the male song consists of phrases that have an average of 4-5 syllables (fig.1) and that females prefer males whose songs include a higher than average number of syllables (Ritchie 1992, 1996, Brunel 2012). During mating male transfers a spermatophylax that comprises over 30 % of his body mass to his mate. He then begins a 48-72-h refractory period when he neither sings nor courts females (cf. Busnel & Durmortier 1955).

Rearing

Our experimental insects were F_1 offspring of individuals collected in the field in July 2009. Eggs were cultured in Petri dishes filled with moist cotton according to standard techniques (Hartley & Dean 1974), which included exposure to 4 ± 2°C during two 150 days intervals separated 120 days at 20 ± 2°C, followed by final placement at 20 ± 2°C. This temperature regime was necessary for the eggs to complete diapause and hatch. We suppressed mould growth by regularly spraying the cotton in the Petri dishes with a 1% Methyl-4-hydroxybenzoate solution.

Upon hatching, nymphs were individually reared in (height x diameter) 8 x 5 cm plastic cages and provided with cabbage, pollen, and fish food *ad libitum*. After the final moult, adults were transferred to individual 10 x 15-cm cages and provided with the same diet. Males and females were kept in separate rooms to prevent female habituation to male songs. Rearing and experiments were both conducted in laboratory chambers kept at 25 ± 2°C and a 16:8 L:D photoperiod.

Experimental procedures

To test the hypothesis that song traits function as predictors of spermatophylax size or quality, we first recorded the song of virgin males who were 24-26 days old (Young males, 1st mating, *N* = 22), as measured from their adult moult. We introduced a virgin female into the cage of each male within the *24* h following the recording in order to collect and measure the features of the spermatophylax transferred (see below). Mating trials were conducted in the males' rearing room wherein insects experienced a constant acoustic environment stimulating mating in both sexes. If mating was not successful on the day of recording, we paired the male again on the following morning with a different female. Only data obtained from successful matings were retained.

In order to evaluate the potential influence of male age and mating history on the relationship between song and spermatophylax traits, we also measured calls and nuptial gifts produced by 33-35 days old virgin males (Old males, 1st mating, *N* = 13) and 33-35 days old males who had already mated once (Old males, 2nd mating, *N* = 15). We sampled these latter 15 males from the group of 22 males who were initially recorded and paired when 24-26 days old. In most cases, 8-9 days elapsed between both mating. As before, mating occurred within 24 h following the recording. All experiments took place between 08:00 and 15:00 h, the main singing and mating period in *E. diurnus* (cf. Busnel and Dumortier 1955). We weighed males and females on a microbalance (±1 mg) prior to each recording and pairing session. The female's age, which ranged from 16-63 days, was also noted.

At the time we conducted our study, fewer females than males were available in the laboratory population. In some cases, we therefore used females that had been previously paired in an earlier trial. In this regard, we point out that the complete spermatophore was always removed from the female immediately after its transfer (see Nuptial Gift Features section below). This prevented the sperm from leaving the ampulla and entering the female's reproductive tract and the female from consuming any part of the spermatophylax. As careful monitoring of the females showed that none of them laid eggs in this condition, we considered these females as still virgin (see Results for the absence of influence of female prior mating experience on the spermatophore transferred by males). To address the possibility that males could adjust the nuptial gift to female quality in regards of their own quality, we also examined the relationship between the quality of the gift transferred and the size difference between the mates (male body weight – female body weight).

Male song traits

We kept males in cages with screen covers for song recording. The insects usually ascended to the top of the cages, and preliminary tests showed that the screen covering did not interfere with the transmission of the song outside of the cage. We recorded the songs with an electret measurement microphone (model 7016; ACO Pacific; Belmont, CA, USA.; frequency response ±2 dB from 0.01-100 kHz, ±6 dB from 0.01-160 kHz) whose output was amplified (preamplifier adaptor; Avisoft Bioacoustics; Berlin, Germany) and sent to a digital tape recorder (model HD-P2; Tascam; Tokyo, Japan). The analogue digital converter in the tape recorder sampled the song at 192 kHz and 16 bits. We note that most of the energy in *E. diurnus* song extends from 10-50 kHz, making the 192-kHz sampling rate more than adequate for an accurate digital recording. *E. diurnus* typically sing in groups, and lone males may sing irregularly at best. To circumvent this problem and provide a uniform acoustic environment, we stimulated each male with a loudspeaker broadcast of *E. diurnus* song during his recording. The song stimulus was the digital recording of an 'average' song from the Col de Mantet population, and we continuously 'looped' this file on a notebook computer using signal processing software (BatSound Pro 4.0; Petterson Elektronik AB; Uppsala, Sweden), converted it to analogue with an input/output card (DAQcard 6062E; National Instruments; Austin, Texas, USA), and sent the analogue output to the broadcasting loudspeaker (model ScanSpeak; Avisoft Bioacoustics;

frequency response: ±2 dB, 60-120 kHz; amplified by an Avisoft power amplifier). We then used a sound level meter (model CEL-430/2; Casella, Kempston, UK; flat frequency response from 0.03-20 kHz) to adjust the amplitude of the stimulus to that representative of a male ca. 70 cm distant. For recording, we fixed the measurement microphone 15 cm above the cage and oriented it directly toward the male within.

We recorded each male for three 60-s intervals separated by 20-30 min during a recording session. These repetitions ensured that our recordings captured the variation inherent in a given male's signalling. We later analyzed the recordings with digital signal processing software (SASLab Pro; Avisoft Bioacoustics; Berlin, Germany) designed for acoustic signals. From this analysis we determined the call rate (song phrases$\cdot$min^{-1}), the mean number of syllables per song phrase (Fig. 1), and the mean peak frequency. The latter parameter was determined by randomly sampling 3 song phrases from each of the three 60-s recording intervals in a given male's session and then averaging the frequency having maximum energy in a middle syllable of each sampled phrase. We chose to measure the first 2 parameters because they represent acoustic energy or power and may thus reflect male condition. Moreover, previous studies showed that females in various *E. diurnus* populations prefer male songs delivered at a faster call rhythm and whose phrases include a higher number of syllables (Ritchie 1992, 1996). Importantly, females from the Col de Mantet population pay more attention to an elevated number of syllables than to a faster call rate when choosing males (Brunel 2012). We measured the mean peak frequency because, like call rate and syllable number, it does not change over distance from a singing male. It thus has the potential to serve as reliable information about the male quality. We did not determine call amplitude because *E. diurnus* males move while they sing, and our measurement microphone therefore recorded a different level every time the male changed of position or orientation within the cage. Moreover, as the song amplitude perceived by a female can reflect the distance to the male as well as his absolute acoustic energy, we considered that it may not be a reliable indication of male quality when the female remains distant. There is, to our knowledge, no evidence that acoustic insects can 'range' the distance to a sound source by features of the song alone (cf. Gerhardt & Huber 2002) and thus determine its absolute amplitude.

Nuptial gift features

Spermatophores were removed from the female's genitalia immediately after their transfer. The ampulla was separated from the spermatophylax and both parts were weighed fresh on a microbalance (±1 mg). We measured the water content of the spermatophylax by dividing it in 2 halves, desiccating one half by freeze-drying (primary drying: 1h at -10°C, ≈ 25 mbar; secondary drying: 12 h at -76°C, ≈ 0, 0010 mbar), and comparing fresh and dry mass of this half. To establish the protein content of the spermatophylax, we ground dried subsamples and suspended 10 mg of the resulting powder in methanol. The concentration of protein in this suspension was then determined spectrometrically at 595 nm using the Bradford protein assay (Bradford 1976).

Statistical analysis

Prior to any analysis, normality of the data distribution was examined using Kolmogorov-Smirnov tests. The relationships between male body mass, song and spermatophore traits, as well as those between spermatophore traits and female traits, were investigated using Spearman's correlation tests. To account for the number of comparisons being performed and avoid false positives, the alpha value was adjusted using the Holm-Bonferroni sequential method (Holm, 1979, Rice 1989).

Comparisons of spermatophore quality between the three categories of males were conducted using parametric (t-test) or non-parametric (Mann-Whitney U-tests) depending on the normality of the data. Differences in the structure of female age in the different groups were analysed with Kruskal-Wallis tests. Relationship between nuptial gift and size difference between mates was investigated using Pearson's correlation test. All statistics were carried out using SigmaStat® 3.5 software.

RESULTS

General song and nuptial gift traits

Of the 47 recorded males, 35 successfully mated with a female. These 35 *E. diurnus* males produced call phrases that averaged 4.2 ± 0.4 syllables (mean ± SD). Call rhythm averaged 15.3 ± 7.2 phrases·min^{-1}, and call peak frequency averaged 19.1 ± 2.5 kHz. For males mated twice, male song did not differ from the first mating to the second one (syllable number, call rate and peak frequency, paired t-test, respectively, $t_{14} = 0.299$, $P = 0.77$; $t_{14} = -1.255^{E-14}$, $P = 1$; $t_{14} = -0.855$, $P = 0.77$, table 1). Similarly, at their first mating, male song did not differ between young and old males (syllable number, call rate and peak frequency, Mann-Whitney U-test, respectively, $U = 120$, $N1=13$, $N2= 22$, $P = 0.441$; $U= 174$, $N1=13$, $N2= 22$, $P = 0.297$; $U= 105$, $N1=13$, $N2= 22$, $P = 0.199$, table 1).

The combined mass of the spermatophore (spermatophylax + ampulla) averaged 822.56 ± 138 mg, which represented 28 ± 4.96 % of male body mass, while the spermatophylax mass accounted for 23 ± 4.14 % of male body mass. Spermatophylax water content represented 85.3 ± 1.6 % of the spermatophylax fresh mass, while the spermatophylax protein content averaged 13.9 ± 1.7 µg, accounting for 13.8 ± 7.1 % of the fresh mass. Spermatophore and spermatophylax mass were strongly correlated (Spearman Rank correlation, $r_s = 0.937$, $N = 35$, $P < 0.0001$). We therefore only retained the spermatophylax mass for the following analyses in order to avoid reporting results suffering from autocorrelation. Moreover, the spermatophylax is the part consumed by the female and thus represents the potential direct benefits donated by the male.

Males mated twice transferred significantly less protein at their second mating than they did at their first mating (Paired t-test, $t_{14} = 2.75$, $P = 0.016$, table 1), although the size of the spermatophylax did not differ between both matings (paired t-test, $t_{14} = 0.198$, $P = 0.846$, table 1). However, males mating for the first time at 33-35 days and same-age males mating for a second time did not transfer significantly different gifts to females (t-test, $t_{26} = 0.156$, $P = 0.30$ for spermatophylax mass, and , $t_{26} = 0.192$, $P = 0.24$ for protein content, table 1). Similarly we found no difference between the quality of the spermatophylax transferred by young and old males at their first mating (t-test, $t_{33} = 0.142$, $P = 0.26$ for spermatophylax mass, Mann-Whitney U-test, $U = 0.161$, $N_1 = 22$, $N_2 = 13$, $P = 0.24$ for protein content, table 1).

Relationship between song, body mass, and spermatophylax traits

In young males at their first mating, male body mass averaged 2886 ± 316 mg. In this group, none of the song traits that we measured predicted body mass (Table 2). Male body mass appeared to predict the size, but not the protein content of the spermatophylax (Table 2). Among the measured song traits, only the mean syllable number was correlated with the spermatophylax mass (Table 3), but this effect disappeared after correction for multiple tests. Similarly, none of the song traits predicted spermatophylax protein content (Table 3).

In older males at their first mating, male body mass averaged 3036 ± 413 mg. Again, none of the measured song traits predicted body mass (Table 2). In this group heavier males did not produce a larger spermatophylax (Table 2), and male body mass was not correlated with spermatophylax protein content (Table 2). None of the song traits predicted either spermatophylax mass or protein content once corrections for multiple tests were made (Table 3).

Older males at their second mating averaged 2797 ± 376 mg. In this group, however, heavier individuals did produce a larger spermatophylax (Table 3) that had higher protein content (Table 2). But as in the 2 previous groups, none of the song traits predicted male body mass (Table 3) or spermatophylax traits (Table 3).

Relationship between spermatophylax traits and features of the receiving female

As 9 females were paired more than once, we compared the quality of the nuptial gift that they received at their first and second pairing trial. We found no effect of prior female mating experience on the gift transferred by males (Wilcoxon signed rank test, $T = -29$, $N = 9$, $P = 0.098$ for spermatophylax weight and $T = 29$, $N = 9$, $P = 0.098$ for protein content).

As above, we analysed separately the data from the 3 categories of males. Although we found no significant influence of the receiving female features (age and body mass) on the traits of the spermatophylax given by young males who were mating for their first time, we observed that when older males were mating for their first time they provided larger spermatophylaces with higher protein content to older females (Table 4, Fig 2). However, when older males were mating for their second time, they provided larger spermatophylaces with higher protein content to heavier females (Table 4, Fig 3). It should be noted that female age structure did not differ between the three male categories (Kruskal-Wallis test, $H_{50} = 0.484$, $P = 0.785$), and female body mass was not correlated with female age (Spearman Rank correlation, $r_s = 0.008$, $N = 22$, $P = 0.971$).

Finally, we found no significant relationship between the nuptial gift quality (size and protein contents) and the size difference between the mates, regardless of the category of males considered (Pearson correlation, young males at first mating: $r = 0.00$, $P = 0.98$ for spermatophylax size, $r = 0.12$, $P = 0.34$ for protein content, $N = 22$. Old males at first mating: $r = 0.25$, $P = 0.42$ for spermatophylax size, $r = -0.32$, $P = 0.28$ for protein content, $N = 13$. Old males at second mating: $r = 0.05$, $P = 0.\ 85$ for protein content, $r = -0.02$, $P = 0.\ 0.91$ for spermatophylax size, $N = 15$).

DISCUSSION

Does male song indicate nuptial gift quality?

Because of the strong preferences that female *E. diurnus* exhibit for certain male song traits and the potential value of a male's spermatophylax as paternal investment, we expected that females would use one or more of a male's song traits as an indication of the spermatophylax to be transferred. However, results from the joint examination of song and spermatophylax traits in given males failed to reveal any support for this expectation. Our confidence in this finding is increased by having treated the data from 3 male categories separately and by having examined multiple song and spermatophylax traits. The examined song traits included features that females evaluate during mate choice (mean syllable number, call rate, Ritchie 1992, 1996, Brunel 2012). These traits do not vary over distance from a sound source and could thus provide reliable information about a singing male. The traits could also reflect the energy expended during signalling (mean syllable number, call rate) and male body size (carrier frequency), and thus be related to a male's condition and his capacity to produce and transfer a large spermatophylax. Although males were reared on a standard laboratory diet, condition as indicated by body mass varied among individuals (see Results) and had the potential to influence spermatophore quality.

Despite having taken various song measurements to ensure a valid examination of the relationship between a male's song and his spermatophylax, other song traits might be used by females to anticipate the nuptial gift quality. For instance, song amplitude, despite changing over distance from a singing male, might inform females about certain general aspects of the singer's quality. Song parameters displayed by males only when interacting with neighbouring callers, could also give critical information to females. *Ephippiger diurnus* females from monosyllabic populations exhibit a rather pronounced precedence effect in which they prefer male calls that lead their neighbour's calls by a short interval (Greenfield et al. 1997, Greenfield & Snedden 2003, Greenfield et al. 2004). This preference has probably selected for the temporal adjustments that male *E. diurnus* make in their calling rhythm such that the likelihood of broadcasting leading calls increases (Greenfield & Snedden 2003). At this point we do not know whether certain males are more proficient at producing leading calls and whether such proficiency might indicate a superior spermatophylax (cf. Richardson et al. 2008). Regarding spermatophylax traits, we measured protein contents although other substances might be considered. For example, the *E. diurnus* spermatophylax is likely to contain carotenoid compounds which may serve as critical anti-oxidants, such as those found in the ampulla of a related *Ephippiger* species (Heller et al. 2000).

If male song traits do not indicate features of the spermatophylax to be transferred, why do females bother to evaluate song? The most likely explanation is that the various song characters are heritable traits that would influence the attractiveness of male offspring, and possibly the general viability of offspring as well. Earlier findings showing that the syllable number in male song and female preference for syllable number are both heritable traits in *E. diurnus* (Ritchie 1996, 2000) support the first of these hypotheses, the Fisherian (arbitrary) sexual selection.

Male choice and the confounding of song as an indicator trait

Considering the high cost of producing a spermatophore representing up to 29.7 % of body mass in the present experiments, a male's capacity to transfer a nuptial gift is likely to be limited. Therefore, it would be advantageous for them to adjust their donation to the reproductive value of the female that they met and her likelihood of remating in a near future. Moreover, a male might calibrate these adjustments based on his own physiological state (age and mating status). Such differential allocation of nuptial gifts in relation to female quality has been found in other Orthopteran species (e.g. Wedell 1992, Simmons & Kvarnemo 1997). In our study younger males did not practice differential transfer, but older males gave less protein to females at the second mating than at the first one. This difference could reflect greater energy available to younger males and possible depletion of these nutrients as a result of a previous donation. Thus, males mated twice appear to allocate limiting resources to heavier females by giving them larger spermatophores containing more protein. This may represent a greater investment in females that are likely to produce a greater number of offspring. In many insect species, female body mass is a good indicator of the number of eggs they can lay (Ridley 1983, Honek 1993). This relationship however remains to be confirmed in *E. diurnus*. Old males at first mating invested differently and offered larger spermatophylaces containing more proteins to older females. This result is surprising, because males are expected to favour younger females, which normally have a greater reproductive value (Roff 1992). However, age may also indicate good survival abilities (indirect genetic benefits) as well as a reduced probability of further matings. This issue is critical in *E. diurnus* because of the precedence of the last-mating male's sperm in fertilizing the female's eggs (Hockham et al. 2004). Such choosy males thus increase their chances that their own progeny will benefit from the spermatophylax they transferred. Currently, we do not know which sensory mechanisms might allow male evaluation of female size and age, but courtship and copulation in this species is a lengthy procedure with many opportunities for close-range assessment (See for example Vahed et al. 2011). The fact that the two categories of males seem to prefer females on the basis of different criteria is surprising, but it is analogous to the way females may modify their choice of mates according to their own physiological state (Anjos-Duarte et al. 2011, Ligout et al. 2012). Further experiments are required to explain this effect in *E. diurnus* males.

In summary, *E. diurnus* males appear to exercise mate choice under a range of environmental conditions. Males reared on a restricted diet reject females often, which may reflect a high level of pre-copulatory choosiness when the cost of producing a spermatophore is particularly high (Ritchie et al. 1998). In our study males were reared on an *ad libitum* diet but they retained some choice by adjusting the quantity and quality of their nuptial gift in accordance with certain attributes of the female. Such adjustment may represent a form of cryptic male choice, and it suggests that male choosiness is a general feature of *E. diurnus* that is expressed at different intensities and stages of pair formation.

Conclusion

Can male mate choice account for the apparent absence of male song traits, especially those attracting and evaluated by females during mate choice, as predictors of nuptial gift quality? Yes, as male choice implies that the quality of the spermatophylax transferred by a given male is not predetermined but can be modified according to the female's features (age and body mass). The only way a female could make a prior evaluation of a nuptial gift to come, would be by assessing her own 'value' and thereby anticipating the adjustment a male broadcasting a particular song would make in his donation. This possibility, which is very unlikely, is even more improbable due to the adjustments that males make according to their own physiological state. Thus, male mate preferences render pre-mating assessment of direct benefits transferred by males effectively impossible for *E. diurnus* females.

On a more general level, the combination of female and male mate choice, commonly termed 'mutual mate choice', generates an elevated level of complexity in sexual selection. Not only do certain assessments of mating partners become unreliable, but male and female mating success may differ markedly from those occurring when mate choice is unidirectional. Sexual selection studies may need to consider this phenomenon during experimental design and subsequent analysis.

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Figure legends

Figure 1: Oscillogram of male calling song (a) and phrase detail (b) in *Ephippiger diurnus*. Each syllable consists in a short component (opening the forewings) and a longer component (closing the forewings). Female preference is mainly based on this second movement (adapted from Ritchie 1996).

Figure 2: Influence of female age on the quality of the spermatophylax provided by *E. diurnus* males. Relationship (Spearman Rank correlation) between female age and the mass (a) or the protein content (b) of the spermatophylax given by old males at second mating.

Figure 3: Influence of female body mass on the quality of the spermatophylax provided by *E. diurnus* males. Relationship (Spearman Rank correlation) between female body mass and the mass (a) or the protein content (b) of the spermatophylax given by old males at first mating.

Table 1: Mean values (± SD) of song and spermatophore traits according to males' age and mating status.

	Male type		
	Young male, 1st mating	**Old male, 1st mating**	**Old male, 2nd mating**
Song traits			
Syllable number	4.16 ± 0.32	4.32 ± 0.39	4.28 ± 0.40
Call rate	16.21 ± 4.67	13.84 ± 6.50	16.04 ± 4.63
Peak frequency	19.11 ± 0.68	19.12 ± 0.84	20.14 ± 0.48
Spermatophore traits			
Spermatophylax mass	701.44 ± 120	661.08 ± 109	698.08 ±122
Protein content	151.20 ±75	127.08 ± 71	98.83 ± 41

Table 2: Relationship between male body mass, song and spermatophore traits in *E. diurnus.*

	Male body mass		
	Young male, 1st mating	**Old male, 1st mating**	**Old males, 2nd**
Song traits			
Average syllable number	r_s=-0.35, p=0.10	r_s=0.43, p=0.14	r_s=0.15, p=0.59
Call rate	r_s=0.27, p=0.23	r_s=-0.39, p=0.17	r_s=0.10, p=0.73
Mean peak frequency	r_s=-0.10, p=0.66	r_s=0.44, p=0.13	r_s=-0.25, p=0.38
Spermatophylax traits			
Mass	**r_s=0.58, p=0.005** [a]	r_s=-0.28, p=0.34	**r_s=0.79, p< 0.0001** [a]
Protein content	r_s=0.09, p=0.67	r_s=-0.45, p=0.12	**r_s=0.72, p=0.002** [b]

r_s: Spearman Rank correlation coefficient. *p*: p-value. Significant results are in bold.

[a] α-value following sequential Holm-Bonferroni adjustment: 0.01,

[b] α-value following sequential Holm-Bonferroni adjustment: 0.0125.

Table 3: Relationship between song and spermatophylax traits according to male age and mating status in *E. diurnus.*

	Spermatophylax traits	
Song traits	Mass	Protein content
Young males, 1st mating		
Average syllable number	r_s=-0.46, p=0.03 [a]	r_s=0.08, p=0.72
Call rate	r_s=0.23, p=0.29	r_s=0.07, p=0.75
Mean peak frequency	r_s=-0.19, p=0.40	r_s=-0.07, p=0.77
Old males, 1st mating		
Average syllable number	r_s=0.02, p=0.94	r_s= 0.24, p=0.41
Call rate	r_s=0.38, p=0.19	r_s=0.58, p=0. 04 [a]
Mean peak frequency	r_s=0.00, p=0.99	r_s=-0.40, p=0.17
Old males, 2nd mating		
Average syllable number	r_s=-0.07, p=0.78	r_s=-0.2, p=0.47
Call rate	r_s=-0.07, p=0.80	r_s=-0.04, p=0.87
Mean peak frequency	r_s=-0.08, p=0.76	r_s=0.11, p=0.69

r_s: Spearman Rank correlation coefficient. p: p-value. Significant results are in bold.

[a] α-value following sequential Holm-Bonferroni adjustment: 0.008

Table 4: Influence of female traits on the quality of the spermatophylax transferred by males according to their age and mating status in *E. diurnus.*

	Spermatophylax traits	
Female traits	**Mass**	**Protein content**
Young males, 1st mating		
Female body mass	r_s=0.50, p=0.02 [a]	r_s=0.09, p=0.69
Female age	r_s=0.24, p=0.29	r_s=-0.05, p=0.83
Old males, 1st mating		
Female body mass	r_s=-0.33, p=0.28	r_s=-0.15, p=0.62
Female age	**r_s=0.74, p=0.004** [b]	**r_s=0.61, p=0.03** [c]
Old males, 2nd mating		
Female body mass	**r_s=0.70, p=0.003** [b]	**r_s=0.72, p=0.002** [c]
Female age	r_s=-0.004, p=0.99	r_s=0.004, p=0.98

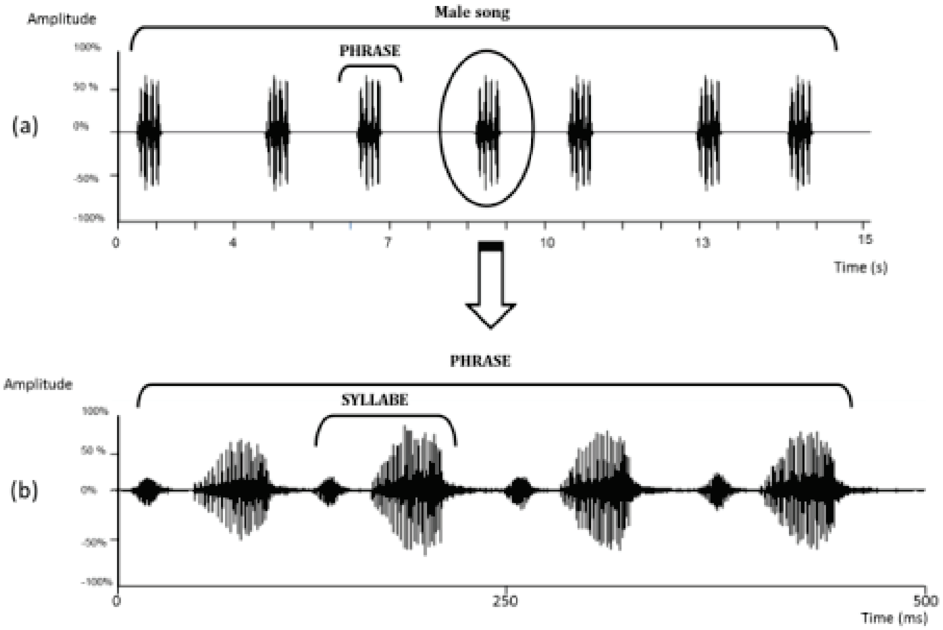
r_s: Spearman Rank correlation coefficient. p: p-value. Significant results are in bold.

[a] α-value following sequential Holm-Bonferroni adjustment: 0.017

[b] α-value following sequential Holm-Bonferroni adjustment: 0.025

[c] α-value following sequential Holm-Bonferroni adjustment: 0.05

Figure 1



Male song
Amplitude
PHRASE
(a)
100%
50 %
0%
-50%
-100%
0
4
7
10
13
15
Time (s)
PHRASE
Amplitude
SYLLABE
(b)
100%
50 %
0%
-50%
-100%
0
250
500
Time (ms)

Figure 2

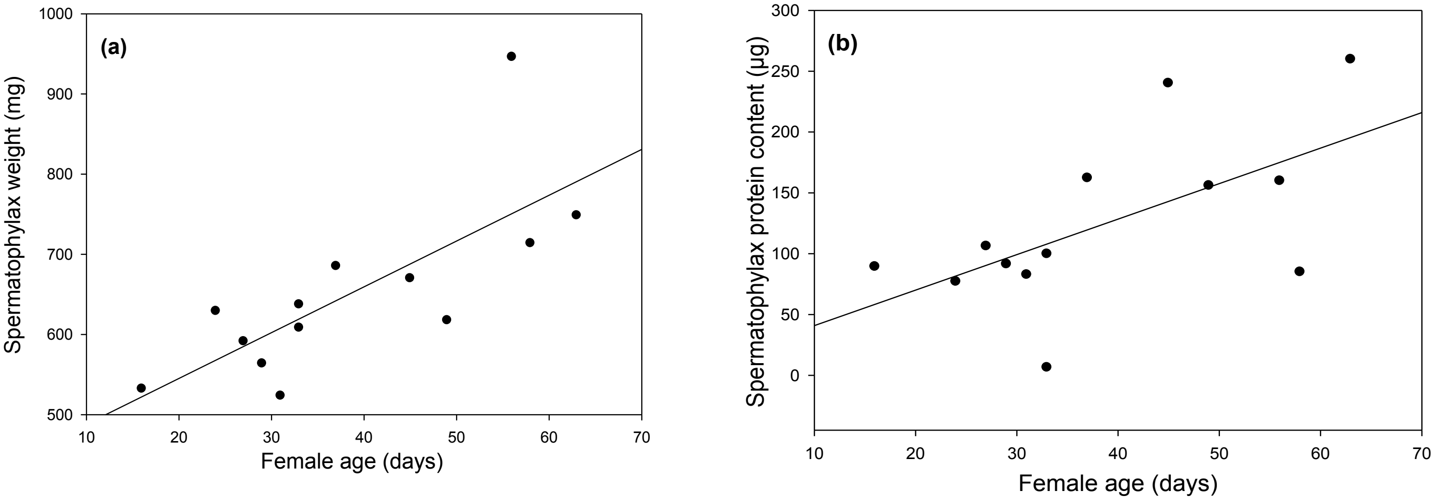
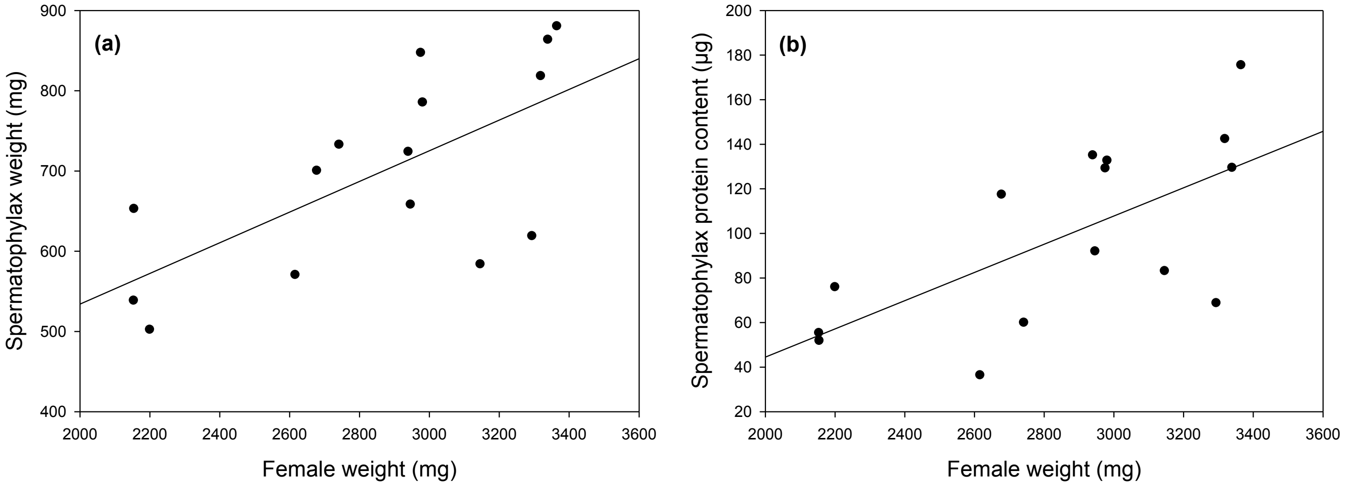

(a)
Spermatophylax weight (mg)
1000
900
800
700
600
500
10
20
30
40
50
60
70
Female age (days)
(b)
Spermatophylax protein content (µg)
300
250
200
150
100
50
0
10
20
30
40
50
60
70
Female age (days)

Figure 3


(a)
Spermatophylax weight (mg)
900
800
700
600
500
400
2000
2200
2400
2600
2800
3000
3200
3400
3600
Female weight (mg)
(b)
Spermatophylax protein content (µg)
200
180
160
140
120
100
80
60
40
20
2000
2200
2400
2600
2800
3000
3200
3400
3600
Female weight (mg)

Protocol for dissection of male and female *Achroia grisella* and eupyrene sperm bundle count

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A- **Material and equipment:**

Dissection:

- Dissection pool: Petri dish with stained candle wax (to buy from any hobby candle making shop) (Fig. 1)
- Binocular
- Two very sharp dissection forceps (Fig. 2)
- Needles
- Scalpel
- Wash bottle filled with distilled water
- Pipette and cones

Fig.1 Dissection pool

Fig.2 Dissection forceps

Eupyrene sperm bundle count:

- Microscopic glass slides
- Microscope with UV light

- DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride, Sigma Aldrich) – handle with care and use protective clothing as this chemical stains DNA, separate waste disposal required.
- Two hand counters

General advice for dissection:

Always prepare and check your tools before you start. While dissecting, always take time to re-adjust the light and the focus of your binocular. If the water is too blurred, do not hesitate to rinse with distilled water. If you have to do so i) first check for the presence of sperm bundles before rinsing and ii) rinse with a mild spurt from the wash bottle (avoid too strong spurts that might damage tissues). And…be patient, distinguishing the different organs from the messy abdominal mass and successfully removing them will definitely require several trials!

B- Female dissection

Female bundle counts will be achieved by removing the female *bursa copulatrix* (hereafter *bursa*), an intermediate sperm storage organ where the male spermatophore (containing the sperm bundles) is deposited. Within the *bursa*, the eupyrene sperm bundles from the spermatophore will be disrupted and single spermatozoa will migrate toward the spermatheca, where they will be stored until oviposition.

1. Subsequent to experimental copulations, place the female in the freezer for two minutes. This should be done immediately after mating (<< 5 minutes). If not respected, bundles might start to disrupt and migrate from the *bursa* to the spermatheca, thus creating bias in your count. For this reason, if you have to dissect both males and females, females should always be dissected first.
2. Place the female on dorsal side in the dissection pool (Fig. 3)
3. Place a needle in the thorax and decapitate with a scalpel (Fig. 3)
4. Spread the wings and place a needle to maintain each wing perpendicularly from the body (Fig. 3)
5. Remove the legs with a pair of forceps. This can be done under the binocular magnification with a x16 to x20 magnification (Fig. 4)

Fig.3

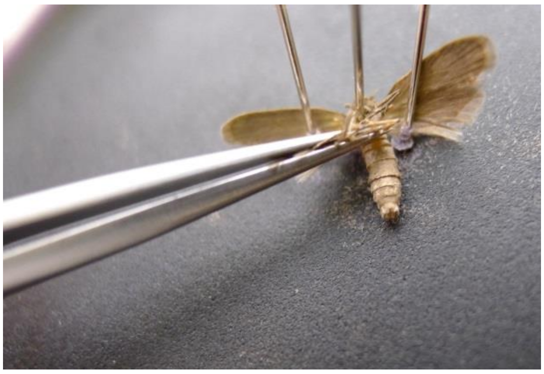


Fig.4

6. Under x16 to x20 binocular magnification, proceed to ventral opening, by inserting your forceps under the second sternite (= second ventral segment starting from the tip of the abdomen) and by gently tearing the cuticula apart (Fig. 5 a). Here the objective is only to rupture the cuticula, do not pull too much on the edge of the opening. Then proceed to the next segment and repeat the procedure until you reach the thorax (Fig. 5 b).

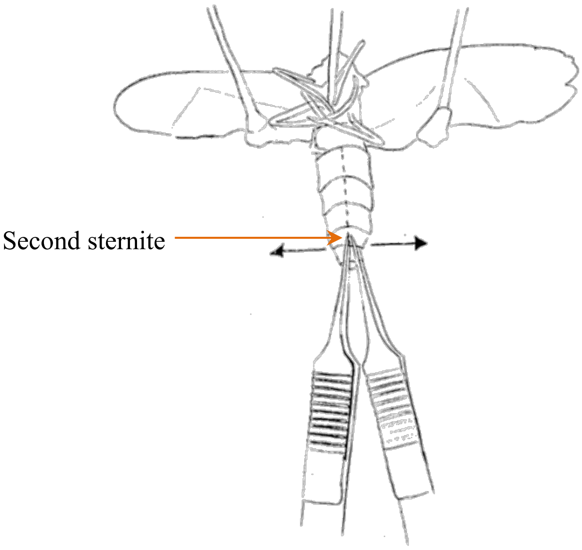


Fig.5 a

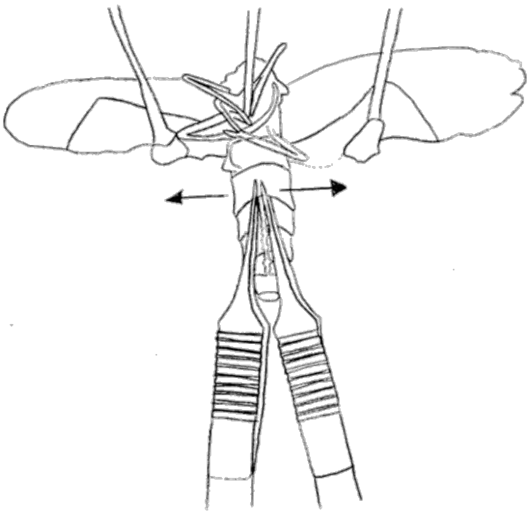


Fig.5 b

7. Once ventral opening is achieved, use the wash bottle to cover the abdomen with distilled water. This will make the organs float and ease localization of the *bursa*.

8. With your forceps, gently move the ovaries aside in order to locate the *bursa*. When the female has been mated, the *bursa* appears as a pearly white, round shaped organ with a curly channel (Fig. 6), located toward the tip of the abdomen. However be aware that this appearance might be

slightly different in virgin females as it will appear as an empty, relatively transparent membrane with a more or less straight channel (and is sometimes more difficult to locate).

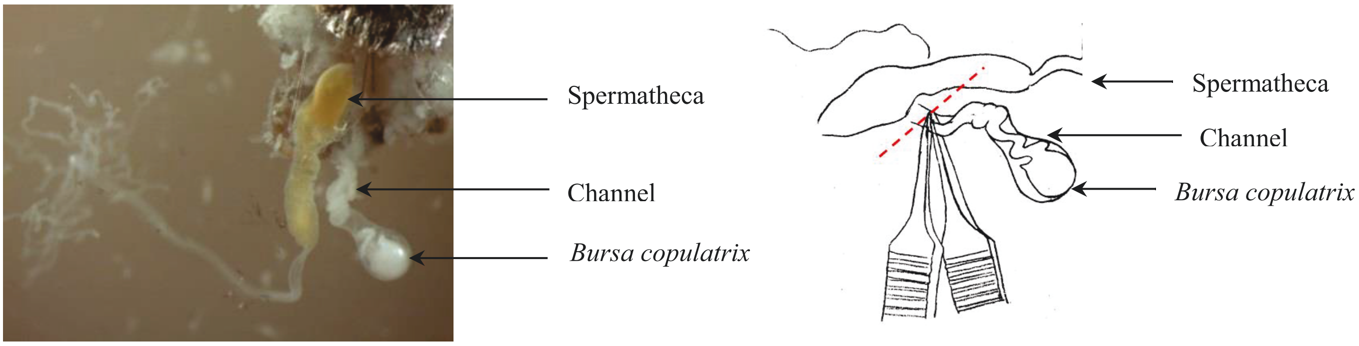


Fig.6 (a)

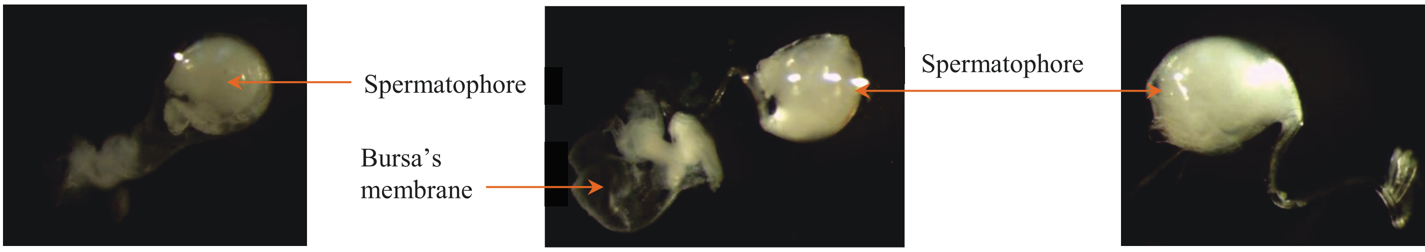
(b)

9. Once you located the *bursa*, prepare a glass slide with two 5 µl drops of 10^{-4} M DAPI.

10. With your forceps, grab the *bursa*'s channel at its basis (Fig. 6 b) and remove the bursa by rupturing the channel.

11. Place the *bursa* on the first drop of DAPI.

12. Remove the membrane of the *bursa*. To do so, gently grab the *bursa's* most external layer with your forceps and pull it out. This will release the spermatophore (Fig. 7).



Bursa copulatrix

Fig.7

13. Transfer the spermatophore in the second drop of DAPI, and disrupt it with a needle. You should avoid disrupting the spermatophore with the forceps, because the sperm bundles might be sucked between the forceps arms which results in huge downward bias in counting!

14. Count eupyrene sperm bundles under UV light microscopy at x 500 (see below D –Sperm Bundle Counting).

C- **Male dissection**

Counting the number of eupyrene sperm bundles in the male will require the removal of the entire male genital tract, as remaining bundles might be found in various location within the tract: in the testes, in the seminal vesicles and in the duplex (this latter case is rare in mated males but common in virgin males).

1. Steps 1 to 7: similar as in females.
2. Gently remove parts of the fat body in order to find the (nearly fused) testes, usually located centrally in the upper part of the abdomen. Here, you should progress very carefully: the male tract is relatively transparent and fragile and will easily break (should this occur your dissection is definitively lost). Once the testes are found, try to locate the thin, whitish adherences that loosely maintain the testes attached to the wall of the abdomen (see Fig. 8).

Note: in some rare cases, the testes are not fused but appear as two clearly separated testes situated in each side of the abdomen. In such case proceed as described above for each testis separately but be aware that this particularity might make the dissection more difficult.

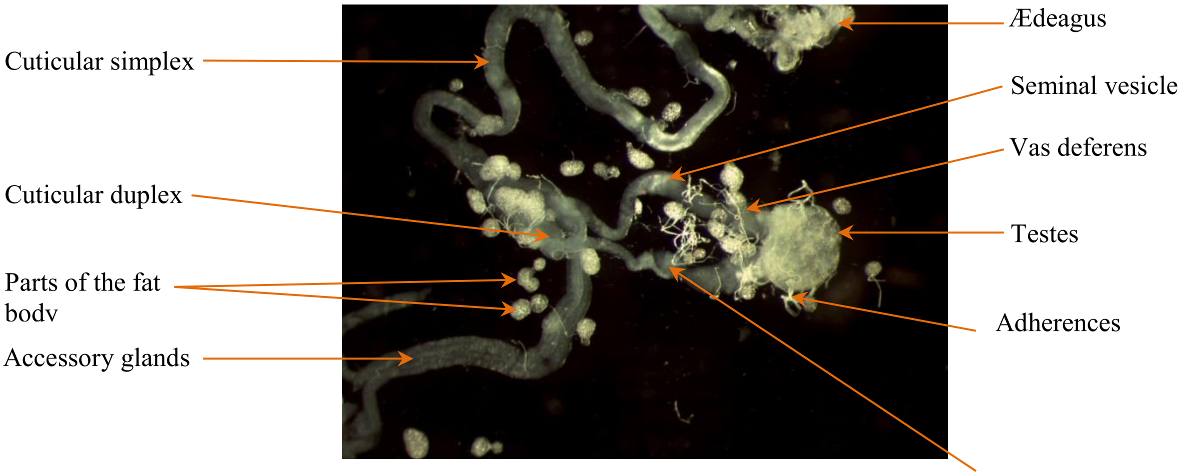


Fig. 8 (a) Male genital tract

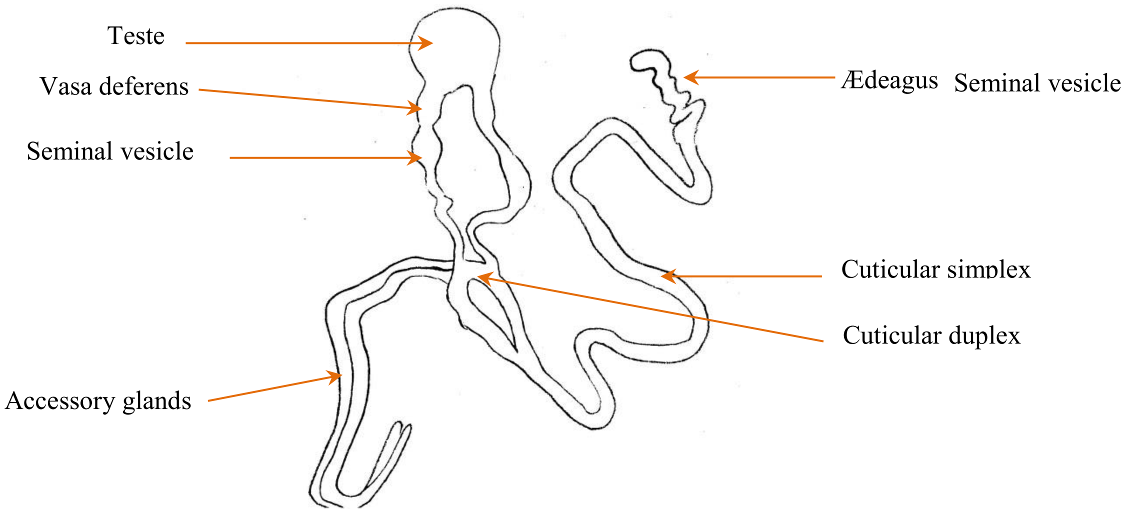


Fig. 8 (b) Schematic representation of male genital tract

3. Starting from the testes and all the way down to the ædeagus (i.e. the male reproductive apparatus located at the tip of the male abdomen), use the forceps to rupture all adherences holding the tract to the intern walls of the abdomen. During this process, you should never grab or pull the tract itself. At the end of this step, the tract should be entirely loose from the abdomen at the exception of the ædeagus.

4. Prepare a glass slide with three 5 µl drops of 10^{-4} M DAPI

5. With the forceps, grab and rupture the genital tract at the level of the ædeagus, and place it on the first DAPI drop on the glass slide.

6. Check for tract integrity. Because any rupture of the tract could lead to a significant loss of bundles and subsequent bias in counting, you should be absolutely certain that the tract has not been damaged during the preceding dissection steps.

    6.1. First, check that there are no bundles in the water remaining in the dissection pool. To do so, you should investigate all the depth of the water layer by adjusting the focus of your binocular (in order to see accurately from the bottom to the surface of the water). Even if this is not very comfortable for the eyes, don't skip this step and always do it very carefully, because really, it is easy to rupture the tract without noticing it in the first place.

    6.2. Secondly, gently move the tract in your DAPI drop. The tract should appear intact, and sperm bundles in the seminal vesicles should be visible. Here again, you should not see any bundles floating outside of the tract.

7. Rupture the tract between the seminal vesicles and the duplex (Fig. 9 line A and Fig. 10), and transfer the seminal vesicles and the testes in the second drop of DAPI. To do so, clamp both canals between the seminal vesicles and the duplex simultaneously with one pair of forceps, and tear it apart from the rest of the genital tract with the second pair of forceps.

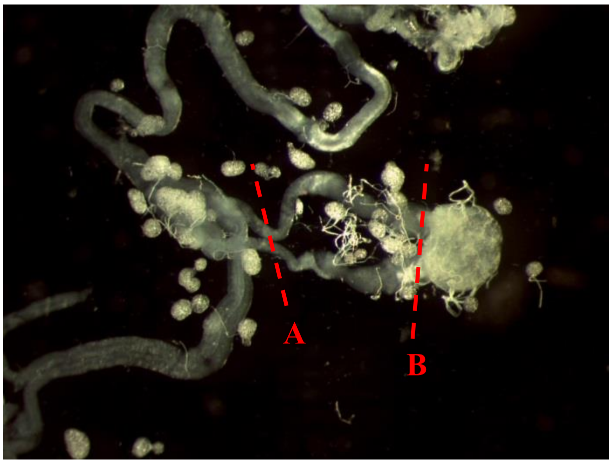


Fig. 9

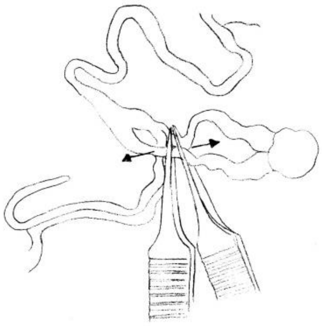


Fig. 10

8. Isolate the testes into the third drop of DAPI by clamping simultaneously both *vasa deferentia* below the testes with one pair of forceps while tearing it apart from the seminal vesicles with the other forceps (Fig. 9 line B).

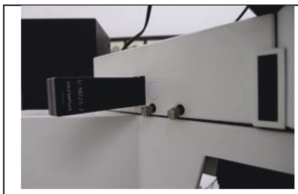
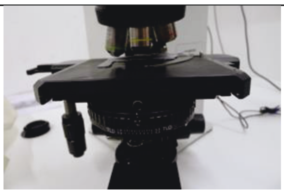
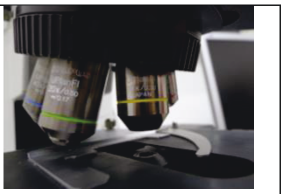
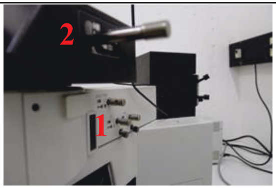
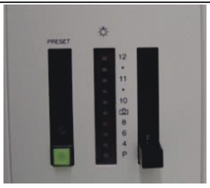
In addition to counting the number of bundles in the male genital tract as a whole, this method will allow you to record which part of the genital tract the bundles originated from.

Always proceed exactly as described above. For example, taking the seminal vesicles (instead of the testis) from the second to the third DAPI drop will result in bias in determining the origin of the sperm, because sperm bundles passively leak from the vesicles, but not from the testes. Therefore, the first drop in which you place the seminal vesicles will contain bundles from this part. However, if something goes wrong in steps 7 and 8, note that you should still be able to reliably count the total number of eupyrene bundles present in the male genital tract, only their origin will not be determined.

D- Sperm Bundle Counting

Microscope settings for microscope Olympus BX50 at Department of Evolutionary Biology, Bielefeld University:

Turn on the UV lamp 10-15 minutes before starting to count to heat the UV bulb.
Illustrated below are the settings used for bundle counting during the internship project by Alicia Jarrige in 11/12 2014:

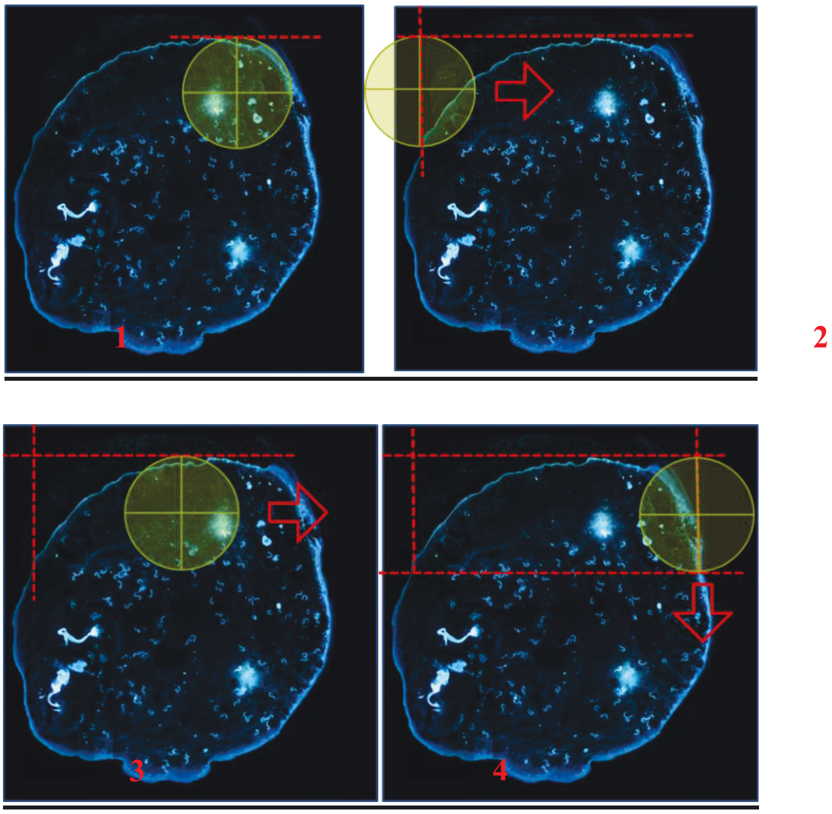
 Both filter U-ND6-2 and U-ND25-2 out	 Upper filter: position WU	 Lower filter: position DP10	 Objective x10
 All shutter in (1) If you need to take pictures, pull the first shutter in mid position (2)	 No standard light	 As in the picture !	 Filter U-DICT out.

Counting methodology:

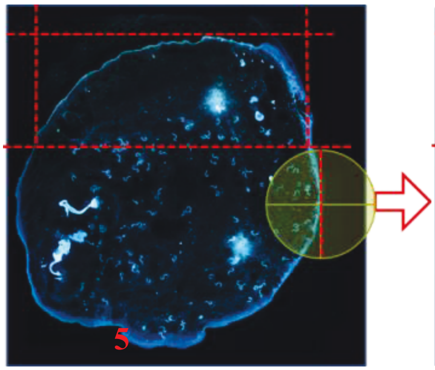
Sperm bundles after DAPI staining

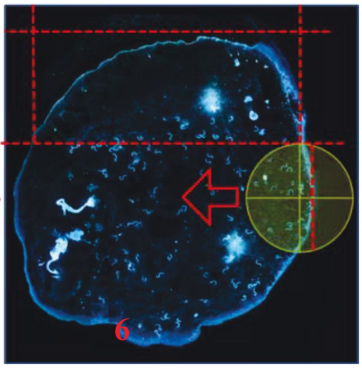
The pictures below (1 to 8) represent a drop of DAPI under UV light 200x magnification. As counting will be performed at 500x magnification, the bundles will appear larger. The focus of the microscope is depicted by the yellow circle. Start by making the largest vertical extension of the focus (vertical diameter of your focus, symbolized by the yellow cross) coincide with the upper part of the drop (1). This step is necessary to make sure you cover all the upper part of your drop (here the drop is very round, but drops will often be more irregular).

Then, move the focus to the left to make the horizontal diameter of your focus coincide with the greatest extension of the drop as shown in (2). The focus should move only on the horizontal axis: be careful not to displace it on the vertical axis as you would not anymore be aligned with the upper part of the drop. This is a general rule: always align the diameter of your focus with the greatest extension of the drop. You are now ready to start counting.



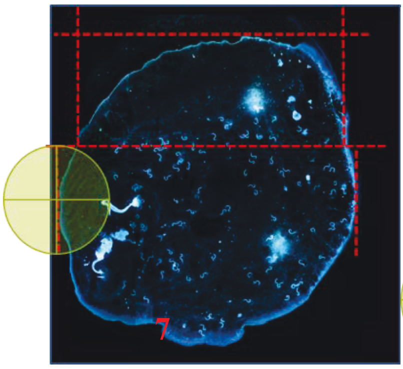
Slowly move the focus to the right and count sperm bundles entering your focus with a hand counter (3). Here again, you should be very cautious to move the focus only on the horizontal axis. Count all the bundles that do not entirely appear in the lowest part of your focus (that is, those that would overlap on the red line in (4)). Continue until the diameter of the focus coincides with the largest part of the right side of the drop. Then, move the focus down until the upper part of the focus reach the point immediately located under the lowest part of your previous position (5). Be very precise, as most measurement error in counts originates from this operation: moving the focus too much down would result in neglected bundles in your count, and moving not enough might result in bundles counted twice. Use water bubbles/impurities as points of reference to reach the right position with the upper part of the focus.

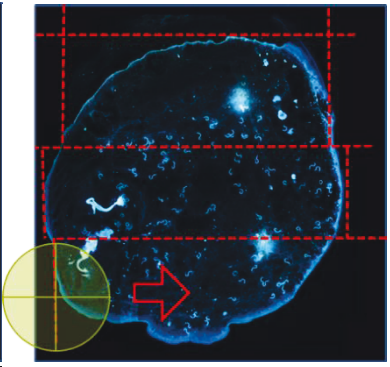




Then, move the focus to the right to realign the largest part of your focus with the largest part of the drop (5). Then, you can start again counting by moving your focus to the left and until you reach the left side of the drop with the focus diameter. Doing so, do not count the bundles that are half way in the upper part of your focus, as those have already been counted. However, count those that are half way out of the lower part of your focus.

Proceed the same way until the drop has been covered completely. At the beginning, I suggest that you count every drop twice blindly using two counters. Once you are done counting the first time, turn your slide and count it a second time, your final count will be the average of both counts (but record both individual counts for the purpose of repeatability analysis). When repeatability is high in a large enough number of test counts, you may count only once for the actual experiment. However, never change your counting methodology once engaged in an experiment.





8

Allocations stratégiques des ressources reproductives, gamétiques et non-gamétiques, chez deux espèces d'insectes.

Pour les mâles, non seulement la reproduction est une activité particulièrement couteuse, mais les ressources dont ils disposent pour y faire face s'avèrent souvent largement contraintes. En effet, la production de l'éjaculat (sperme et substances non gamétiques) peuvent générer des couts énergétiques substantiels, voire être limités au cours de la vie des mâles. Par ailleurs, les traits impliqués dans l'attraction des femelles ou la compétition entre mâles rivaux peuvent également être liés à d'importantes dépenses énergétiques et/ou des risques de dommages physiques. Enfin, la sélection sexuelle post-éjaculat (compétition spermatique et choix femelle cryptique) peut fortement affecter leurs chances de paternité. Sous ces conditions, il est attendu que les mâles allouent stratégiquement leurs ressources reproductives au regard (i) des opportunités d'accouplement présentes et futures, (ii) de la qualité reproductive de la femelle et (iii) du risque et/ou de l'intensité de la compétition spermatique. Toutefois, la nature des allocations stratégiques chez les mâles et les contraintes qui influencent leur évolution restent encore mal comprises, en particulier parce que les interactions potentielles entre sélection sexuelle pré- et post-copulatoire peuvent soumettre les mâles à des épisodes sélectifs plus complexes que ceux existants chez les femelles.Dans le cadre de ce travail de thèse, j'ai examiné les allocations de ressources reproductives chez les mâles de deux espèces d'insectes : le papillon *Achroia grisella* et la sauterelle *Ephippiger diurnus*. Les mâles de ces deux espèces émettent des chants d'appel sexuels ciblés par les préférences des femelles, et subissent de ce fait d'intenses pressions de sélections pré-copulatoire intra et intersexuelles. En revanche, ces deux modèles diffèrent grandement dans la contribution des mâles aux femelles lors de l'accouplement : tandis que le transfert des mâles *A. grisella* se résume aux spermatozoïdes, les mâles *E. diurnus* transfèrent le produit de leurs glandes accessoires sous la forme d'un large spermatophylax que les femelles consomment à l'issue de l'accouplement. J'ai donc plus spécifiquement étudié les variations d'allocation i) spermatique chez A. *grisella* et ii) du spermatophylax chez *E. diurnus*, au regard de la variance de qualité des femelles et/ou de la compétition inter-mâle pré- et post-copulatoire. Les résultats obtenus dans le cadre de cette étude révèlent des allocations stratégiques complexes et variées au sein des deux espèces étudiées, notamment en réponse aux indices sociaux révélateurs de compétition pré et/ou post copulatoire. En revanche, la qualité des femelles semble avoir un effet marginal sur les variations de réponse sexuelle des mâles *A. grisella* et *E. diurnus*. Nos résultats fournissent un apport empirique intéressant dans la compréhension des facteurs sous-jacents à l'évolution d'allocations stratégiques chez les mâles sous pressions conjointes de sélection pré et post-copulatoire. Ils mettent en lumière que la combinaison des processus sélectifs peut contraindre l'évolution d'allocations stratégiques associées à la qualité de la partenaire. Ces résultats sont ensuite discutés dans le contexte de la théorie de la sélection sexuelle et des rôles sexuels.

Contrasting with a long assumed assumption, reproduction is particularly costly for males. Not only can ejaculate production (sperm and other non gametic materials) induce substantial energetic costs and be drastically limited along males' life, but the traits involved in mate attraction or competition between rival males might further incur energetic cost and/or risks of injuries. In addition, post-copulatory mechanisms such as sperm competition and cryptic female choice might strongly bias male's chances of paternity. Under these conditions, males are expected to strategically allocate their reproductive resources according to i) present and future mating opportunities, ii) the female reproductive quality and iii) the risk or intensity of sperm competition. However, the means by which males allocate their reproductive resources as well as the factors affecting their evolution remains poorly understood, in particular because potential interactions between pre and post-copulatory sexual selection makes the selective episodes in males often far more complex than those existing in females. Therefore, during my PhD, I investigated the strategic allocations of male reproductive resources in two insect species: the moth *Achroia grisella* and the bushcricket *Ephippiger diurnus*. In both species, males experience strong precopulatory sexual selection, as males display sexual courtships songs targeted by female choice. However, these two models are contrasting when it comes to the male contribution to females during copulation: while males *A. grisella* appear to transfer only sperm, males *E. diurnus* provide females with the product of their accessory glands under the form of a large spermatophylax, that females readily consumes after mating. Thus, I more specifically investigated the variations of male investment in i) fertilizing sperm in *A. grisella*, and ii) spermatophylax in *E. diurnus*, in regards to female quality and/or pre and post-copulatory intra-sexual competition.The results obtained in this study revealed complex and multimodal allocations of reproductive resources in the two model species, in particular according to social cues indicative of pre and/or post copulatory competition. However, considering both species, variance in female quality appears to have a marginal effect on males' sexual response. Our results provide interesting empirical insight on the factors affecting the evolution of male strategic allocation under joint pre and post-copulatory selective pressures. In particular, they shade light on the combination of selective pressure that might constrain the evolution of strategic allocation toward female quality. These results are further discussed within the frame of sexual selection theory and sex roles.