JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12252

Genetic architecture of sensory exploitation: QTL mapping of female and male receiver traits in an acoustic moth

S. ALEM*, R. STREIFF†, B. COURTOIS‡, S. ZENBOUDJI§, D. LIMOUSIN¶ & M. D. GREENFIELD*

*Centre National de la Recherche Scientifique (CNRS), UMR 7261 (IRBI), Université François Rabelais de Tours, Tours, France
†Institut National de la Recherche Agronomique (INRA), UMR CBGP (INRA–IRD–CIRAD–Montpellier SupAgro), Montferrier sur Lez, France
‡Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR AGAP, Montpellier, France
§Centre National de la Recherche Scientifique (CNRS), UMR 5175 (CEFE), Montpellier, France
¶Institut National de la Recherche Agronomique (INRA), UMR 1272, Physiologie de l'Insecte Signalisation et Communication, Versailles, France

Keywords:

acoustic communication;
predatory–prey interactions;
receiver bias;
sensory ecology;
sexual selection.

Abstract

The evolution of extravagant sexual traits by sensory exploitation occurs if males incidentally evolve features that stimulate females owing to a pre-existing environmental response that arose in the context of natural selection. The sensory exploitation process is thus expected to leave a specific genetic imprint, a pleiotropic control of the original environmental response and the novel sexual response in females. However, females may be subsequently selected to improve their discrimination of environmental and sexual stimuli. Accordingly, responses may have diverged and the original genetic architecture may have been modified. These possibilities may be considered by studying the genetic architecture of responses to male signals and to the environmental stimuli that were purportedly 'exploited' by those signals. However, no previous study has addressed the genetic control of sensory exploitation. We investigated this question in an acoustic pyralid moth, *Achroia grisella*, in which a male ultrasonic song attracts females and perception of ultrasound likely arose in the context of detecting predatory bats. We examined the genetic architecture of female response to bat echolocation signals and to male song via a cartographic study of quantitative trait loci (QTL) influencing these receiver traits. We found several QTL for both traits, but none of them were colocalized on the same chromosomes. These results indicate that – to the extent to which male *A. grisella* song originated by the process of sensory exploitation – some modification of the female responses occurred since the origin of the male signal.

Introduction

The evolution of extravagant sexual traits by sensory exploitation occurs if males incidentally evolve features that stimulate females owing to a pre-existing environmental response (West-Eberhard, 1979, 1984; Ryan, 1990, 1998; Enquist & Arak, 1993; Ryan & Rand, 1993; Shaw, 1995; Endler & Basolo, 1998). This response is assumed to have arisen in the context of natural selection, and it may be found in both males and females (Kirkpatrick & Ryan, 1991; Ryan, 1998; Fuller *et al.*, 2005; Kokko *et al.*, 2006). For example, when a particular response to visual or olfactory cues of food or habitat exists, males who happen to produce a visual or olfactory display that imitates these cues may experience enhanced female encounter rates and mating success. This trait will then be favoured by sexual selection and become part of the male signalling repertoire. Because of this historical sequence of events, a phylogenetic pattern in which a female environmental response precedes the evolution of the corresponding male signal traits has been interpreted as evidence of

Correspondence: S. Alem, Centre National de la Recherche Scientifique (CNRS), UMR 7261 (IRBI), Université François Rabelais de Tours, Parc de Grandmont, 37200 Tours, France.
Tel.: +33 247 367 355; fax: +33 247 366 966;
e-mail: sylvain.alem@etu.univ-tours.fr

sensory exploitation (Basolo, 1990; Ryan, 1990; Ryan & Rand, 1993; Basolo, 1995; Shaw, 1995; Endler & Basolo, 1998; Smith *et al.*, 2004; Fernandez & Morris, 2007; Egger *et al.*, 2011).

Biologists have typically studied the possibility of sensory exploitation in signal evolution by employing the comparative methods of phylogenetic analysis. But the process of sensory exploitation may also leave a specific genetic imprint, a pleiotropic control of the original environmental response and the novel sexual response in females (Kirkpatrick & Ryan, 1991; Ryan, 1998; Fuller *et al.*, 2005; Fuller, 2009). This pleiotropy results from the evolutionary history of the environmental response, a trait originally selected for food or habitat recognition in females that later acquired an additional, sexual function once the novel male signal appeared. *De facto*, some loci would have a dual function and influence both the environmental response that evolved in the context of natural selection and the sexual response exhibited by females during mating.

A strict definition of the sensory exploitation process assumes that female responses remain unchanged following the initial appearance of the male signal. Full pleiotropic control of female environmental and sexual responses would be consistent with this narrow-sense definition, which leaves no option for coevolutionary mechanisms of sexual selection to function subsequent to the origin of the male signal (Via & Lande, 1985; Basolo & Endler, 1995; Christy & Backwell, 1995; Fuller, 2009). However, several authors have considered sensory exploitation from a broader perspective and have emphasized that the process does not necessarily preclude simultaneous or subsequent action by other, coevolutionary mechanisms (Ryan & Rand, 1993; Holland & Rice, 1998; Phelps & Ryan, 2000; Jennions & Brooks, 2001; Ryan *et al.*, 2001; Rodriguez & Snedden, 2004; Arnqvist, 2006). In addition, sexual signals that have evolved via sensory exploitation are often exaggerations of the imitated environmental cues and thus represent supernormal stimuli. Consequently, signal discrimination may also be essential to retain the ability to respond to environmental cues (Greenfield, 2002; Macias-Garcia & Ramirez, 2005) and/or to evolve resistance to the exploitative trait (Bradbury & Vehrencamp, 2000; Arnqvist, 2006). As an example, male courtship pheromones in the Lepidoptera are often derived from host plant substances which evoke feeding responses in both sexes or oviposition responses in females. While these pheromones may have originated via sensory exploitation, females currently respond differently to the male pheromones and the cues from the host plant (e.g. in the moth *Utetheisa ornatrix*; Eisner & Meinwald, 1995). Such discrimination would be critical if females are to avoid inappropriate environmental responses to sexual cues, while exhibiting unambiguous responses to males during courtship. Effective discrimination is perhaps most important in the case of male signals exploiting defensive responses to natural enemies. Here, discrimination may be essential for a female's immediate survival. In turn, selection for female discrimination is expected to favour the evolution of male signals that diverge increasingly from predator cues.

With an increasing divergence between environmental and sexual response traits, due to selection for discrimination and/or the action of coevolutionary mechanisms, independent adaptations such as new components of sexual display or new criteria in mate choice may arise (Greenfield, 2002; Macias-Garcia & Ramirez, 2005; Arnqvist, 2006; Fuller, 2009; Greenfield & Hohendorf, 2009). Some of these changes might entail modifications of the sensory system in which the environmental and sexual response traits acquire separate genetic control. The process of gene duplication represents an evolutionary mechanism that is potentially conducive to such separation, as gene copies may diverge in their functional specialization (Innan & Kondrashov, 2010). Genetic studies that focus specifically on pleiotropy can help resolve the fundamental question regarding putative cases of sensory exploitation: Have sexual responses remained unchanged since their origin as environmental responses, the situation predicted if pleiotropic control of both response traits is found, or have other processes intervened and led to evolutionary divergence between the responses? Whereas it is now acknowledged that investigations of the 'genetic architecture' of response traits would be critical for disentangling these possibilities (Fuller, 2009), to date no empirical study has addressed the issue.

Here, we present the findings of a study of the genetic architecture of female response traits in an acoustic pyralid moth, *Achroia grisella*. As in most pyralid moths, both sexes of *A. grisella* perceive ultrasound with a pair of tympanal ears located on the first abdominal segment, and it is assumed that this capability evolved approximately 60 million years ago in the context of avoiding insectivorous bats (cf. Spangler, 1988; Hoy, 1992; Hoy & Robert, 1996; Conner & Corcoran, 2012; Yager, 2012). In *A. grisella*, specialized defensive behaviour has been documented in both males and females in response to synthetic bat echolocation signals (Greenfield & Weber, 2000; Greenfield & Baker, 2003; Greig & Greenfield, 2004; Rodriguez & Greenfield, 2004; Alem & Greenfield, 2010) as well as to live bats (Spangler *et al.*, 1984; Alem *et al.*, 2011). Unlike the majority of pyralid moth species, however, male *A. grisella* also broadcast an intense ultrasound advertisement song that attracts receptive females (Spangler *et al.*, 1984; Conner, 1999). Because sensitivity to ultrasound originated as an environmental response to bat predation and is widespread within the Pyralidae, whereas the use of ultrasound in mating communication is only found in several isolated genera, it is inferred that male ultrasonic sexual signal evolved subsequently and that acoustic sexual communication in the family originated via the sensory exploitation pro-

cess (Greenfield, 2002). In the specific case of *A. grisella*, the general overlap in sound frequency between male song and bat echolocation signals that elicit sexual and defensive responses in females is consistent with the sensory exploitation inference (Greenfield & Hohendorf, 2009).

Previous genetic studies and molecular analyses conducted in *A. grisella* indicated that male song and female sexual preferences are heritable and independent traits (Collins *et al.*, 1999; Jang & Greenfield, 2000; Zhou *et al.*, 2011; Limousin *et al.*, 2012). These results are consistent with sensory exploitation, as genetic linkage between sexual signal and preference traits is not expected under this process (Fuller, 2009). The genetics of defensive behaviour have not been investigated as thoroughly, but a recent study using inbred lines suggested that sexual and defensive responses, in both males and females, might also be genetically independent (Greenfield & Hohendorf, 2009). This finding differs from the expectation under the narrower sense of sensory exploitation, where some level of pleiotropy is predicted (Fuller, 2009). Thus, female sexual responses appear to have diverged from their original, defensive function in *A. grisella*.

To examine the genetic architecture of female sexual and defensive responses, we bred hybrid and backcross generations from two inbred lines issued from two geographically distant populations of *A. grisella*, and we phenotyped both sexual and defensive behaviours in females taken from these generations. We also phenotyped these behaviours in males because the perceptual bias, detection and response to bat echolocation signals, presumably existed in both sexes (Greenfield, 2002; Greenfield & Hohendorf, 2009). In parallel, we genotyped the backcross generation with amplified fragment-length polymorphism (AFLP) molecular markers. We performed a standard quantitative trait loci (QTL) analysis to identify the loci involved in the sexual and defensive traits as well as several developmental traits that had been measured incidentally. Thus, we determined the number of loci that influence female sexual and defensive responses as well as the corresponding male responses, the distribution of these loci within the genome, and whether the QTL for the female sexual and defensive responses colocalized on the same linkage groups (chromosomes) and in the same region within a linkage group. To our knowledge, this study represents the first attempt to investigate the genetic architecture of sensory exploitation.

Materials and methods

Natural history and acoustic behaviour of *Achroia grisella*

Achroia grisella are symbionts of the western honeybee (*Apis mellifera*) and are currently distributed in most regions of the world where apiculture is practised (Milum, 1940). The moth larvae feed on combs and organic detritus from honeybees, and they normally infest colonies with low worker populations (Künike, 1930). *A. grisella* adults often remain in the vicinity of their natal honeybee colony, and mating activities occur in/or the colony or on the surrounding vegetation. The adults have atrophied mouthparts, neither feed nor drink, and have a markedly brief lifespan. In the laboratory at 25 °C, females survive approximately 1 week and males several days longer (Greenfield & Coffelt, 1983).

Male *A. grisella* broadcast an advertisement song that is attractive to receptive females up to 1 m distant (Dahm *et al.*, 1971; Spangler *et al.*, 1984). The song is produced more or less continuously for 6–10 h on each night from adult eclosion until morbidity and death. Males produce their song while remaining stationary on the substrate, and they do so by fanning their wings, which causes a pair of tymbal structures at the forewing bases to resonate and emit a continuous train of brief (approximately 100 μs) pulses of high-frequency (70–130 kHz) sound. Tymbal resonations occur twice during a cycle of wing movement, once during the upstroke and once during the downstroke. Because the resonations of the left and right tymbals are not fully synchronous, each upstroke and downstroke is typically represented by a pair of pulses separated by a short (200–500 μs) 'asynchrony interval'. At 25 °C, male wingstroke rates during singing may range from 35 to 50 s^{-1} within a population, implying that pulse-pair rates in male song range from 70 to 100 pulse pairs per second (Spangler *et al.*, 1984; Jang & Greenfield, 1996). The song is relatively loud [approximately 90–95 dB SPL (Sound Pressure Level) as measured at a 1-cM distance; 0 dB re 20 μPa] and is broadcasted rather omnidirectionally from the male (Snedden *et al.*, 1994). We provide these acoustic details because they are critical in the contexts of sexual selection and interactions with predatory bats.

Both male and female *A. grisella* hear with a pair of abdominal tympana that is broadly sensitive to sound frequencies ranging from 20 to 120 kHz (Spangler & Takessian, 1983) and that exhibits a peak sensitivity between 80 and 100 kHz (Rodriguez *et al.*, 2005). Importantly, the peak sensitivity matches the dominant frequencies found in male song (Jang & Greenfield, 1996). From the perspective of defensive behaviour, the overall sensitivity range encompasses the frequencies of most echolocation signals broadcasted by insectivorous bats (Neuweiler, 1989; Miller & Surlykke, 2001; Russo *et al.*, 2007). This broad frequency sensitivity indicates that *A. grisella* could readily perceive bat species that glean their prey from the substrate as well as those that hunt aerially. However, during mating activities, *A. grisella* may be particularly vulnerable to substrate gleaners that use inadvertent sounds – either calling song or sound produced during movement – to

localize and capture their prey (Faure & Barclay, 1992; Arlettaz *et al.*, 2001; Siemers & Güttinger, 2006; Goerlitz *et al.*, 2008; Alem *et al.*, 2011; Siemers *et al.*, 2012).

Laboratory tests using synthetic male song stimuli show that female *A. grisella* exhibit clear preferences for certain features of male song. In general, females prefer songs that are delivered at a faster pulse-pair rate, that include louder and longer pulses and that have longer asynchrony intervals within the pulse pairs (Jang & Greenfield, 1996; Limousin & Greenfield, 2009). Differences exist between populations and between individuals regarding the 'preference function' that defines the relative importance of these several parameters in overall evaluation of male song (Jang & Greenfield, 1998; Alem & Greenfield, 2010; Limousin *et al.*, 2012). Female responses to male song also include thresholds, parameter values below which no response is elicited even when only a single stimulus or male is present. Response thresholds are particularly evident for pulse-pair rate and song amplitude (Brandt *et al.*, 2005; Greenfield & Hohendorf, 2009; Zhou *et al.*, 2011). In the latter case, females will generally not respond to male song delivered at an amplitude lower than 50 dB in SPL. As in song preferences, differences in response thresholds exist between individuals and populations. Substantial variation is also found in the male song parameters (Jang & Greenfield, 1996; Limousin & Greenfield, 2009), and tests using live males confirmed that variation in song accounts for a significant proportion of variation in male mating success (Jang & Greenfield, 1998; Reinhold *et al.*, 1998).

As in many acoustic animals, *A. grisella* males respond to conspecific song in a competitive manner. Males who happen to be temporarily silent will recommence singing immediately if a neighbour begins (Greenfield & Coffelt, 1983). A neighbour's song will also elicit a 3–6% increase in a male's pulse-pair rate for 15–20 min. This increase is presumably a form of song matching in which a male must equal or exceed the attractiveness of a neighbour's song to compete for local females (Jia *et al.*, 2001).

Both sexes of *A. grisella* respond defensively to bat echolocation signals when in flight as well as on the substrate. Flying moths cease beating their wings and drop to the ground in response to ultrasound pulses (40–100 kHz) that exceed 1 ms in length and 75 dB in SPL (Rodriguez & Greenfield, 2004). Here, a single pulse will elicit the response, particularly if it is long. Responses while on the substrate show finer discrimination and differ between the sexes. Males will stop singing and females will interrupt movement, as during their attraction towards a male, when exposed to ultrasound pulses that exceed 1 ms in length, 80 dB in SPL, and that are delivered at a rate below 30–40 s^{-1} (Greenfield & Weber, 2000; Greenfield & Baker, 2003; Greig & Greenfield, 2004). This latter criterion appears to be the means with which *A. grisella* discriminate between male song and the echolocation signals of bats in the searching phase, which normally are repeated at 10–25 s^{-1} (see Waters & Jones, 1995; Russo *et al.*, 2007). Experiments with the greater horseshoe bat, *Rhinolophus ferrumequinum*, a species that includes gleaning in its hunting repertoire, showed that *A. grisella* exhibit both silence and arrestment responses in the presence of live bats. Moreover, the bats oriented towards singing male *A. grisella* held within cages in a flight room (Alem *et al.*, 2011).

A series of half-sib/full-sib breeding experiments and tests with random inbred lines demonstrated that the various male song parameters are heritable traits (Jang *et al.*, 1997; Collins *et al.*, 1999; Brandt & Greenfield, 2004; Zhou *et al.*, 2011). A more restricted amount of testing indicated that the female preference function and the response thresholds for song amplitude and pulse-pair rate are also heritable traits (Jang & Greenfield, 2000; Zhou *et al.*, 2011). QTL analysis has identified several loci that have moderate to major influences on the various song and preference traits, as well as on developmental parameters. The locus with the strongest influence is associated with the pulse-pair rate in male song, a finding that agrees with earlier results from an artificial selection experiment (Limousin *et al.*, 2012).

Populations studied and breeding design

We used random inbred lines developed from *A. grisella* populations collected near Baton Rouge, Louisiana (USA; LA: 30°27′N, 91°8′W) and in Tours, Indre et Loire (France; IL: 47°19′N, 0°46′E) in 2007. Sampled insects were reared in the laboratory on a standard diet of flours, honey, beeswax, glycerol, nutritional yeast and water (see Jang & Greenfield, 1996) and were kept at 25 ± 1 °C and a 12:12 h photoperiod. The random inbred lines were bred via brother–sister mating over 18–20 consecutive generations, a regime that is predicted to reduce heterozygosity by at least 95% (see Crow & Kimura, 1970). We, then, chose two inbred lines, one from each of the populations, for our experimental analysis. These two lines, hereafter designated LA and IL, exhibited markedly different developmental traits (Table 1). Due to logistic constraints inherent to the development and the selection of inbred lines, receiver traits were, however, not possible to measure in parental lines.

We crossed one IL female (♀B, P_0) with one LA male (♂2, P_0) to produce male hybrids (HY males, F_1). In parallel, we crossed two full siblings (♂1 and ♀A, P_0) of the IL female (♀B, P_0) to produce F_1 IL females whose development was synchronized with the F_1 HY males. We then crossed HY males with F_1 IL females to produce our backcross generation, BC_1 (Fig. 1).

Combining male and female progeny has proved to be a powerful strategy in some cases for QTL discovery, but female *A. grisella*, like other Lepidoptera, do not

recombine. Thus, females do not help to produce a linkage map based on recombination events (Heckel *et al.*, 1999). We therefore restricted our study to BC_1 progeny issued from F_1 HY males and did not use F_1 HY females in our crossing scheme.

The information forthcoming from a QTL analysis depends on the number of BC_1 individuals analysed (Falconer & Mackay, 1996; Beavis, 1998; Xu, 2003), but this number is limited by the fecundity of individual females. To circumvent this problem, we set up a series of crosses that increased the number of BC_1 individuals sampled while keeping the same pedigree and expected segregation. This plan relied on the high level of consanguinity within our lines and the capacity of males to mate multiple times, approximately once per 24–48 h. In total, we backcrossed 15 HY F_1 males, each with 2–5 IL females from the same F_1 generation, to generate a pool of BC_1 progeny (Fig. 1). Owing to consanguinity, these F_1 females were nearly clones of one another. We then kept the BC_1 progeny from the HY F_1 male that produced the greatest number of individuals surviving to the adult stage, that is, 129 males and 79 females. To increase the number of BC_1 females, we also kept the 55 female BC_1 progeny of a second HY F_1 male (Fig. 1). Immediately following adult eclosion, the 129 BC_1 males and 134 females were individually isolated in 30-mL plastic cups to ensure that each one experienced a similar social environment. This isolation was particularly critical for females because they usually mate only once and become sexually unreceptive thereafter.

Table 1 Developmental traits (±SD) of males and females from the IL and LA lines in the parental generation (P_0).

Parental generation (P_0)	Males		Females	
	IL	LA	IL	LA
Developmental traits				
Body mass (mg)	12.25 (±1.94)	10.14 (±2.59)	31.95 (±4.41)	22.17 (±2.28)
Development duration (d)	58 (±3.55)	52 (±2.99)	59 (±2.32)	51.25 (±2.40)

Body mass was measured the day of adult emergence, and development duration indicates the time between oviposition and adult eclosion.

Phenotyping

We measured sexual and defensive responses in the 129 males and 134 females of the BC_1 generation. These responses included (1) the phonotactic response of females to male song (SEX_f), (2) the arrestment response of females to bat echolocation signals (DEF_f), (3) the competitive response of males to male song (SEX_m) and (4) the silence response of males to bat echolocation signals (DEF_m) (Table 2). In each case, we determined the threshold sound pressure level (SPL) required for a positive (1, 3) or negative (2, 4) response. We chose to measure threshold levels because they reveal a fundamental aspect of behaviour that may have been involved in the process of sensory exploitation in *A. grisella*. That is, at the presumed origin of the male song, the female response to this novel sexual signal and to bat echolocation signals must have had equivalent sensitivity. Thus, measurement of the two threshold values in contemporary moths could help to ascertain whether the sexual and defensive

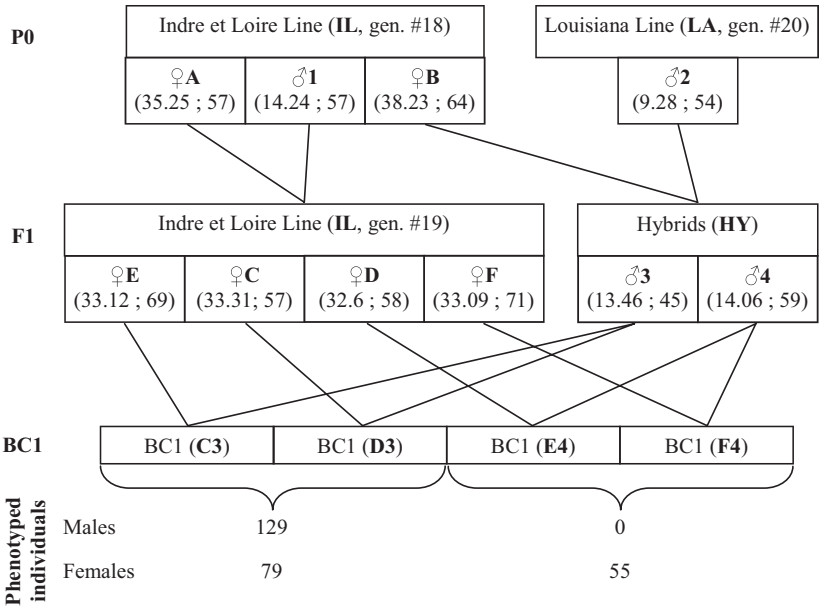

Fig. 1 Diagram of crosses. P_0: the parental generation, two inbred lines from full-sib crossing over 18 (IL) and 20 (LA) generations. Female individuals (body mass, mg; development duration, days) are indexed with letters, male individuals with numbers. F_1: first filial generation. BC_1: backcross generation (female index–male index). Phenotyped individuals indicate the total number of BC_1 males and females tested in our behavioural assay.

Table 2 Trait code, description and unit of measurement.

Trait code	Description and unit of measurement
Response traits	
SEX_f	Amplitude threshold of response to the sexual signal; dB
SEX_m	
DEF_f	Amplitude threshold of response to the predatory signal; dB
DEF_m	
Male song traits	
PR_m	Pulse-pair rate; pairs per second
PA_m	Average peak amplitude; arbitrary linear units
AI_m	Asynchrony interval; μs
Developmental traits	
BM_f	Body mass of male (m) and female (f) at day test; mg
BM_m	
DEV_f	Development duration from oviposition to adult eclosion; d
DEV_m	

$Trait_f$: female phenotype; $Trait_m$: male phenotype.

responses remain under pleiotropic control or shifted since the original pleiotropy. We measured thresholds in males as well as females because bat perception probably originated in both sexes and should have constituted a general sensory bias in *A. grisella* (Greenfield, 2002).

We tested the sexual and defensive responses of all BC_1 individuals in a 2 × 2 × 2-m (length × width × height) chamber that was lined with acoustic insulation foam which minimized echoes. The chamber temperature was held at 25 ± 1 °C, equivalent to that during rearing, and illumination was provided by an overhead red bulb (incandescent; 25 W) that did not disturb the nocturnal behaviour of the insects. All behavioural tests were made during the initial 6 h of the insects' photoperiodic night, which coincides with the peak in mating behaviours in natural populations as well as in the laboratory colony and lines (Greenfield & Coffelt, 1983). Individuals were allowed 30 min to acclimate to the chamber prior to testing.

In all four tests of response threshold, we presented sound stimuli from an ultrasonic loudspeaker (model ScanSpeak; Avisoft Bioacoustics; frequency response: ±2 dB, 60–120 kHz; see Data S1 for the playback methods). Playback experiments were designed to measure the threshold sound pressure level (peSPL) required to elicit a response to the broadcast of a male sexual signal or a bat echolocation signal (see Greenfield & Hohendorf, 2009). These signals were, respectively, edited from original recordings of a male *A. grisella* calling song and an echolocation call of the greater horseshoe bat, *Rhinolophus ferrumequinum*. The male song stimulus lasted 60 s and was made from the uninterrupted calling song of an HY male whose parameters were average among the HY F_1 generation (pulse-pair rate = 77 s^{-1}, asynchrony interval = 535 μs; Fig. 2a). The bat echolocation stimulus lasted 2 s and corresponded to a hunting sequence of the greater horseshoe bat recorded while approaching a prey (*Tenebrio molitor* larvae) in a flight room. The sequence consisted of 24 echolocation calls (constant frequency = 80 kHz, average call length = 57 ms, duty cycle = 69%; Fig. 2b). In addition to sexual and defensive response traits, we measured male song traits (pulse-pair rate, PR_m; peak amplitude, PA_m; asynchrony interval, AI_m) and two developmental traits (body mass, BM; development duration, DEV) in all individuals (Table 2). Specific protocols for trait measurements in males and females are detailed in supporting information (Data S2).

Genotyping

DNA extraction and AFLP markers

Immediately after phenotyping, all BC_1 males and females were killed and stored at −80 °C to preserve their DNA for later extraction. We removed the wings of each specimen and extracted DNA using the DNeasy Tissue Kit (QIAGEN, Venlo, Netherlands), following manu-

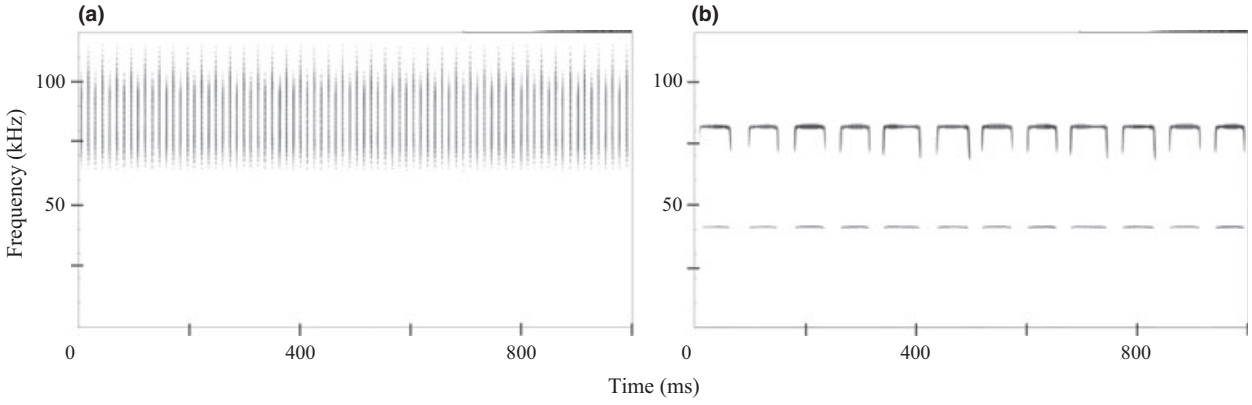

Fig. 2 Spectrograms of the stimulus signals used for measurements of phenotypes. Signal duration is 1 s for both spectrograms. (a) Sexual signal of the selected *A. grisella* HY male. (b) Echolocation signal of *R. ferrumequinum*. Sound frequency ranges of both signals overlap broadly.

facturer recommendations. The DNA concentration and purity were estimated with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Afterwards, we used a slightly modified version of the AFLP protocol described in the study by Midamegbe *et al.* (2011), see Data S3. We then electrophoresed the AFLP products on an ABI 3130XL (Applied Biosystems, Foster City, CA, USA) sequencer and analysed the raw data with GENEMAPPER©, version 4.0 (Applied Biosystems). We scored BC_1 individuals and F_1 parents for the presence or absence of the AFLP bands between 80 and 550 bp. The GENEMAPPER automatic band detection based on peak intensity and size was carefully validated, or corrected, by visual inspection of the individual profiles.

Linkage map

We built a linkage map on the basis of the segregation pattern in the BC_1 offspring of markers present in the two HY males (+/−) and absent in the four F_1 IL sisters (−/−). We used JOINMAP®4 software (Kyazma B.V., Wageningen, Netherlands) to perform the linkage analysis. We first removed the markers that deviated strongly (chi-square tests, $P < 0.0001$) from the expected Mendelian segregation ratio of 1 : 1 in BC_1. Linkage groups were then identified based on a LOD score (logarithm10 of odds) of 6. To determine the ordering of the markers within linkage groups, we used JOINMAP regression mapping algorithm (see Data S4 for parameters). To optimize the ordering, we manually compared the results obtained with the regression mapping algorithm and the maximum likelihood mapping algorithm, and we removed from further consideration those markers that exhibited order reversals between the two algorithms. Recombination values were converted to map distances (in cM) using the Kosambi mapping function (Kosambi, 1944).

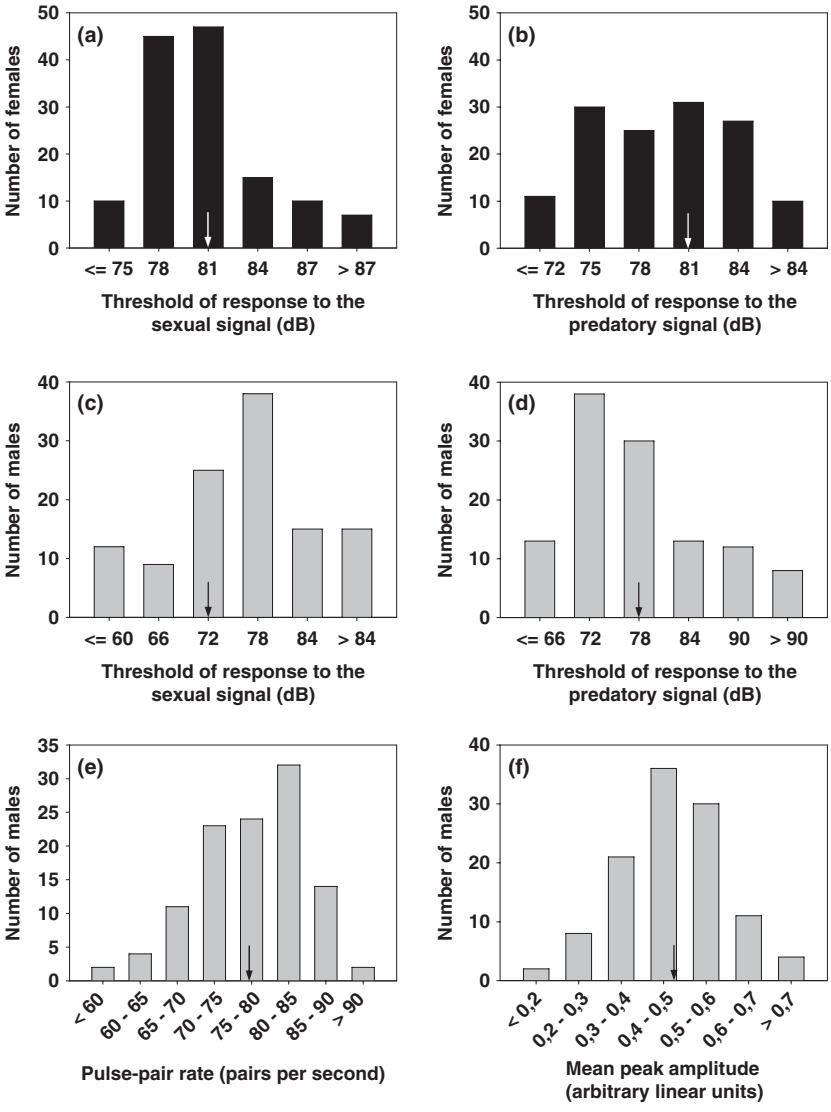

Fig. 3 Distribution of the phenotypic traits. Dark bars correspond to female measures and grey bars to males. (a) Female threshold of response to the sexual signal, SEX_f; (b) Female threshold of response to the predatory signal, DEF_f; (c) Male threshold of response to the sexual signal, SEX_m; (d) Male threshold of response to the predatory signal, DEF_m; (e) Pulse-pair rate of male song, PR_m; (f) Mean peak amplitude of male song: average maximum amplitude reached in a pulse pair, PA_m; (g) Mean asynchrony interval of male song: mean interval of silence between the two pluses of a pair, AI_m; (h) Development duration, DEV_f and DEV_m; (i) Body mass, BM_f and BM_m. Black and white arrows indicate medians of the threshold of responses and means of other measured traits.

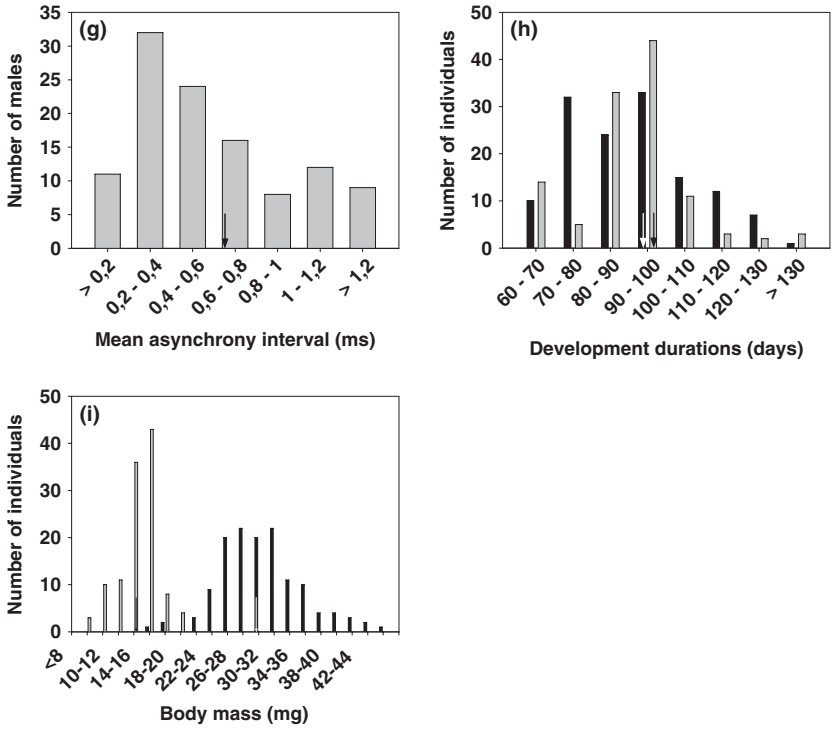

Fig. 3 (Continued)

QTL detection

We used numerical values for all traits, and we carried out the computational analysis with MAPQTL®6 software (Kyazma B.V.). The list of analysed traits is given in Table 2. We conducted a preliminary simple marker analysis based on ranks (SMA; Kruskal–Wallis rank sum test, $P < 0.001$) on all measured individuals to detect simple marker/trait association. We then performed interval mapping (hereafter IM) on raw quantitative values and selected the QTL positions with significant LOD scores for each trait. A correction for multiple testing was applied to determine the significance threshold (number of tests equal to the number of markers). The threshold to be used for each individual test for declaring a QTL significant with an error risk of 5% at the whole-genome level was therefore determined using a permutation test with 1000 iterations (Churchill & Doerge, 1994). We ran a final analysis using composite interval mapping (CIM) with a maximum of 5 cofactors to account for the possibility that several QTL might be segregating in the populations. These cofactors represented the markers nearest to each QTL detected with CIM. We also looked for putative QTL (1.8 < LOD score < genome-wide threshold of significance) because similar QTL studies showed that LOD scores of QTL influencing complex behavioural traits can be relatively low (Oxley *et al.*, 2010; Limousin *et al.*, 2012). We identified several significant and putative QTL with both the SMA and the CIM methods. The confidence interval for each QTL was calculated by projecting the map positions on either side of its LOD peak that corresponded to a decrease in the LOD score by 1 unit (Fig. S2). A colocalization of two QTL was defined as overlapping of the QTL confidence intervals.

Results

Phenotypic variation in the backcross generation

Sexual and defensive responses in females and males, as well as developmental traits in females and males, all exhibited variation among the BC1 individuals measured (Fig. 3). In females, the sensitivity thresholds to the predatory signal (DEF_f; median: 81 dB; range: 72–84 dB) and to the sexual signal (SEX_f; median: 81 dB; range: 75–87 dB) were significantly different (Wilcoxon signed rank test, $N = 134$, $W = -1317$, $P \leq 0.001$; Fig. S1A). Conversely, we did not observe a difference between sexual (SEX_m; median: 78 dB; range: 60–84 dB) and defensive response thresholds (DEF_m; median: 78 dB; range: 66–90 dB) in males (Wilcoxon signed rank test, $N = 115$, $W = 403$, $P = 0.077$; Fig. S1B). Sensitivity thresholds to both sexual and predatory signals were significantly higher in females than in males (Mann–Whitney tests, $U_{SEX} = 7831.5$, $U_{DEF} = 6584.5$, $P \leq 0.001$), which is consistent with some previous findings (Greenfield & Hohendorf, 2009). Sexual and defensive response thresholds were not correlated with one another in either females or

Table 3 Significant and putative QTL detected with simple marker analysis and composite interval mapping for response, song and developmental traits.

	LG	QTL name	Position	Nearest marker	Nearest marker position	Lod score	Additive effect	R^2 (%)
SEX_f	**18**	**SEX_f.1**	**42.67**	**eATCmGAC410.11**	**45.47**	**2.5**	**2.10**	**7.7**
	28	SEX_f.2	34.04	eATGmGCT295.13	31.04	2.0	1.77	6.1
SEX_m	16	SEX_m.1	8.17	eAGCmGCG368.43	8.24	2.0	−3.84	6.4
	13	SEx_m.2	21.23	eACGmGTA255.19	21.23	1.8	3.63	5.7
DEF_f	**5**	**DEF_f.1**	**51.47**	**eACTmGAA135.96**	**51.47**	**2.7**	**−2.22**	**7.8**
	2	**DEF_f.2**	**8.61**	**eATAmGCC320.44**	**8.61**	**1.8**	**−1.83**	**5.3**
	15	DEF_f.3	0.00	eATAmGCC239.94	0.00	1.8	−1.80	5.1
DEF_m	1.2	DEF_m.1	2.00	eATCmGAC197 66	0.00	2.5	4.69	8.5
	11	**DEF_m.2**	**16.68**	**eATCmCTT220.13**	**16.68**	**2.4**	**−4.32**	**7.9**
	16	DEF_m.3	7.17	eAGCmGCG368 43	8.24	1.9	3.96	6.2
PR_m	**23**	**PR_m.1**	**24.90**	**eATCmGCC150.81**	**24.90**	**4.1**	**−5.16**	**12.4**
	13	**PR_m.2**	**20.44**	**eACCmGCC350.23**	**19.44**	**3.5**	**4.80**	**10.2**
	27	PR_m.3	21.11	eAGAmCAG 77.98	15.11	2.4	4.28	7.0
PA_m	19	PA_m.1	7.82	eATGmGAT144.33	7.82	2.3	73.19	8.4
	7	PA_m.2	28.30	eAGAmGCT73.28	28.30	2.0	66.19	7.0
BM_f	**6**	**BM_f.1**	**34.41**	**eAGAmCAC125.51**	**34.41**	**2.2**	**−2.37**	**6.4**
	1.1	**BM_f.2**	**19.25**	**eATGmCAG220.2**	**19.25**	**2.1**	**−2.36**	**6.1**
	4	**BM_f.3**	**0.00**	**eATTmGCG405.35**	**0.00**	**1.9**	**−2.34**	**5.6**
BM_m	**27**	**BM_m.1**	**8.05**	**eACAmGCA167.93**	**7.05**	**2.0**	**1.21**	**7.2**
DEV_f	**15**	**DEV_f.1**	**10.10**	**eAGAmGAC231.23**	**10.10**	**5.3**	**−22.12**	**16.7**
	15	DEV_f.2	13.98	eAGCmCTT130.47	13.98	3.0	16.05	9.0
DEV_m	18	DEV_m.1	44.67	eATCmGAC410.11	45.47	3.1	4.61	10.4
	14	DEV_m.2	19.27	eATTmGCC359 21	19.83	2.6	4.35	8.9
	27	**DEV_m.3**	**15.11**	**eAGAmCAG77.98**	**15.11**	**2.6**	**4.12**	**8.7**
	24	DEV_m.4	59.10	eATAmGCC426.92	59.10	2.0	3.60	6.7
	21	DEV_m.5	15.09	eACGmGCT284.14	15.09	2.0	−3.60	6.5

Quantitative trait loci (QTL) detected with both methods are shown in boldface; significant QTL are shaded in grey; putative QTL are in ordinary font. See Table 2 for trait descriptions.

males (Pearson correlation, $d.f._{females} = 132$, $d.f._{males} = 127$, $P > 0.25$).

All three critical male song traits also exhibited variation among BC_1 individuals (Fig. 3e–g). Only one of these song traits (pulse-pair rate, PR_m) was correlated with a response trait (defensive response, DEF_m; Pearson correlation, d.f. = 127, $P = 0.0454$, $r = 0.19$). Similarly, developmental traits in females and males varied among individuals (Fig. 3h,i). In both females and males, body mass was positively correlated with development duration (Pearson correlation, $d.f._{females} = 132$, $d.f._{males} = 127$, $P < 0.0001$, $r = 0.67$). Moreover, female body mass (BM_f) was positively correlated with their defensive response (DEF_f; Pearson correlation, d.f. = 127, $P = 0.0049$, $r = 0.0241$), and male body mass (BM_m) was positively correlated with the mean peak amplitude (PA_m) of their song (Pearson correlation, d.f. = 127, $P = 0.0006$, $r = 0.31877$). We observed a unimodal distribution of values for all measured traits in both females and males.

Linkage map

Among the 263 phenotyped BC_1 individuals, 249 were genotyped at 475 AFLP markers; DNA extracts from fourteen males were too low in concentration to allow accurate genotyping. Some of these markers exhibited a strongly distorted segregation ratio ($n = 76$) or were removed in the optimization process of the ordering ($n = 61$), leaving 338 informative markers that could be positioned on the linkage map. Using an LOD score of 6, these 338 markers were distributed among 29 linkage groups. We tested more stringent LODs, but the number of linkage groups stayed the same up to a LOD of 10. This number of linkage groups is largely consistent with that reported previously by Limousin *et al.* (2012), who used both cytological analysis and linkage map construction.

In linkage group 1, the markers treated at the beginning of the optimization process of ordering were found to be distributed in two separate segments with only one marker pair linking the two segments. This specificity did not enable us to order one segment relative to the other (at least two marker pairs are needed for a correct ordering). Consequently, two different maps of equivalent robustness were produced, 1.1 and 1.2, although these maps were definitely two parts of the same linkage group (i.e. two segments of the same chromosome). The total length of the map is 1263 cM, with linkage group length ranging from 12.9 cM to 66.4 cM (or 99.6 cM if we sum the lengths of groups

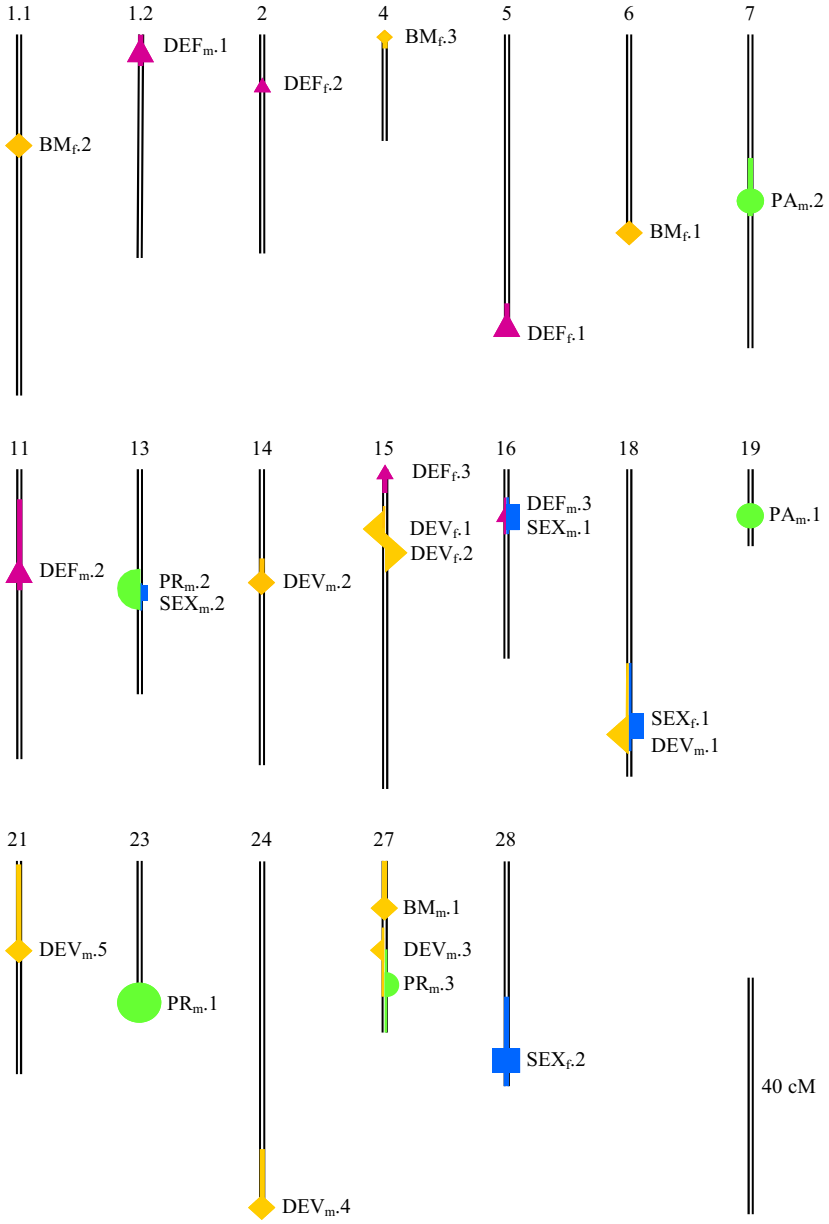

Fig. 4 Map of the QTL detected. Both significant and putative QTL are represented. QTL detected for developmental traits, acoustic features of the male song, sexual response traits and defensive response traits are respectively represented with diamonds (◆), circles (●), rectangles (■) and triangles (▲). The symbols indicate the positions of the QTL; thickness of the symbols is proportional to the LOD score and trait names are indicated nearby. Vertical bars show, when possible, the QTL confidence intervals. Linkage group numbers are shown above each graph; map distances (cM) were estimated with the Kosambi mapping function (Kosambi, 1944).

1.1 and 1.2). The average interval distance between markers was 4.1 cM (see Fig. S2). The markers were not evenly distributed among and within the linkage groups. Some regions included clusters of markers (e.g. linkage group 2), whereas in others, intervals longer than 20 cM occurred between consecutive markers (e.g. linkage group 11, Fig. S2).

QTL detected

We found rather similar QTL with SMA and CIM (Table 3). According to the traits, the threshold to declare a QTL significant was 2.9 or 3. We identified a total of 26 QTL: 5 significant (LOD score $\geq$ 3.0) and 21 putative ($1.8 \leq$ LOD score ≤ 2.9). The 5 significant QTL identified had a mean LOD score of 3.8 (range from 3.0 to 5.3), and the 21 putative QTL identified had a mean LOD score of 2.17 (range from 1.8 to 2.7). Significant QTL explained a mean of 11.7% (range from 9% to 16.7%) of the phenotypic variation, and putative QTL explained a mean of 6.9% (range from 5.1% to 8.9%) of the phenotypic variation. All detected QTL are mapped on their linkage group in Fig. 4. Detailed maps for the 29 linkage groups and the AFLP markers are presented in Fig. S2.

We identified QTL for each of the four response traits, all putative (Table 3). For female responses, we identified 5 QTL: two for the sexual response (SEX_f),

positioned on linkage groups 18 and 28 (R^2 = 7.7% and 6.1%, respectively), and three for the defensive response (DEF_f), located on linkage groups 5, 2 and 15 (R^2 = 7.8%, 5.3% and 5.1%, respectively). In males, we also found 5 QTL influencing receiver traits: two for the sexual response (SEX_m), located on linkage groups 16 and 13 (R^2 = 6.4% and 5.7%, respectively), and three for the defensive response (DEF_m), positioned on linkage groups 1.2, 11 and 16 that explained from 6.2% to 8.5% of the phenotypic variation.

For male song traits, we detected 5 QTL. We found 2 significant QTL for pulse-pair rate (PR_m). These were detected on linkage groups 23 and 13 and explained 12.4% and 10.2% of the phenotypic variation, respectively (Table 3). We detected another putative QTL for pulse-pair rate on linkage group 27 (R^2 = 7%). For mean peak amplitude (PA_m), we only found putative QTL. These two QTL were located on linkage groups 19 and 7 and explained 8.4%, 7.0% of the phenotypic variation, respectively. We did not detect any QTL for asynchrony interval (AI_m).

QTL were detected for every developmental trait measured (Table 3). We found 3 significant QTL associated with development duration: two in females (DEV_f), both located on linkage group 15 (R^2 = 16.7% and 9%), and one in males (DEV_m), positioned on linkage group 18 (R^2 = 10.4%). We detected in addition 4 putative QTL associated with development duration in males, located on linkage groups 14, 27, 24 and 21 and that explained from 6.5% to 8.9% of the phenotypic variation. For body mass, we found 4 putative QTL: three in females (BM_f), positioned on linkage group 6, 1.1 and 4 (R^2 = 6.4%, 6.1% and 5.6%, respectively), and only one in males, located on linkage group 27 (R^2 = 7.2%).

Colocalization of QTL

We found no incidence of colocalization of QTL, defined as overlapping of the QTL confidence intervals, for sexual and defensive responses (SEX_f, DEF_f) in females. In males, however, we found one colocalization on linkage group 16 of putative QTL influencing sexual and defensive responses (SEX_m.1, DEF_m.3; Fig. 4). We also found a colocalization on linkage group 13 of putative QTL associated with song pulse-pair rate and defensive response (PR_m.2, SEX_m.2; Fig. 4). Several additional colocalizations appear on Fig. 4 that involve QTL, putative and significant, for the several developmental traits in males and females.

Discussion

Sexual and defensive responses

As in previous studies (Greenfield & Hohendorf, 2009; Lafaille *et al.*, 2010), we found that the behavioural threshold to bat signals was lower in males than in females. This gender difference in sensitivity may reflect differential selection pressures that arise because gleaning bats are potentially attracted to leks of singing male *A. grisella* (Alem *et al.*, 2011). Similarly, we also found that the behavioural threshold to male song was lower in males than in females. The gender difference in sensitivity of the sexual response may occur because selection pressure to discriminate against 'inferior' males causes females to ignore low amplitude song. On the other hand, male–male competition may have selected for a high level of male sensitivity to the courtship of rival individuals (Cremer & Greenfield, 1998). Relatively high/low sensitivity in males/females to both predator and conspecific signals could be interpreted as a correlation between defensive and sexual responses. However, our QTL analyses indicate that this is rather not the case, as the 2 response traits are mostly independent in both females and males (see also Greenfield & Hohendorf, 2009).

QTL for sexual and defensive responses, signal and developmental traits

Our analyses indicated QTL of moderate to major influence (LOD scores ranged from 1.8 to 5.3; mean = 2.5) for most of the traits measured in males as well as in females. Several QTL were detected for all traits except for asynchrony interval, the sole trait for which no QTL were found. The QTL identified in our study were distributed among 19 of the 29 linkage groups in *A. grisella* genome. We did not find any marked clustering of QTL in certain linkage groups, either for all traits or for any of the three trait types (see Table 3 and Fig. 4). Most of the QTL identified (19/26) had a LOD score between 2 and 4. These values are comparable to those found in QTL studies of complex behavioural traits performed on similar population sizes of nonmodel organisms (Velthuis *et al.*, 2005; Oxley *et al.*, 2010; Sasabe *et al.*, 2010).

Considering the population size that we sampled and the traits measured, the LOD scores of the detected QTL are relatively high. These scores are similar to the values reported in a previous QTL study of sexual behaviour that was performed on a smaller population of *A. grisella* (mean LOD score = 2.6, range: from 1.3 to 4.4; Limousin *et al.*, 2012). Morevover both QTL studies performed on *A. grisella* showed that male pulse rate (PR) was influenced by QTL of major effect. Importantly, we found that QTL associated with complex response traits had LOD scores comparable (mean 2.1) to QTL associated with developmental traits (mean 2.6). Overall, the large number of informative markers used to develop our linkage map, as well as the numbers of males and females sampled in the BC_1 generation, suggest that both significant and putative QTL shown in Fig. 4 represent the main genetic factors

influencing sexual and defensive response traits, developmental traits and male song in *A. grisella*.

Our data (Table 3) suggest that most traits have polygenic control and are influenced by several QTL of moderate effect rather than few of large effect. The remaining phenotypic variation, unaccounted for by the identified QTL, may be explained by environmental influence (i.e. inevitable differences between different rearing containers), QTL not detected in this study and genetic factors having undetectable effects in our specific experimental design. Similar levels of polygenic influence have been observed in other QTL studies on sexual traits in acoustic insects (Gleason *et al.*, 2002; Shaw *et al.*, 2007; Limousin *et al.*, 2012; Singh & Shaw, 2012) and may be a general feature of sexual behaviour in insects (Ritchie & Phillips, 1998; Arbuthnott, 2009). Our study represents one of the few applications of QTL mapping to investigate defensive behaviour (Blumstein *et al.*, 2010), and we found a similar level of polygenic control in this domain as in sexual behaviour (see Table 3 and Fig. 4).

On the evolution of receiver traits subsequent to sensory exploitation

Contrary to the prediction of the sensory exploitation hypothesis *sensu stricto*, we found that QTL that influenced sexual and defensive responses in *A. grisella* females were independent. Thus, female responses to male song and to predatory bats are not likely to be pleiotropically controlled (see Table 3). In accordance with our initial expectation, this finding indicates that the genetic architecture of these traits has evolved since the origin of the male song via sensory exploitation. Our result is consistent with recent theoretical and experimental studies on sensory exploitation that reported that environmental and sexual receiver traits have the potential to evolve independently despite sharing a common sensory system (Greenfield, 2002; Macias-Garcia & Ramirez, 2005; Arnqvist, 2006; Fuller, 2009). It is also consistent with the specific finding in *A. grisella* that separate 'auditory streams' appear to process sexual and bat signals (Greenfield & Hohendorf, 2009; Lafaille *et al.*, 2010). In comparison, independent control of defensive and sexual responses might be less likely in moth species that do not discriminate conspecific courtship song from bat echolocation calls (e.g. the Asian corner borer, *Ostrinia furnicalis*, Nakano *et al.*, 2013).

What factors might have led to the independent evolution of sexual and environmental (= defensive) response traits? In the case of *A. grisella*, the ability to discriminate male song from the signals of predatory bats may have been crucial. We can imagine that at its origin, the novel male song – a train of ultrasonic pulses that bore some crude resemblance to bat echolocation signals – may have stimulated females to land and/or cease all movement: this is the basic response to pulsed ultrasounds, as broadcast in the echolocation signals of bats. Males who had been advertising with another signalling modality, for example pheromonal, may have benefited from the female response to the sound pulses because courtship would have been easier with a female who remained stationary (Greenfield, 2002; Nakano *et al.*, 2013). The sexual selection process, including Fisherian, good genes and chase-away mechanisms, could then have led to an evolutionary exaggeration of the song. These various processes are not mutually exclusive with one another (Rowe & Houle, 1996; Kirkpatrick & Barton, 1997; Chapman *et al.*, 2003; Kokko *et al.*, 2003, 2006), or with sensory exploitation (Ryan & Rand, 1993; Holland & Rice, 1998; Phelps & Ryan, 2000; Jennions & Brooks, 2001; Ryan *et al.*, 2001; Rodriguez & Snedden, 2004; Arnqvist, 2006). As a consequence of male song exaggeration, signal discrimination by female receivers may have been favoured because of the importance of responding to the echolocation signals of predatory bats as well as to the evolving male song. In parallel, the divergence of male song and predator signals may also have been selectively favoured because males who imitated bat echolocation signals too closely would have had less and less of an advantage in mate attraction. But mechanisms other than sexual selection, for example genetic drift and gene flow, may have been equally important in the evolution of discrimination and divergence of signals.

Sensory exploitation has generally been treated as a phenomenon restricted to female receivers (Shaw, 1995; Rodriguez & Snedden, 2004; Arnqvist, 2006; Fuller, 2009). However, the ancestral perceptual bias was a response to environmental stimuli that was shared by both sexes, and some male responses to a novel male signal may be expected. For example, when the terminal yellow band evolved on the tail of male Mexican splitfin fish (Goodeinae), purportedly via exploitation of a pre-existing feeding response, this signal may have evoked responses in males as well as females (Macias-Garcia & Ramirez, 2005). In *A. grisella*, males that evolved the ability to produce acoustic stimuli may have increased their female encounter rate and mating success. They may have also eliminated their rivals by silencing them with bat-like signals. In that case, at the origin, male responses to male song (sexual response) and to bat echolocation stimuli (defensive response) were controlled by the same genes (pleiotropy). But male–male competition could have then led to exaggeration of the song and consequent evolution of perceptual discrimination by males of male and bat signals, analogous to that proposed above for females. In our study, we investigated this potential parallel process by studying the genetic architecture of the male response traits. As predicted and contrary to the female response traits, we found evidence of partial pleiotropy:

a colocalization was observed between two QTL for male sexual and defensive responses on linkage group 16 (SEXm.1 and DEFm.3; Fig. 4). Moreover, we also detected a colocalization between two QTL that influence the pulse rate of the male song (PRm.2) and the sexual response trait of males (SEXm.2) on linkage group 13 (Fig. 4). These findings support the hypothesis that sensory exploitation may also occur in male receivers and that male–male competition may drive subsequent evolution either by coevolution (Fisher, 1958; West-Eberhard, 1983; Kirkpatrick & Ryan, 1991; Andersson, 1994) or by genetic coupling (Alexander, 1962; Butlin & Ritchie, 1989) involving sexual response and signal traits (see colocalization between SEXm.2 and PRm.2 on linkage group 13, Fig. 4). Thus, origin via sensory exploitation followed by subsequent evolution may have affected receiver traits in males as well as females.

Conclusion

In our study, we attempted to shed light on the genetic architecture underlying sensory exploitation and on the evolution of acoustic communication in *A. grisella*. Based on phylogenetic inference, there is little doubt that acoustic perception in *A. grisella*, as in most Lepidoptera, originated in the specific antipredator context of avoiding insectivorous bats (Greenfield, 2002). However, the circumstances under which male song arose and the specific trajectory along which female attraction towards that song evolved remain unknown. The results presented here offer some clues to the missing links in this evolutionary process (see also Nakano *et al.*, 2013). But other questions pertaining to the historical appearance of male song and its perception by females require further investigation. Male song and bat echolocation signals are processed by the same sensory system, and we therefore ask whether some aspects of sexual and defensive response traits are still subject to pleiotropic control. If yes, to what extent would this level of pleiotropic control differ from that which might exist for other traits that share the same sensory system but did not originate via sensory exploitation? Moreover, in what sequence did (1) female discrimination of male song from predator signals and (2) the active orientation of females towards male song evolve? Whereas we can imagine possible scenarios through which acoustic communication might have evolved in *A. grisella*, we cannot at present propose a definitive timeline of events. Such precision will probably demand phylogenetic information and further genetic study.

Because the evolution of response traits subsequent to the origin of signals via sensory exploitation may be widespread among animal species (Greenfield, 2002; Rodriguez & Snedden, 2004; Macias-Garcia & Ramirez, 2005), we propose that the complementary use of phylogenetic analysis and genetic mapping be applied in various cases to help resolve fundamental questions on the evolution of signal and response traits. Nonetheless, some current limitations of this approach should be recognized. QTL data may overestimate pleiotropy because the co-inheritance of phenotypic traits arising due to tight physical linkage cannot be distinguished from actual pleiotropy with the present method (resolution determined by the population size and marker density; Wagner & Zhang, 2011). It is hoped that continuing refinements in molecular genetics and mapping, combined with more relevant behavioural assays, will overcome these difficulties.

Acknowledgments

We are most grateful to Yihong Zhou and Jean-Pierre Chartier for providing the Louisiana and Indre-et-Loire natural populations of *A. grisella*, Guy Bourdais and Fabrice Vannier for their technical assistance in the development of the inbred lines and Florence Deau for helping in the analysis of quantitative data. AFLP data used in this work were produced by means of the molecular genetic analysis technical facilities of the SFR 'Montpellier Environnement Biodiversité'. We also thank Dominique Allainé, Jean-François Ferveur, Elisabeth Huguet, Anders Moller and Michel Renou for their constructive input and valuable criticisms. The project was supported financially by the Agence Nationale de la Recherche of France (contrat ANR-07-BLAN-0113-01), the Centre National de la Recherche Scientifique (CNRS), the Université François Rabelais de Tours and the Ministère de l'Education of France.

References

Alem, S. & Greenfield, M.D. 2010. Economics of mate choice at leks: do female waxmoths pay costs for indirect genetic benefits? *Behav. Ecol.* **21**: 615–625.

Alem, S., Koselj, K., Siemers, B.M. & Greenfield, M.D. 2011. Bat predation and the evolution of lek in acoustic moths. *Behav. Ecol. Sociobiol.* **65**: 2105–2116.

Alexander, R.D. 1962. Evolutionary change in cricket acoustical communication. *Evolution* **16**: 443–467.

Andersson, M.B. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.

Arbuthnott, D. 2009. The genetic architecture of insect courtship behavior and premating isolation. *Heredity* **103**: 15–22.

Arlettaz, R., Jones, G. & Racey, P.A. 2001. Effect of acoustic clutter on prey detection by bats. *Nature* **414**: 742–745.

Arnqvist, G. 2006. Sensory exploitation and sexual conflict. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **361**: 375–386.

Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**: 808–810.

Basolo, A.L. 1995. Phylogenetic evidence for the role of a preexisting bias in sexual selection. *Proc. R. Soc. Lond. B* **259**: 307–311.

Basolo, A.L. & Endler, J.A. 1995. Sensory biases and the evolution of sensory systems. *Trends Ecol. Evol.* **10**: 489.

Beavis, W.D. 1998. QTL analyses: power, precision, and accuracy. In: *Molecular Dissection of Complex Traits* (A.H. Paterson, ed.), pp. 145–162. CRC Press, Boca Raton, FL.

Blumstein, D.T., Lea, A.J., Olson, L.E. & Martin, J.G.A. 2010. Heritability of anti-predatory traits: vigilance and locomotor performance in marmots. *J. Evol. Biol.* **23**: 879–887.

Bradbury, J.W. & Vehrencamp, S.L. 2000. Economic models of animal communication. *Anim. Behav.* **59**: 259–268.

Brandt, L.S.E. & Greenfield, M.D. 2004. Condition-dependent traits and the capture of genetic variance in male advertisement song. *J. Evol. Biol.* **17**: 821–828.

Brandt, L.S.E., Ludvar, B.C. & Greenfield, M.D. 2005. Co-occurrence of acceptance thresholds and preference functions in female choice: mate discrimination in the lesser wax moth. *Ethology* **111**: 609–625.

Butlin, R.K. & Ritchie, M.G. 1989. Genetic coupling in mate recognition systems: what is the evidence? *Biol. J. Linn. Soc.* **37**: 237–246.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003. Sexual conflict. *Trends Ecol. Evol.* **18**: 41–47.

Christy, J.H. & Backwell, P.R.Y. 1995. The sensory exploitation hypothesis. *Trends Ecol. Evol.* **10**: 417.

Churchill, G.A. & Doerge, R.W. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* **138**: 963–971.

Collins, R.D., Jang, Y., Reinhold, K. & Greenfield, M.D. 1999. Quantitative genetics of ultrasonic advertisement signalling in the lesser waxmoth, *Achroia grisella* (Lepidoptera: Pyralidae). *Heredity* **83**: 644–651.

Conner, W.E. 1999. 'Un chant d'appel amoureux': acoustic communication in moths. *J. Exp. Biol.* **202**: 1711–1723.

Conner, W.E. & Corcoran, A.J. 2012. Sound strategies: the 65-million-year-old battle between bats and insects. *Annu. Rev. Entomol.* **57**: 2139.

Cremer, S. & Greenfield, M.D. 1998. Partitioning the components of sexual selection: attractiveness and agonistic behavior in male wax moths, *Achroia grisella* (Lepidoptera: Pyralidae). *Ethology* **104**: 1–9.

Crow, T.F. & Kimura, M. 1970. *An Introduction to Population Genetics Theory*. Harper and Row, New York, NY.

Dahm, K.H., Meyer, D., Finn, W.E., Reinhold, V. & Roller, H. 1971. The olfactory and auditory mediated sex attraction in *Achroia grisella* (Fabr.). *Naturwissenschaften* **58**: 265–266.

Egger, B., Klaefiger, Y., Theis, A. & Salzburger, W. 2011. A sensory bias has triggered the evolution of egg-spots in cichlid fishes. *PLoS ONE* **6**: e25601.

Eisner, T. & Meinwald, J. 1995. The chemistry of sexual selection. *Proc. Natl. Acad. Sci. USA* **92**: 50–55.

Endler, J.A. & Basolo, A.L. 1998. Sensory ecology, receiver biases and sexual selection. *Trends Ecol. Evol.* **13**: 415–420.

Enquist, M. & Arak, A. 1993. Selection of exaggerated male traits by female aesthetic senses. *Nature* **361**: 446–448.

Falconer, D.S. & Mackay, T.F.C. 1996. *Introduction to Quantitative Genetics*. Prentice Hall, Harlow, UK.

Faure, P.A. & Barclay, R.M.R. 1992. The sensory basis of prey detection by the long-eared bat, *Myotis evotis*, and the consequences for prey selection. *Anim. Behav.* **44**: 31–39.

Fernandez, A.A. & Morris, M.R. 2007. Sexual selection and trichromatic color vision in primates: statistical support for the preexisting-bias hypothesis. *Am. Nat.* **170**: 10–20.

Fisher, R.A. 1958. *The Genetical Theory of Natural Selection*, 2nd edn. Dover Publications, Mineola, NY.

Fuller, R.C. 2009. A test of the critical assumption of the sensory bias model for the evolution of female mating preference using neural networks. *Evolution* **63**: 1697–1711.

Fuller, R.C., Houle, D. & Travis, J. 2005. Sensory bias as an explanation for the evolution of mate preferences. *Am. Nat.* **166**: 437–446.

Gleason, J.M., Nuzhdin, S.V. & Ritchie, M.G. 2002. Quantitative trait loci affecting a courtship signal in *Drosophila melanogaster*. *Heredity* **89**: 1–6.

Goerlitz, H.R., Greif, S. & Siemers, B.M. 2008. Cues for acoustic detection of prey: insect rustlings sounds and the influence of walking substrate. *J. Exp. Biol.* **211**: 2799–2806.

Greenfield, M.D. 2002. *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford University Press, Oxford, UK.

Greenfield, M.D. & Baker, M. 2003. Bat avoidance in non-aerial insects: the silence response of signaling males in an acoustic moth. *Ethology* **109**: 427–442.

Greenfield, M.D. & Coffelt, J.A. 1983. Reproductive behaviour of the lesser wax moth, *Achroia grisella* (Pyralidae: Galleriinae): signalling, pair formation, male interactions, and mate guarding. *Behaviour* **84**: 287–315.

Greenfield, M.D. & Hohendorf, H. 2009. Independence of sexual and antipredator perceptual functions in an acoustic moth: implications for the receiver bias mechanism in signal evolution. *Ethology* **115**: 1137–1149.

Greenfield, M.D. & Weber, T. 2000. Evolution of ultrasonic signalling in wax moths: discrimination of ultrasonic mating calls from bat echolo- cation signals and the exploitation of an anti-predator receiver bias by sexual advertisement. *Ethol. Ecol. Evol.* **12**: 259–279.

Greig, E.I. & Greenfield, M.D. 2004. Sexual selection and predator avoidance in an acoustic moth: discriminating females take fewer risks. *Behaviour* **141**: 799–815.

Heckel, D.G., Gahan, L.J., Liu, Y.B. & Tabashnik, B.E. 1999. Genetic mapping of resistance to *Bacillus thuringiens*is toxins in diamondback moth using biphasic linkage analysis. *Proc. Natl. Acad. Sci. USA* **96**: 8373–8377.

Holland, B. & Rice, W.R. 1998. Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution* **52**: 1–7.

Hoy, R.R. 1992. The evolution of hearing in insects as an adaptation to predation from bats. In: *The Evolutionary Biology of Hearing* (D.B. Webster, R.R. Fay & A.N. Popper, eds), pp. 115–129. Springer-Verlag, New York, NY.

Hoy, R.R. & Robert, D. 1996. Tympanal hearing in insects. *Ann. Rev. Entomol.* **41**: 433–450.

Innan, H. & Kondrashov, F. 2010. The evolution of gene duplications: classifying and distinguishing between models. *Nat. Rev. Gen.* **11**: 97–108.

Jang, Y. & Greenfield, M.D. 1996. Ultrasonic communication and sexual selection in wax moths: female choice based on energy and asynchrony of male signals. *Anim. Behav.* **51**: 1095–1106.

Jang, Y. & Greenfield, M.D. 1998. Absolute versus relative measurements of sexual selection: assessing the contributions of ultrasonic signal characters to mate attraction in lesser wax moths, *Achroia grisella* (Lepidoptera: Pyralidae). *Evolution* **52**: 1383–1393.

Jang, Y. & Greenfield, M.D. 2000. Quantitative genetics of female choice in an ultrasonic pyralid moth, *Achroia grisella*:

variation and evolvability of preference along multiple dimensions of the male advertisement signal. *Heredity* **84**: 73–80.

Jang, Y., Collins, R.D. & Greenfield, M.D. 1997. Variation and repeatability of ultrasonic sexual advertisement signals in *Achroia grisella* (Lepidoptera: Pyralidae). *J. Insect Behav.* **10**: 87–98.

Jennions, M.D. & Brooks, R. 2001. A sense of history. *Trends Ecol. Evol.* **16**: 113–115.

Jia, F.-Y., Greenfield, M.D. & Collins, R.D. 2001. Ultrasonic signal competition between male wax moths. *J. Insect Behav.* **14**: 19–33.

Kirkpatrick, M. & Barton, N.H. 1997. The strength of indirect selection on female mating preferences. *Proc. Natl. Acad. Sci. USA* **94**: 1282–1286.

Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**: 33–38.

Kokko, H., Brooks, R., Jennions, M.D. & Morley, J. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B* **270**: 653–664.

Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.

Kosambi, D.D. 1944. The estimation of map distance from recombination values. *Ann. Eugen.* **12**: 172–175.

Künike, G. 1930. Zur biologie der kleinen wachsmotte, *Achroia grisella* (Fabricius). *Zeitschrift für angewandte entomologie* **16**: 304–356.

Lafaille, M., Bimbard, G. & Greenfield, M.D. 2010. Risk trading in mating behavior: forgoing anti-predator responses reduces the likelihood of missing terminal mating opportunities. *Behav. Ecol. Sociobiol.* **64**: 1485–1494.

Limousin, D. & Greenfield, M.D. 2009. Evaluation of amplitude in male song: female waxmoths prefer fortissimo notes. *J. Exp. Biol.* **212**: 4091–4100.

Limousin, D., Streiff, R., Courtois, B., Dupuy, V., Alem, S. & Greenfield, M.D. 2012. Genetic architecture of sexual selection: QTL mapping of male song and female receiver traits in an acoustic moth. *PLoS ONE* **7**: e44554.

Macias-Garcia, C. & Ramirez, E. 2005. Evidence that sensory traps can evolve into honest signals. *Nature* **434**: 501–505.

Midamegbe, A., Vitalis, R., Malausa, T., Delava, E., Cros-Arteil, S. & Streiff, R. 2011. Scanning the European corn borer (*Ostrinia* spp.) genome for adaptive divergence between host-affiliated sibling species. *Mol. Ecol.* **20**: 1414–1430.

Miller, L.A. & Surlykke, A. 2001. How some insects detect and avoid being eaten by bats: tactics and countertactics of prey and predator. *Bioscience* **51**: 570–581.

Milum, V.G. 1940. Larval pests common to the nests of bumblebees and combs of the honeybees. *J. Econ. Entomol.* **33**: 81–83.

Nakano, R., Takanashi, T., Surlykke, A., Skals, N. & Ishikawa, Y. 2013. Evolution of deceptive and true courtship songs in moths. *Sci. Rep.* **3**: 2003.

Neuweiler, G. 1989. Foraging ecology and audition in echolocating bats. *Trends Ecol. Evol.* **4**: 160–166.

Oxley, P.R., Spivak, M. & Oldroyd, B.P. 2010. Six quantitative trait loci influence task thresholds for hygienic behaviour in honeybees (*Apis mellifera*). *Mol. Ecol.* **19**: 1452–1461.

Phelps, S.M. & Ryan, M.J. 2000. History influences signal recognition: neural network models of tungara frogs. *Proc. R. Soc. Lond. B* **267**: 1633–1639.

Reinhold, K., Greenfield, M.D., Jang, Y. & Broce, A. 1998. Energetic cost of sexual attractiveness: ultrasonic advertisement in waxmoths. *Anim. Behav.* **55**: 905–913.

Ritchie, M.G. & Phillips, S.D.F. 1998. The genetics of sexual isolation. In: *Endless Forms: Species and Speciation* (D.J. Howard, S.H. Berlocher, eds), pp. 291–304. Oxford University Press, New York, NY.

Rodriguez, R.L. & Greenfield, M.D. 2004. Behavioral context regulates dual function of hearing in ultrasonic moths: bat avoidance and pair formation. *Physiol. Entomol.* **29**: 159–168.

Rodriguez, R.L. & Snedden, W.A. 2004. On the functional design of mate preferences and receiver biases. *Anim. Behav.* **68**: 427–432.

Rodriguez, R.L., Cocroft, R.B., Schul, J. & Greenfield, M.D. 2005. The contribution of tympanic transmission to fine temporal signal evaluation in an ultrasonic moth. *J. Exp. Biol.* **208**: 4159–4165.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**: 1415–1421.

Russo, D., Jones, G. & Arlettaz, R. 2007. Echolocation and passive listening by foraging mouse-eared bats *Myotis myotis* and *M. blythii*. *J. Exp. Biol.* **210**: 166–1763.

Ryan, M.J. 1990. Sexual selection, sensory systems and sensory exploitation. *Oxf. Surv. Evol. Biol.* **7**: 157–195.

Ryan, M.J. 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* **281**: 1999–2003.

Ryan, M.J. & Rand, A.S. 1993. Sexual selection and signal evolution—the ghost of biases past. *Philos. Trans. R. Soc. B* **340**: 187–195.

Ryan, M.J., Phelps, S.M. & Rand, A.S. 2001. How evolutionary history shapes recognition mechanisms. *Trends Cogn. Sci.* **5**: 143–148.

Sasabe, M., Takami, Y. & Sota, T. 2010. QTL for the species-specific male and female genital morphologies in Ohomopterus ground beetles. *Mol. Ecol.* **19**: 5231–5239.

Shaw, K.L. 1995. Phylogenetic tests of the sensory exploitation model of sexual selection. *Trends Ecol. Evol.* **10**: 117–120.

Shaw, K.L., Parsons, Y.M. & Lesnick, S.C. 2007. QTL analysis of a rapidly evolving speciation phenotype in the Hawaiian cricket Laupala. *Mol. Ecol.* **16**: 2879–2892.

Siemers, B.M. & Güttinger, R. 2006. Prey conspicuousness can explain apparent prey selectivity. *Curr. Biol.* **16**: 157–159.

Siemers, B.M., Kriner, E., Kaipf, I., Simon, M. & Greif, S. 2012. Bats eavesdrop on the sound of copulating flies. *Curr. Biol.* **14**: R563–R564.

Singh, N.D. & Shaw, K.L. 2012. On the scent of pleiotropy. *Proc. Natl. Acad. Sci. USA* **109**: 5–6.

Smith, C., Barber, I., Wootton, R.J. & Chittka, L. 2004. A receiver bias in the origin of three-spined stickleback mate choice. *Proc. R. Soc. Lond. B* **271**: 949–955.

Snedden, W.A., Tosh, C.R. & Ritchie, M.G. 1994. The ultrasonic mating signal of the male lesser wax moth. *Physiol. Entomol.* **19**: 367–372.

Spangler, H.G. 1988. Hearing in tiger beetles (Cicindelidae). *Physiol. Entomol.* **13**: 447–452.

Spangler, H.G. & Takessian, A. 1983. Sound perception by two species of wax moths (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* **76**: 94–97.

Spangler, H.G., Greenfield, M.D. & Takessian, A. 1984. Ultrasonic mate calling in the lesser wax moth. *Physiol. Entomol.* **9**: 87–95.

Velthuis, B.J., Yang, W., Van Opijnen, T. & Werren, J.H. 2005. Genetics of female mate discrimination of heterospecific males in Nasonia (Hymenoptera, Pteromalidae). *Anim. Behav.* **69**: 1107–1120.

Via, S. & Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**: 505–522.

Wagner, G.P. & Zhang, J. 2011. The pleiotropic structure of the genotype-phenotype map: the evolvability of complex organisms. *Nat. Rev. Genet.* **12**: 204–213.

Waters, D.A. & Jones, G. 1995. Echolocation call structure and intensity in five species of insectivorous bats. *J. Exp. Biol.* **198**: 475–489.

West-Eberhard, M.J. 1979. Sexual selection, social competition, and evolution. *Proc. Am. Phil. Soc.* **123**: 222–234.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**: 155–183.

West-Eberhard, M.J. 1984. Sexual selection, competitive communication and species-specific signals in insects. In: *Insect Communication* (T. Lewis, ed.), pp. 283–324. Academic Press, New York, NY.

Xu, S. 2003. Theoretical basis of the Beavis effect. *Genetics* **165**: 2259–2268.

Yager, D.D. 2012. Predator detection and evasion by flying insects. *Curr. Opin. Neurobiol.* **22**: 201–207.

Zhou, Y., Kelly, J.K. & Greenfield, M.D. 2011. Testing the fisherian mechanism: examining the genetic correlation between male song and female response in waxmoths. *Evol. Ecol.* **25**: 307–329.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Playback methods.

Data S2 Specific protocols for trait measurements.

Data S3 AFLP protocol.

Data S4 Regression mapping algorithm.

Figure S1 Threshold of response to the sexual and predatory signals.

Figure S2 Mapping of QTL for female and male response traits, male song traits and developmental traits among linkage groups.

Data deposited at Dryad: doi:10.5061/dryad.8k0d8

Received 31 May 2013; revised 9 August 2013; accepted 18 August 2013