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Sexual dimorphism in epicuticular compounds despite similar sexual selection in sex role-reversed seed beetles

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Abstract

Sexual selection imposed by mating preferences is often implicated in the evolution of both sexual dimorphism and divergence between species in signalling traits. Epicuticular compounds (ECs) are important signalling traits in insects and show extensive variability among and within taxa. Here, we investigate whether variation in the multivariate EC profiles of two sex role-reversed beetle species, *Megabruchidius dorsalis* and *Megabruchidius tonkineus*, predicts mate attractiveness and mating success in males and females. The two species had highly distinct EC profiles and both showed significant sexual dimorphism in ECs. Age and mating status in both species were also distinguishable by EC profile. Males and females of both species showed significant association between their EC profile and attractiveness, measured both as latency to mating and as success in mate-choice trials. Remarkably, the major multivariate vector describing attractiveness was correlated in both species, both sexes, and in both choice and no-choice experiments such that increased attractiveness was in all cases associated with a similar multivariate modification of EC composition. Furthermore, in both sexes this vector of attractiveness was associated with more male-like EC profiles, as well as those characterizing younger and nonvirgin individuals, which might reflect a general preference for individuals of high condition in both sexes. Despite significant sexual selection on EC composition, however, we found no support for the proposition that sexual selection is responsible for divergence in ECs between these species.

Introduction

Sexual dimorphism is the result of differing natural and/or sexual selection on males and females. For signalling traits, which can carry information about an individual's state, quality or class membership, sex-specific selection is often attributed to mate preferences in the opposite sex (Andersson, 1994). Commonly, one sex experiences sexual selection, resulting in the evolutionary elaboration of a trait, such as ornamentation, in that sex. The nonornamented sex sometimes experiences natural selection directly opposing the evolution of this trait; for example, selection for crypsis is the foremost hypothesis for the often relatively drab appearance of females in bird species with brightly coloured, ornamented males (Amundsen, 2000; Dale *et al.*, 2015). Alternatively, a simple lack of selection to maintain a trait may explain its reduced expression in one sex, although intersexual genetic correlations will slow this route to phenotypic divergence.

In contrast, when males and females express mating preferences for similar phenotypes, sexual monomorphism is expected. Such a scenario could arise when the information content of a signalling trait is similar in both sexes. For example, in sexually monomorphic black guillemots (*Cepphus grylle*), which mutually display their brightly coloured feet during courtship, the intensity of foot coloration is associated with a putative measure of the ability to withstand physiological stress

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(Fasanello *et al.*, 2015). Both male and female crested auklets (*Aethia cristatella*) bear an elaborate crest during the breeding season, and both sexes prefer mates with longer crests (Jones & Hunter, 1993). Similarly, mutually preferred feather ornaments predict social dominance, which is correlated with parental quality, in both male and female black swans (*Cygnus atratus*) (Kraaijeveld *et al.*, 2004). However, the strength and the direction of selection will affect the degree to which ornaments are sexually dimorphic, and while mutual ornamentation is relatively common, identical male and female mate preferences are seemingly rare. Additionally, differential natural selection against the preferred trait between the sexes could result in dimorphism despite similar sexual selection in both sexes.

Preferences for signalling traits can also contribute to the evolution and maintenance of reproductive isolation between related species (Lande, 1981; West-Eberhard, 1983; Panhuis *et al.*, 2001; Servedio & Noor, 2003). In theory, disruptive preferences may lead to divergence between subsets of a population and eventual population differentiation, although empirical evidence for such sympatric divergence is limited (Uy & Borgia, 2000; Panhuis *et al.*, 2001). More commonly, the coevolution of traits and preferences may proceed along different trajectories within allopatric populations due to random factors, such as the order of mutations (Mendelson *et al.*, 2014) and/or to differences in local environmental factors (Boughman, 2002). Upon secondary contact, population-specific mating preferences reduce the likelihood of mating between the populations and mating preferences can be further reinforced when hybridization is costly (Servedio & Noor, 2003). Although preferences in only one sex are sufficient to then maintain reproductive isolation, mutual mate choice is particularly efficient in generating assortative mating. For example, mutual mate choice targeting the same signalling trait in both sexes is known to reinforce premating isolation among lacewings from the *Chrysoperla carnea* species complex (Noh & Henry, 2010).

Chemical signalling is an important mode of communication in arthropods, contributing to species recognition (e.g. Buellesbach *et al.*, 2013), social cohesion (e.g. nestmate recognition, van Wilgenburg *et al.*, 2010), sex discrimination (e.g. Fernandez & Kravitz, 2013), mate location (e.g. Linn *et al.*, 1987; Svensson, 1996; Umbers *et al.*, 2015) and individual mating preferences (review: Johansson & Jones, 2007). In diverse arthropod taxa, chemical signals advertising mate quality or fecundity are a major determinant of an individual's attractiveness and mating success. There is abundant evidence that complex and dynamic signalling of mate quality, condition or receptivity (i.e. mating status) is in part mediated through cuticular hydrocarbons, lipids and their derivatives – nonvolatile compounds that make up a waxy layer on the epicuticle (e.g. Everaerts *et al.*, 2010; Delcourt & Rundle, 2011; Ingleby *et al.*, 2013; Bonduriansky *et al.*, 2015). However, natural selection clearly also acts on these epicuticular compounds (ECs). The main function of cuticular hydrocarbons in many insects is to reduce water transpiration, thus avoiding desiccation, and to provide protection against microorganisms and pathogens (Blomquist *et al.*, 1987; Hadley, 1981; Howard & Blomquist 2005, Blomquist & Bagneres, 2010). In fact, experimental studies in *Drosophila* have demonstrated EC evolution in response to environmental humidity (e.g. Kwan & Rundle, 2010).

The mating behaviour of the honey-locust beetles *Megabruchidius dorsalis* and *Megabruchidius tonkineus* is strongly suggestive of a role for chemical signalling, although sex pheromones have not been investigated in these beetles. There is marked sexual dimorphism in both species: only females possess two large, dark-pigmented oval patches on the pygidial plate of the abdomen. Under the microscope, these patches are dense with pores, apparently exuding a pheromonal substance. Both species show reversed courtship roles, whereby females perform stereotypic displays when they encounter a male (Takakura, 1999, 2001, 2006; Salehialavi *et al.*, 2011). The male and female antennate one another's carapace head-to-head, and the female then turns on the spot by 180° to present her pygidial patches to the male, who palpates and antennates the surface of the patches. The female continues to turn back and forth, repeatedly presenting her abdomen, until the male attempts to mount and copulation occurs. Males prefer females that court for longer and perform more turns during courtship (Salehialavi *et al.*, 2011; Fritzsche *et al.*, 2016). The central role of palpation and antennation of the pygidial patches in this courtship display strongly suggests that males assess females via a largely nonvolatile chemical signal. Females also exhibit choosiness, commonly rejecting male mating attempts (Salehialavi *et al.*, 2011). Male body size is the only trait known to be associated with female rejection behaviour, but female antennation of males during courtship again suggests a possible role for the chemical assessment of potential mates.

Here, we investigated the contribution of ECs to sexual signalling in these two courtship role-reversed honey-locust beetle species. We first documented variation in ECs along axes of species, sex, age and mating status. We then used mating trials to examine whether and how ECs are related to male and female attractiveness. Given patterns of variation for sexual traits in other species (Arnqvist, 1998; Ritchie, 2007), we expect (1) sexual dimorphism in ECs and that (2) species should show distinct EC profiles. Further, we expect that (3) EC profile will be related to attractiveness in both sexes, as mutual antennation forms a key component of courtship and antennae are not sexually dimorphic in *Megabruchidius* (Tuda & Morimoto, 2004). As only female *Megabruchidius* bear the pore-rich pygidial ornament, however, we also expect that (4) females will show a stronger relationship between ECs and attractiveness than males. Finally, if sex-specific sexual selection underlies the evolution of sexual dimorphism in these species, we predict that (5) the EC axis of mate attractiveness should align with that of sexual dimorphism. Our analyses, from both choice and no-choice mating trials, revealed a multivariate vector of EC attractiveness that, surprisingly, was largely shared by males and females of both species. Furthermore, this attractiveness vector was significantly related to the main vector discriminating sex, age and mating status, such that in both species individuals with EC profiles characteristic of younger, mated males were more attractive to partners of both sexes.

Materials and methods

Variation in epicuticular compound profile by species, sex, age and mating status

We extracted the ECs of individual adult *M. dorsalis* and *M. tonkineus* to quantify variation with respect to species, sex, mating status (virgin vs. recently mated) and age (9 vs. 27 days post-eclosion). We collected individuals for extraction from large ($N > 1000$) laboratory stock populations of each species. Beetles were maintained in controlled environment chambers at 26 °C and 70% relative humidity on a 14 : 10 hour light : dark cycle. Honey-locust beetle larvae develop inside single seeds of the legume *Gleditsia* spp, usually individually, and emerge after pupation. By distributing seeds from the breeding substrate of the populations among individual wells of 24-well culture plates, we were able to obtain individual virgin adults of known emergence date (hereafter, adult 'age' is measured in days from the date of emergence from the seed). We individually maintained 60 virgin males and 60 virgin females of each species in 3-cm-diameter plastic Petri dishes until EC extraction at age 9 ('young': $N = 30$ per sex) or 27 ('old': $N = 30$ per sex) days. Extractions were performed over several days for each age group, and we note that effects of age in our analyses may be influenced by the effect of day of EC extraction. For another set of males and females of each species, we used controlled matings between eight-day-old males and females to create once-mated individuals. To do this, male–female pairs were placed together in a 3-cm-diameter Petri dish and observed until mating was confirmed visually. Mated pairs ($N = 60$) were then separated and housed individually until EC extraction at age 9 ($N = 30$ per sex) or 27 ($N = 30$ per sex) days. Thus, for each species we extracted ECs from eight classes of individuals: young unmated males, young unmated females, young mated males, young mated females, old unmated males, old unmated females, old mated males and old mated females (N for each class ≈ 30).

Mating trials – sexual selection on ECs

We also extracted ECs from individual male and female *M. dorsalis* and *M. tonkineus* that differed in their attractiveness (here, we use 'attractiveness' to indicate mating success largely mediated by choice/acceptance by a mating partner). Attractiveness was assayed in two ways. First, we placed trios of 14-day-old virgin adults – either one male and two females ('male choice': $N = 40$ trios per species) or one female and two males ('female choice': $N = 40$ trios per species) – in 3-cm-diameter plastic Petri dishes. Dishes were observed until a mating occurred (within 1 h – if no mating occurred, the trio was discarded: this was the case for eight of 48 *M. dorsalis* 'female choice' trials, one of 41 *M. dorsalis* 'male choice' trials, 0 of 40 *M. tonkineus* 'female choice' trials and two of 42 *M. tonkineus* 'male choice' trials). Once mating commenced, the 'rejected' individual (not involved in mating) was removed from the dish and weighed to the nearest μg on a microbalance (Sartorius, Germany). When mating finished, we collected and weighed the 'chosen' individual (of the same sex as the rejected individual). The third individual, that is the one exercising choice, was discarded. The chosen and rejected beetles were then housed individually overnight, and on day 15, their ECs were extracted. These paired samples allowed us to compare EC profiles of relatively attractive and unattractive (successful/unsuccessful) same-sex, age-matched beetles.

Second, to avoid potential confounding effects of mating status (mated or unmated) when measuring the EC profile of more- or less-attractive individuals, we also performed 'no-choice' mating trials. For this assay, we paired 9-day-old males and females in 3-cm-diameter Petri dishes until mating was visually confirmed ($N = 100$ pairs per species). Pairs were then separated and housed individually for 2 days. On day 11, these mated individuals were randomly reshuffled into new pairs and allowed to mate a second time. From this second pairing, we recorded the latency to mate as a measure of the attractiveness/mating success of both members of the pair. Pairs that failed to mate within the 30-min time limit (six of 100 *M. dorsalis* and seven of 101 *M. tonkineus* pairs) were assigned a mating latency of 30 min, a conservative approach given mean mating latencies of 3 min 16 s (*M. dorsalis*) and 4 min 12 sec (*M. tonkineus*). Pairs were separated and housed individually overnight, and on day 12, their ECs were extracted.

Both types of mating assay were carried out during the light hours of the daily cycle at the same temperature and humidity as their normal maintenance routine.

Epicuticular compound extraction and gas chromatography

To extract epicuticular compounds, each individual was separately washed in 200 μl hexane for 4 min including 1 min of vortex mixing to thoroughly dissolve the hydrocarbons. Beetles were then removed from the vials, and the EC samples were stored at −20 °C until analysis. Gas chromatography with flame ionization detection was performed on 1 μl liquid sample injections using a dual-channel Agilent Technologies 6890N gas chromatograph with a fast oven (198–231 V power supply), fitted with an HP-5 5% phenyl methyl siloxane column of 30 m length and 0.25 mm internal diameter and pulsed splitless inlets (at 200 °C with a pulse of 206.8 kPa (30 psi) for the first 1.4 min). The flame ionization detectors were at 310 °C. Hydrogen was used as the carrier gas with a constant column flow of 2 ml min^{-1}. The temperature programme began at 180 °C and was immediately increased at 12 °C min^{-1} to 280 °C, then increased at the maximum rate (set point of 120 °C min^{-1}) to 315 °C and holding for 9 min. The total run time was just under 18 min. We note that this extraction procedure focusses on the nonvolatile components of the individual's pheromone profile; volatile components were not quantified.

The EC profile of each sampled individual was measured by integration of the area under 20 putatively homologous compounds (i.e. matched by retention times; Fig. S1) that were reliably present in at least one sex of one of the species, although all appeared to be shared between both sexes and species (see Results). These compounds are referred to by number in order of their retention time. Kovats retention indices were calculated for each compound by comparison with a standard C7–C40 saturated n-alkane mixture (Sigma-Aldrich, Oakville, ON, Canada, product #49452-U) (Table S1). We calculated the relative abundance of each compound in a sample by dividing the area under each peak by the total area under all 20 peaks. Relative concentrations such as this are a form of compositional data to which standard statistical analyses should not be applied (Aitchison, 1986). To address this, proportions were transformed into centred log-ratios by dividing each proportion by the geometric mean of all 20 proportions and taking the natural logarithm of this value; that is $\ln\left(p^i/\prod_{i=1}^{n} p_i\right)^{1/n}$. Centred log-ratios (CLRs) have a zero-sum constraint, but this transformation avoids the use of one arbitrarily chosen peak as a divisor. We calculated Mahalanobis distances in the multivariate analysis procedure of JMP v.10 (SAS Institute, Cary, NC) but did not detect multivariate outliers in our data sets.

Statistical analyses

Data on EC composition are inherently multivariate, and we found strong multicollinearity among the CLR-transformed traits. We therefore used partial least squares (PLS) models to examine whether and how multivariate EC profiles varied between the species, and with sex, age class and mating status. A PLS model aims to find the multivariate relationships between two matrices by modelling their covariance structures in a latent variable approach and can accommodate collinear variables (Abdi, 2007). We coded dichotomous classifiers as −1 and 1 for symmetry. PLS models involving dichotomous variables yield correct latent variables (i.e. multivariate axes), although tests of these dimensions using cross-validation should be interpreted with caution (Nguyen & Rocke, 2002). We note that we are here primarily interested in characterizing and comparing the various latent variables that describe how multivariate EC composition differs between our classifiers. To compare latent EC variables in order to assess the degree to which, for example, the EC axis that best distinguishes the sexes is similar to the axis that best distinguishes the species, we inspected the correlation between (1) the loadings, and (2) the PLS regression coefficients, of the two latent variables. Loadings capture direction and the extent of covariance between the original and latent variable, while coefficients reflect only direction. We first fitted a global model including all four classifiers (species, sex, age and mating status) and then proceeded to model each species separately. We then ran eight independent PLS models using ECs to predict attractiveness in the eight different attractiveness data sets: female attractiveness measured by male choice, male attractiveness measured by female choice, female attractiveness measured by mating latency and male attractiveness measured by mating latency, in each species. Finally, we compared the major EC vectors (i.e. latent variables) that best predicted attractiveness, sex, age and mating status in each species. Analyses were performed in SYSTAT v. 13 (Systat Software, San Jose, CA) and GENSTAT v. 18.1 (VSNi, Hemel Hempstead, UK). The SIMPLS algorithm (de Jong, 1993) was used to fit PLS models, and we employed the method of Osten (1988) for cross-validation and testing of PLS dimensions.

Results

Variation in epicuticular compound profile by species, sex, age and mating status

Epicuticular compound profiles of males and females of the two species were qualitatively similar with all 20 compounds that were reliably present in a given sex and species being shared with the other sex and species (Fig. S1). Kovats retention indices ranged from 2700 to 3162 (Table S1), indicating these compounds contain from 27 to 31 or 32 carbons. Although shared, there were significant quantitative differences in the relative abundances of these ECs among different classes of individuals (Fig. 1). Our global PLS model identified nine significant (Osten's F-test; $P < 0.05$) dimensions of covariation between ECs and our four classifiers. The first was dominated by species differences (94.8%), the second by sex differences (26.5%), the third by age effects (15.6%), the fourth by mating status (5.2%) and the fifth again by sex differences (24.9%). The two species were very well separated along the first PLS axis of variation in ECs (Fig. 2). Separate PLS models in the two species found that sex, age and mating status covaried significantly with ECs in both species (Table 1): there was pronounced sexual dimorphism in ECs in both species, beetles of different ages had distinct CHC profiles, but mated and virgin beetles differed less.

The vectors of PLS coefficients that best described between-sex (Fig. 2) and between-age variation in ECs were both similar between the two species ($r = 0.75$ and 0.71, respectively), indicating similar multivariate patterns of EC sexual dimorphism and changes with age in *M. dorsalis* and *M. tonkineus*. The vectors of EC coefficients in each species that best described variation due to mating status were less strongly correlated ($r = 0.47$), indicating moderate similarity between the species in how ECs changed with mating.

Predicting attractiveness from EC profile

The amount of variance in attractiveness explained by the first PLS axis of variation in ECs ranged from 3.9% to 16.8% depending on species, sex and type of mating trial. Discrimination of chosen vs. rejected individuals by their EC profile was only significant for *M. dorsalis* females, but mating latency was significantly predicted by the first PLS axis for males and females of both species (Table 2). The PLS axis best predicting species was only weakly associated with the vectors of attractiveness (mean $|r| = 0.38$; absolute values are used to compare the magnitude of positive and negative relationships), suggesting that sexual selection is not associated with species differences in EC profile.

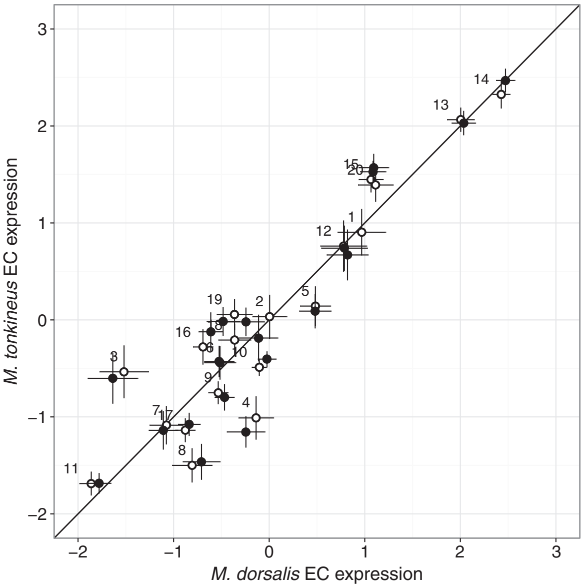

Figure 1 Variation in the relative concentration of the 20 ECs in *Megabruchidius tonkineus* and *Megabruchidius dorsalis* females (filled circles) and males (open circles). The figure shows CLR-transformed values, sex- and species-specific means with associated standard deviations. Labels indicate the number of the EC peak. The line depicts a 1 : 1 relationship.

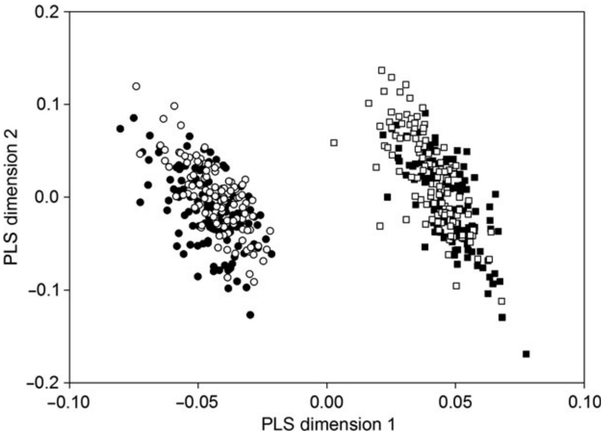

Figure 2 Ordination of samples along the 1st and 2nd PLS dimension, illustrating the distinct EC profiles of the two species. Circles, *Megabruchidius dorsalis*; squares, *Megabruchidius tonkineus*; filled symbols, females; open symbols, males.

Table 1 Osten's F and the per cent variation explained by the first PLS axis for models of 20 CLR-transformed ECs including a single classifier – age, sex or mated status – for *Megabruchidius dorsalis* and *Megabruchidius tonkineus* separately

Species	Classifier	Osten's *F*	df1	df2	*P*	% variation explained
M. dorsalis	Age	104.45	20	4760	< 0.001	31.5
	Sex	112.2	20	4760	< 0.001	35.8
	Mated	2.53	20	4760	< 0.001	7.0
M. tonkineus	Age	55.61	20	4820	< 0.001	25.9
	Sex	101.45	20	4820	< 0.001	30.1
	Mated	28.84	20	4820	< 0.001	11.3

Remarkably, the vector of EC loadings on the axis of attractiveness was significantly correlated across the eight different PLS models in Table 2 (Bartlett's $X^2_{28} = 219.5$, $P < 0.001$), as was the vector of EC coefficients (Bartlett's $X^2_{28} = 99.6$, $P < 0.001$), indicating that the multivariate EC vector predicting attractiveness shares a similar direction across species and sexes and that a similar vector is identified by both two-choice and no-choice tests of mate attractiveness (Figs 3, S2). As expected, the effect of EC variation in females was

Table 2 Osten's *F* and the per cent variation explained by the first axis for PLS models of the 20 CLR-transformed ECs, including a single classifier – attractiveness – for each species, sex and mating-trial type separately. Shown also are F values from conventional ANOVAS of PLS regressions including only a single PLS dimension, as a measure of relative effect size

Species	Sex	Classifier	Osten's F	df1	df2	*P*	% variation explained	$F_{\mathrm{ndf,ddf}}$
Megabruchidius dorsalis	Male	Chosen	1.41	20	1580	0.105	3.9	$3.68_{1,78}$
	Female	Chosen	2.43	20	1540	< 0.001	16	$13.45_{1,76}$
	Male	Latency	12.52	20	1980	< 0.001	12.8	$12.3_{1,98}$
	Female	Latency	9.0	20	1960	< 0.001	16.8	$23.83_{1,97}$
Megabruchidius tonkineus	Male	Chosen	1.5	20	1580	0.071	5.2	$3.87_{1,78}$
	Female	Chosen	1.41	20	1560	0.108	5.5	$4.53_{1,77}$
	Male	Latency	1.65	20	2000	0.035	5.7	$5.45_{1,99}$
	Female	Latency	2.97	20	1980	< 0.001	8.6	$8.63_{1,98}$

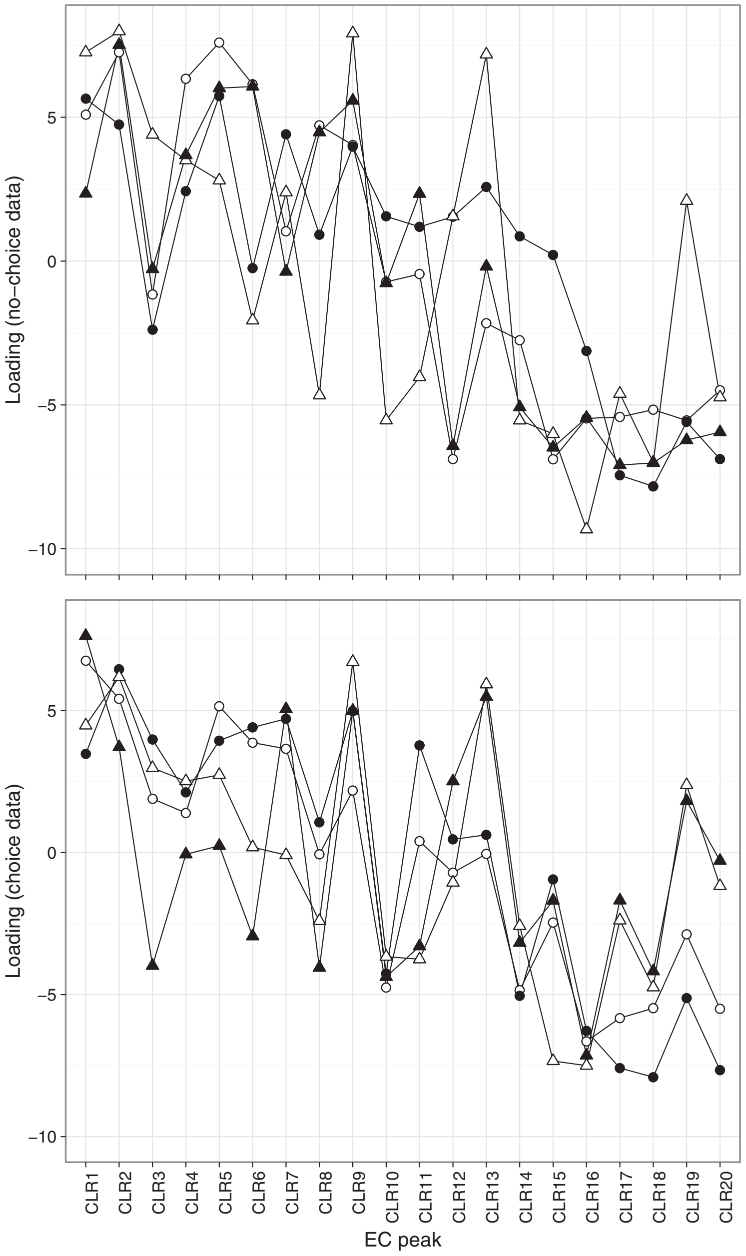

Figure 3 Loadings of the 20 CLR-transformed ECs onto the first PLS axes of eight independent PLS models describing attractiveness, measured by latency to mate (upper panel) or by mate choice (lower panel), in males and females of the two species. The sign of loadings for mating latency has been reversed for ease of comparison. Filled circles, *Megabruchidius dorsalis* females; open circles, *M. dorsalis* males; filled triangles, *Megabruchidius tonkineus* females; open triangles, *M. tonkineus* males.

more closely related to their attractiveness than it was in males, for both species and both measures of attractiveness (Table 2). A closer examination of the pairwise correlations found strong positive correlations between the sexes, as well as correlations between the two mating-trial methods (Table 3). In 24 of 24 cases, values were positive when correlating a choice vector with another choice vector but were negative in 31 of 32 cases when correlating a choice vector with a latency vector (Table 3), as expected. This is important as, given mutual mate choice in these species, mating-latency trials provide imperfect measures of the attractiveness of either sex; if one member of a pair is very unattractive and the other very attractive, and hence reluctant to mate with the unattractive partner provided, the resulting long latency may not accurately reflect the attractiveness of this choosy individual. However, assuming that attractiveness is normally distributed in both sexes, it can be shown that the expected Pearson product moment correlation between observed latency (our measure) and attractiveness (the underlying causal variable) is $r = 0.6$ in both sexes in paired trials such as ours. This represents the expected correlation between the highest value of a pair of variables with each of the variables that makes up this pair.

Correlating the EC vectors described by success in choice trials and by mating latency allowed us to ascertain whether these methods captured similar aspects of attractiveness, as well as to exclude the possibility that the difference in ECs between chosen and rejected individuals is simply the difference between mated and unmated individuals. In *M. dorsalis*, the correlation between the vector of EC loadings associated with chosen individuals and the vector of EC loadings associated with short mating latencies was −0.8 in both males and females (Table 3, Fig. S2). In *M. tonkineus* males, this correlation was −0.95, and in *M. tonkineus* females, −0.28 (Table 3, Fig. S2). The similarities between these attractiveness vectors indicate that an EC profile that led to a higher likelihood of being chosen in a choice trial was similar to a profile that led to a shorter mating latency. To further confirm that both measures of attractiveness were detecting the same effect, we ran an additional PLS model using the loadings of the four PLS vectors from the mating-latency models to predict the loadings of the four vectors from the choice-trial models. All four choice-trial loading vectors were significantly predicted by the first axis of variation (all four $F_{1,18} > 8.97$, $P < 0.008$). The first pair of latent variables explained 75.5% of the variance in mating-latency loadings and 63.9% of the variance in choice loading. Along this first dimension of covariation, all measures of latency loaded negatively (−4.01 to −3.47) and all measures of choice loaded positively (2.51 to 3.98), as expected.

In a heuristic attempt to use lower-order data to validate the findings of our multivariate analyses, we also ran a series of general/generalized linear models of each of the 20 epicuticular compounds separately. These models all used either (1) latency to mating or (2) chosen or not chosen as their responses and sex, species, EC and all four interaction terms as predictors. These analyses showed that a number of ECs were indeed significantly (at $\alpha = 0.05$) related to overall attractiveness, whether measured as latency to mating (EC 1, 2, 5, 9, 12, 16, 17, 18, 19 and 20) or as chosen/rejected (EC 1, 7, 16, 17 and 18). However, only four of in total 120 interactions terms involving ECs were significant (i.e. even fewer than predicted under type I error alone) and graphical inspections of these four cases suggested potential differences in strength, rather than in direction, across groups. Thus, these analyses confirm that ECs are related to attractiveness in a manner that is, to a large extent, shared across species and sexes.

Relating attractiveness to chemical indicators of sex, age and mating status

Next, we asked how the multivariate EC vectors predicting attractiveness related to those predicting sex,

Table 3 Pairwise correlations between the PLS axes of multivariate EC variation from eight independent models of variation in attractiveness (see Table 2). Correlations using EC coefficients above the diagonal, and using EC loadings below the diagonal

			Megabruchidius dorsalis				*Megabruchidius tonkineus*			
			Choice		Latency		Choice		Latency	
			Male	Female	Male	Female	Male	Female	Male	Female
M. dorsalis	Choice	Male		0.628	−0.475	−0.428	0.380	0.592	−0.685	−0.353
		Female	0.933		−0.392	−0.793	0.163	0.406	−0.518	−0.370
	Latency	Male	−0.804	−0.743		0.548	−0.221	−0.159	0.230	0.871
		Female	−0.769	−0.805	0.684		0.034	−0.416	0.314	0.407
M. tonkineus	Choice	Male	0.695	0.596	−0.587	−0.475		0.486	−0.507	−0.261
		Female	0.597	0.465	−0.291	−0.523	0.749		−0.728	−0.037
	Latency	Male	−0.774	−0.703	0.529	0.592	−0.950	−0.827		0.253
		Female	−0.814	−0.833	0.955	0.715	−0.598	−0.276	0.560	

Table 4 Osten's *F* and the per cent variation explained by the first PLS axis in models predicting the four vectors of EC loadings describing attractiveness (male and female choice and male and female mating latency) with a single classifier – age, sex or mated status – for *Megabruchidius dorsalis* and *Megabruchidius tonkineus* separately

Species	Classifier	Osten's F	df1	df2	*P*	% variation explained
M. dorsalis	Age	3.98	4	76	0.006	37.5
	Sex	22.23	4	76	< 0.001	45.2
	Mated	24.15	4	76	< 0.001	53.6
M. tonkineus	Age	52.30	4	76	< 0.001	76.6
	Sex	129.54	4	76	< 0.001	86.6
	Mated	11.29	4	76	< 0.001	39.0

age and mating status in the two species. For each species, we fitted PLS models using EC loadings of the four attractiveness vectors (Table 2) as independent variables and those best distinguishing age, sex or mating status as single response variables. These models (Table 4) showed that EC profiles distinguishing individuals of different age, sex and mating status are aligned with EC attractiveness. In terms of sex differences, the correlation between EC loadings for the sex dimension and attractiveness ranged between $|r| = 0.44–0.81$ in *M. dorsalis* and $|r| = 0.60–0.88$ in *M. tonkineus*. Thus, in both species, individuals with more attractive EC profiles (i.e. those that were more often chosen or had shorter latencies) were those with EC profiles also characterizing young, mated males.

Discussion

Although they shared the same suite of ECs, we detected marked differences in multivariate EC profiles between the two *Megabruchidius* species, and between males and females of each species. Sexual dimorphism in the EC profiles of *M. dorsalis* and *M. tonkineus* cannot be explained by sex-specific sexual selection through qualitative differences in mutual mate choice, as we found that the vector explaining the most variance in EC attractiveness was correlated between the sexes. Furthermore, this vector of attractiveness is related to the axis that best discriminates sex, age and mating status, such that EC combinations characterizing young, mated males are the most attractive mate phenotypes to both sexes of both species. While our primary aim in this study was to characterize the variation in multivariate EC profile between the species and sexes in relation to their mating success, we note that different compounds may show the same retention time in gas chromatography, and further work to chemically identify the substances represented by the peaks detected here could reveal qualitative, as well as quantitative differences between groups.

Why are preferred EC profiles similar between species?

The two species were completely separated in EC space without overlap along the first dimension of covariation (Fig. 2). However, the pattern of variation and covariation between the 20 epicuticular compounds showed a similar relationship with attractiveness in both species, both when attractiveness was measured as success in two-choice mating trials and as mating latency in no-choice trials. This suggests, firstly, that the attractive phenotype itself is not the result of selection to avoid mating with heterospecifics. Secondly, EC divergence between the two species is unlikely to have been driven by differences in sexual selection. Instead, the different EC compositions of *M. dorsalis* and *M. tonkineus* may reflect neutral divergence or divergent natural selection, for example as could result from adaptation to different humidity regimes or pathogens. The pattern seen in our data could arise if selection in both species acts to maintain the particular covariation of ECs important for mating success, while ECs are free to evolve for other reasons in other multivariate dimensions. Consider, for example, a simple scenario where sexual selection favours having twice as much of compound A as compound B, while the ratio of (A + B) to C evolves upwards in one species and downwards in another. Sex pheromones are largely conserved between related species and diverging populations in several other arthropod taxa as well (e.g. *Drosophila*, Symonds & Wertheim, 2005; Cerambycid beetles, Mitchell *et al.*, 2015; *Schizocosa* wolf spiders, Roberts & Uetz, 2004; *Odontomachus brunneus* trap-jaw ants, Smith *et al.*, 2014). In these examples, the maintenance of species boundaries is attributed to physical separation (allopatry or phenological differences between species), or to other cues providing species identity information, explanations that may both apply to *Megabruchidius*. For example, our study focused on nonvolatile compounds, but volatile pheromones are also common in insects (de Bruyne & Baker, 2008). Volatile pheromones can be detected over longer distances, as well as at close range, and could be more important than nonvolatile surface compounds in the initial location and identification of potential mates (Cardé & Baker, 1984; Cardé & Haynes, 2004).

Why are preferred EC profiles similar in males and females?

Perhaps even more intriguing was our finding that, despite clear sexual dimorphism in EC profiles, the vectors of attractiveness in each sex were strongly correlated. Because honey-locust beetles show courtship role reversal and mutual mate choice with stronger male than female mate choice (Salehialavi *et al.*, 2011), we predicted that the effect of EC variation on attractiveness would be stronger in females than in males, and this prediction was upheld. Moreover, the same direction of change in relative abundances of 20 epicuticular compounds was associated with increased success in mate-choice trials and with shorter mating latency for both males and females. Considering the fact that these beetles show mutual mate choice, this may not be entirely surprising. It is likely, for example, that both sexes prefer mates in high condition (Salehialavi *et al.*, 2011) and that high phenotypic condition is revealed by a particular EC profile in both sexes (Johansson & Jones, 2007). The condition dependence of ECs in one or both sexes is known from several insect taxa, and heightened condition dependence has been linked to sexual selection on male ECs in decorated crickets (Weddle *et al.*, 2012; Rapkin *et al.*, 2017), cockroaches (South *et al.*, 2011), burying beetles (Chemnitz *et al.*, 2015) and *Drosophila serrata* (Delcourt & Rundle, 2011; Gosden & Chenoweth, 2011). In contrast, female attractiveness was not affected by diet treatments mediating condition dependence of female ECs in *Drosophila melanogaster* (Fedina *et al.*, 2012). However, under mutual mate choice, as in the *Megabruchidius* species studied here, sexual signals in both sexes should reflect condition (e.g. South & Arnqvist, 2009). Interestingly, the correlations between EC attractiveness vectors described by choice and by latency measures were extremely high in both sexes of *M. dorsalis* and in *M. tonkineus* males, but somewhat lower in *M. tonkineus* females (Table 3). This pattern is consistent with previous work showing that role reversal and the extent of male mate choice is weaker in *M. tonkineus* than in *M. dorsalis* (Salehialavi *et al.*, 2011). Less pronounced male mate choice in *M. tonkineus* would reduce the dependence of mate-choice outcomes on female EC profiles, leading to a lower correlation between the two attractiveness vectors in females.

Mutual mate preferences acting in a similar direction on the same set of traits are generally expected to lead to sexually monomorphic phenotypes, although this will in theory depend on the strength of selection imposed on each sex, as well as any differences in other forms of selection and the genetic basis of the traits in each sex. Even under uniform selection, if the genetic covariance structure underlying the traits differs between the sexes, then responses may be sex-specific (Wyman *et al.*, 2013). We found that the axes of attractiveness were well aligned with those describing sex differences. A shared EC preference could result in sexual dimorphism in EC expression if, as suggested by female-limited ornamentation in *Megabruchidius*, the strength of sexual selection is indeed stronger in females such that the degree of EC elaboration differs across the sexes. Under this scenario, our results are consistent with sexual selection having caused the sexual dimorphism seen in EC profile. Alternatively, the extent of EC elaboration by sexual selection in females could be limited by trade-offs with naturally selected traits, causing net selection to differ between the sexes. ECs synthesized through common biochemical pathways are known to serve multiple functions within a wide range of insect taxa (Chung & Carroll, 2015). A particularly relevant possibility is a trade-off between mate signalling and female fecundity, which has been demonstrated in *Drosophila* spp. (Kwan & Rundle, 2010; Rundle & Chenoweth, 2011), and likely stems from a requirement for hydrocarbons of both the cuticle and the ovaries (Schal *et al.*, 1994).

Why do both sexes prefer EC profiles associated with young, mated males?

We found that the EC axes explaining the most variance in sex, age and mating status were significantly predicted by the vectors explaining variance in attractiveness, and attractiveness was associated with an EC profile that is characteristic of young, mated males. The fact that male-like phenotypes are associated with female attractiveness may seem puzzling. However, as suggested above, the EC axis of attractiveness may reflect general phenotypic condition in both sexes. This is supported by previous observations of mate choice for behavioural correlates of condition in honey-locust beetles (Salehialavi *et al.*, 2011). In these beetles, males are to a large extent capital breeders that reproduce exclusively on resources gathered as juveniles. Males do feed on pollen and nectar (Takakura, 2004), but ingested food resources do not fully compensate for the successive costs of producing large and nutritious ejaculates (Salehialavi *et al.*, 2011). In contrast, female honey-locust beetles are to a large extent income breeders that rely on the ejaculate resources derived from males for their reproduction (Takakura, 2006), and females can survive and reproduce for many months without food or water if mated regularly. It is thus possible that young males show highest phenotypic condition merely as a result of having unexpended energy reserves and resources, which may explain the EC profile alignment between young males and attractiveness in both sexes. Females are, however, frequently found to prefer older males as mates across a range of insect (and other) taxa (e.g. Lopez *et al.*, 2003; Avent *et al.*, 2008; Judge, 2011; Verspoor *et al.*, 2015; and see Brooks & Kemp, 2001). There are a few cases where younger males are preferred: ageing altered cuticular hydrocarbon profiles towards greater proportions of compounds with longer carbon chains in both males and females of *D. melanogaster*, which decreased the sexual attractiveness of both sexes, although the effect on male attractiveness was contingent on the availability of visual cues during female mate choice (Kuo *et al.*, 2012). In other insect systems (Wedell & Ritchie, 2004; Hartbauer *et al.*, 2015), a preference for younger male mates has been suggested to be due to the correlation of younger ages with virgin status in species where the quality of nuptial gifts (Wedell & Ritchie, 2004) or sperm (Jones *et al.*, 2000) declines with male multiple mating. Such benefits to females of virgin male mates, and consequently the increased attractiveness of virgins, appear to be more common than the reverse among insect taxa.

Attractiveness of younger females as mates is relatively common in arthropods and is usually attributed to (i) their higher fecundity, when fecundity declines with age, and/or (ii) the association of younger ages with increased likelihood of virginity, when sperm competition reduces the value of already-mated females (Bonduriansky, 2001). A male preference for virgin over mated females is also common among insect taxa. There is, however, some evidence for male preferences for mated females in some insect species, for example in the psyllid *Diaphorina citri* (Wenninger *et al.*, 2008) as well as in *M. tonkineus* (Salehialavi *et al.*, 2011). The suggested explanation in *M. tonkineus* is related to male provision of a large, costly and nutritious ejaculate, and an observed delay between a female's first mating and the onset of egg production (Salehialavi *et al.*, 2011). In such cases, with female multiple mating and last-male precedence in sperm competition, the first male to mate with a young virgin female may invest highly but gain little or no paternity. Interestingly, in contrast, virgin females have previously been shown to be preferred by males in *M. dorsalis* (Salehialavi *et al.*, 2011).

We have shown that the ECs of honey-locust beetles are related to mate attractiveness in both sexes. In both *M. dorsalis* and *M. tonkineus*, both males and females with EC compositions that were more male-like, and characteristic of younger or mated individuals, had increased mating success and shorter mating latencies, indicating that significant sexual selection is operating on both sexes through mutual mate preferences in these species. Despite a vector of attractiveness that shared a similar direction in the two sexes, there was clear sexual dimorphism in EC profile and, as predicted based on the presence of a female-only ornament and courtship display, our results suggested stronger sexual selection on female than on male ECs. Our study demonstrates that mutual mate preferences for similar trait values in males and females are not sufficient to constrain sexual dimorphism. This serves as a reminder that even in traits under sexual selection, dimorphism is a product of the complex interactions between the relative strength and direction of selection on each sex, the sex-specific genetic architecture underlying both trait and preference, and other sources of selection. Finally, while *M. dorsalis* and *M. tonkineus* clearly had distinct multivariate EC profiles, they exhibited similar vectors of attractiveness, revealing that sexual selection favours similar EC combinations in both species. Sexual selection is therefore unlikely to be responsible for the divergence in EC profiles between these two honey-locust beetles.

Data archiving

Data is accessible from the DRYAD repository(https://doi.org/10.5061/dryad.pj557).

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References

Abdi, H. 2007. Partial least square regression. In: *Encyclopedia of Measurement and Statistics* (N. Salkin, ed), pp. 740–744. Sage, Thousand Oaks, CA.

Aitchison, J. 1986. *The Statistical Analysis of Compositional Data*. Chapman and Hall, London, UK.

Amundsen, T. 2000. Why are female birds ornamented? *Trends Ecol. Evol.* **15**: 149–155.

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

Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**: 784–786.

Avent, T.D., Price, T.A.R. & Wedell, N. 2008. Age-based female preference in the fruit fly *Drosophila pseudoobscura*. *Anim. Behav.* **75**: 1413–1421.

Blomquist, G.J. & Bagneres, A.G. (eds) 2010. *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. Cambridge University Press, Cambridge, UK.

Blomquist, G.J., Nelson, D.R. & De Renobales, M. 1987. Chemistry, biochemistry, and physiology of insect cuticular lipids. *Arch. Insect Biochem. Physiol.* **6**: 227–265.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.* **76**: 305–339.

Bonduriansky, R., Mallett, M.A., Arbuthnott, D., Pawlowsky-Glahn, V., José Egozcue, J. & Rundle, H.D. 2015. Differential effects of genetic vs. environmental quality in *Drosophila melanogaster* suggest multiple forms of condition dependence. *Ecol. Lett.* **18**: 317–326.

Boughman, J.W. 2002. How sensory drive can promote speciation. *Trends Ecol. Evol.* **17**: 571–577.

Brooks, R.C. & Kemp, D.J. 2001. Can older males deliver the good genes? *Trends Ecol. Evol.* **16**: 308–313.

de Bruyne, M. & Baker, T.C. 2008. Odor detection in insects: volatile codes. *J. Chem. Ecol.* **34**: 882–897.

Buellesbach, J., Gadau, J., Beukeboom, L.W., Echinger, F., Raychoudhury, R., Werren, J.H. *et al.* 2013. Cuticular hydrocarbon divergence in the jewel wasp *Nasonia*: evolutionary shifts in chemical communication channels? *J. Evol. Biol.* **26**: 2467–2478.

Cardé, R.T. & Baker, T.C. 1984. Sexual communication with pheromones. In: *Chemical Ecology of Insects* (W.J. Bell, R.T. Cardé, eds), pp. 355–383. Chapman and Hall, London, UK.

Cardé, R.T. & Haynes, K.F. 2004. Structure of the pheromone communication channel in moths. In: *Advances in Insect Chemical Ecology* (R.T. Cardé, J.G. Millar, eds), pp. 283–332. Cambridge University Press, Cambridge, UK.

Chemnitz, J., Jentschke, P.C., Ayasse, M. & Steiger, S. 2015. Beyond species recognition: somatic state affects long-distance sex pheromone communication. *Proc. R. Soc. Lond. B* **282**: 20150832.

Chung, H. & Carroll, S.B. 2015. Wax, sex and the origin of species: dual roles of insect cuticular hydrocarbons in adaptation and mating. *BioEssays* **37**: 822–830.

Dale, J., Dey, C.J., Delhey, K., Kempenaers, B. & Valcu, M. 2015. The effects of life history and sexual selection on male and female plumage colouration. *Nature* **527**: 367–370.

Delcourt, M. & Rundle, H.D. 2011. Condition dependence of a multicomponent sexual display trait in *Drosophila serrata*. *Am. Nat.* **177**: 812–823.

Everaerts, C., Farine, J.-P., Cobb, M. & Ferveur, J.-F. 2010. *Drosophila* cuticular hydrocarbons revisited: mating status alters cuticular profiles. *PLoS ONE* **5**: e9607.

Fasanello, V.J., Carlton, E.D., Pott, M., Marchetto, N.M., Vaughn, E., McGraw, K.J. *et al.* 2015. Monomorphic ornamentation related to oxidative damage and assortative mating in the black guillemot (*Cepphus grylle*). *Waterbirds* **38**: 106–110.

Fedina, T.Y., Kuo, T.-H., Dreisewerd, K., Dierick, H.A., Yew, J.Y. & Pletcher, S.D. 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. *PLoS ONE* **7**: e49799.

Fernandez, M.P. & Kravitz, E.A. 2013. Aggression and courtship in *Drosophila*: pheromonal communication and sex recognition. *J. Comp. Physiol. A.* **199**: 1065–1076.

Fritzsche, K., Booksmythe, I. & Arnqvist, G. 2016. Sex-ratio bias leads to the evolution of sex-role reversal in honey locust beetles. *Curr. Biol.* **26**: 2522–2526.

Gosden, T.P. & Chenoweth, S.F. 2011. On the evolution of heightened condition dependence of male sexual displays. *J. Evol. Biol.* **24**: 685–692.

Hadley, N.F. 1981. Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure, composition, and waterproofing function. *Biol. Rev.* **56**: 23–47.

Hartbauer, M., Siegert, M.E. & Römer, H. 2015. Male age and female mate choice in a synchronizing katydid. *J. Comp. Physiol. A.* **201**: 763–772.

Howard, R.W. & Blomquist, G.J. 2005. Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* **50**: 371–393.

Ingleby, F.C., Hunt, J. & Hosken, D.J. 2013. Heritability of male attractiveness persists despite evidence for unreliable sexual signals in *Drosophila simulans*. *J. Evol. Biol.* **26**: 311–324.

Johansson, B.G. & Jones, T.M. 2007. The role of chemical communication in mate choice. *Biol. Rev.* **82**: 265–289.

Jones, I.L. & Hunter, F.M. 1993. Mutual sexual selection in a monogamous seabird. *Nature* **362**: 238–239.

Jones, T.M., Balmford, A. & Quinnell, R.J. 2000. Adaptive female choice for middle–aged mates in a lekking sandfly. *Proc. Royal Soc. London B* **267**: 681–686.

de Jong, S.D. 1993. PLS fits closer than PCR. *J. Chemom.* **7**: 551–557.

Judge, K.A. 2011. Do male field crickets, *Gryllus pennsylvanicus*, signal their age? *Anim. Behav.* **81**: 185–194.

Kraaijeveld, K., Gregurke, J., Hall, C., Komdeur, J. & Mulder, R.A. 2004. Mutual ornamentation, sexual selection, and social dominance in the black swan. *Behav. Ecol.* **15**: 380–389.

Kuo, T.-H., Yew, J.Y., Fedina, T.Y., Dreisewerd, K., Dierick, H.A. & Pletcher, S.D. 2012. Aging modulates cuticular hydrocarbons and sexual attractiveness in *Drosophila melanogaster*. *J. Exp. Biol.* **215**: 814–821.

Kwan, L. & Rundle, H.D. 2010. Adaptation to desiccation fails to generate pre- and post-mating isolation in replicate *Drosophila melanogaster* laboratory populations. *Evolution* **64**: 710–723.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl Acad. Sci.* **78**: 3721–3725.

Linn, C.E., Campbell, M.G. & Roelofs, W.L. 1987. Pheromone components and active spaces: what do moths smell and where do they smell it? *Science* **237**: 650–652.

Lopez, P., Aragon, P. & Martin, J. 2003. Responses of female lizards, *Lacerta monticola*, to males' chemical cues reflect their mating preference for older males. *Behav. Ecol. Sociobiol.* **55**: 73–79.

Mendelson, T.C., Martin, M.D. & Flaxman, S.M. 2014. Mutation-order divergence by sexual selection: diversification of sexual signals in similar environments as a first step in speciation. *Ecol. Lett.* **17**: 1053–1066.

Mitchell, R.F., Reagel, P.F., Wong, J.C.H., Meier, L.R., Dias Silva, W., Mongold-Diers, J. *et al.* 2015. Cerambycid beetle species with similar pheromones are segregated by phenology and minor pheromone components. *J. Chem. Ecol.* **41**: 431–440.

Nguyen, D.V. & Rocke, D.M. 2002. Tumor classification by partial least squares using microarray gene expression data. *Bioinformatics* **18**: 39–50.

Noh, S. & Henry, C.S. 2010. Sexually monomorphic mating preferences contribute to premating isolation based on song in European green lacewings. *Evolution* **64**: 261–270.

Osten, D.W. 1988. Selection of optimal regression models via cross-validation. *J. Chemom.* **2**: 39–48.

Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364–371.

Rapkin, J., Jensen, K., House, C.M., Sakaluk, S.K., Sakaluk, J.K. & Hunt, J. 2017. The complex interplay between macronutrient intake, cuticular hydrocarbon expression and mating success in male decorated crickets. *J. Evol. Biol.* **30**: 711–727.

Ritchie, M.G. 2007. Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* **38**: 79–102.

Roberts, J.A. & Uetz, G.W. 2004. Species-specificity of chemical signals: silk source affects discrimination in a wolf spider (Araneae: Lycosidae). *J. Insect Behav.* **17**: 477–491.

Rundle, H.D. & Chenoweth, S.F. 2011. Stronger convex (stabilizing) selection on homologous sexual display traits in females than in males: a multipopulation comparison in *Drosophila serrata*. *Evolution* **65**: 893–899.

Salehialavi, Y., Fritzsche, K. & Arnqvist, G. 2011. The cost of mating and mutual mate choice in 2 role-reversed honey locust beetles. *Behav. Ecol.* **22**: 1104–1113.

Schal, C., Gu, X.P., Burns, E.L. & Blomquist, G.J. 1994. Patterns of biosynthesis and accumulation of hydrocarbons and contact sex pheromone in the female German cockroach, *Blattella germanica*. *Arch. Insect Biochem. Physiol.* **25**: 375–391.

Servedio, M.R. & Noor, M.A.F. 2003. The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.* **34**: 339–364.

Smith, A.A., Vanderpool, W., Millar, J.G., Hanks, L.M. & Suarez, A.V. 2014. Conserved male-specific cuticular hydrocarbon patterns in the trap-jaw ant *Odontomachus brunneus*. *Chemoecology* **24**: 29–34.

South, S.H. & Arnqvist, G. 2009. Morphological variation of an ornament expressed in both sexes of the mosquito *Sabethes cyaneus*. *Evol. Ecol. Res.* **11**: 1–21.

South, S.H., House, C.M., Moore, A.J., Simpson, S.J. & Hunt, J. 2011. Male cockroaches prefer a high carbohydrate diet that makes them more attractive to females: implications for the study of condition dependence. *Evolution* **65**: 1594–1606.

Svensson, M. 1996. Sexual selection in moths: the role of chemical communication. *Biol. Rev.* **71**: 113–135.

Symonds, M.R.E. & Wertheim, B. 2005. The mode of evolution of aggregation pheromones in *Drosophila* species. *J. Evol. Biol.* **18**: 1253–1263.

Takakura, K. 1999. Active female courtship behavior and male nutritional contribution to female fecundity in *Bruchidius dorsalis* (Fahraeus) (Coleoptera: Bruchidae). *Res. Populat. Ecol.* **41**: 269–273.

Takakura, K. 2001. Courtship-role-reversal in the bean weevil, *Bruchidius dorsalis* (Coleoptera: Bruchidae): interplay between male-male competition and cryptic female choice. *Appl. Entomol. Zool.* **36**: 311–316.

Takakura, K.I. 2004. The nutritional contribution of males affects the feeding behavior and spatial distribution of females in a bruchid beetle, *Bruchidius dorsalis*. *J. Ethol.* **22**: 37–42.

Takakura, K.I. 2006. Estimation of relative reproductive expenditure in the courtship-role-reversed bean weevil, *Bruchidius dorsalis* (Fahraeus). *J. Ethol.* **24**: 33–36.

Tuda, M. & Morimoto, K. 2004. A new species *Megabruchidius sophorae* (Coleoptera, Bruchidae), feeding on seeds of *Styphnolobium* (Fabaceae) new to Bruchidae. *Zoolog. Sci.* **21**: 105–110.

Umbers, K.D.L., Symonds, M.R.E. & Kokko, H. 2015. The mothematics of female pheromone signaling: strategies for aging virgins. *Am. Nat.* **185**: 417–432.

Uy, J.A.C. & Borgia, G. 2000. Sexual selection drives rapid divergence in bowerbird display traits. *Evolution* **54**: 273–278.

Verspoor, R.L., Cuss, M. & Price, T.A.R. 2015. Age-based mate choice in the monandrous fruit fly *Drosophila subobscura*. *Anim. Behav.* **102**: 199–207.

Weddle, C.B., Mitchell, C., Bay, S.K., Sakaluk, S.K. & Hunt, J. 2012. Sex-specific genotype-by-environment interactions for cuticular hydrocarbon expression in decorated crickets, *Gryllodes sigillatus*: implications for the evolution of signal reliability. *J. Evol. Biol.* **25**: 2112–2125.

Wedell, N. & Ritchie, M.G. 2004. Male age, mating status and nuptial gift quality in a bushcricket. *Anim. Behav.* **67**: 1059–1065.

Wenninger, E.J., Stelinski, L.L. & Hall, D.G. 2008. Behavioral evidence for a female-produced sex attractant in *Diaphorina citri*. *Entomol. Exp. Appl.* **128**: 450–459.

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

van Wilgenburg, E., Sulc, R., Shea, K.J. & Tsutsui, N.D. 2010. Deciphering the chemical basis of nestmate recognition. *J. Chem. Ecol.* **36**: 751–758.

Wyman, M.J., Stinchcombe, J.R. & Rowe, L. 2013. A multivariate view of the evolution of sexual dimorphism. *J. Evol. Biol.* **26**: 2070–2080.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Example gas chromatograph output from a sample of a single *Megabruchidius* individual.

Figure S2 Pairwise correlations between EC loadings on the PLS axes of multivariate EC variation from 8 independent models of variation in attractiveness (see Tables 2 & 3).

Table S1 Kovats retention index for the 20 epicuticular compounds present in hexane extractions of *Megabruchidius* (see Figure S1).

Data deposited at Dryad: doi: 10.5061/dryad.pj557

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