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The evolution of sex pheromones in an ecologically diverse genus of flies

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In theory, pheromones important in specific mate recognition should evolve via large shifts in composition (saltational changes) at speciation events. However, where other mechanisms exist to ensure reproductive isolation, no such selection for rapid divergence is expected. In *Bactrocera* fruit flies (Diptera: Tephritidae), males produce volatile chemicals to attract females for mating. *Bactrocera* species exhibit great ecological diversity, with a wide range of geographical locations and host plants used. They also have other mechanisms, including temporal and behavioural differences, which ensure reproductive isolation. Therefore, we predicted that their sex pheromones would not exhibit rapid divergence at speciation events. In the present study, we tested this idea by combining data on male sex pheromone composition for 19 species of *Bactrocera* with a phylogeny constructed from DNA sequence data. Analyses of the combined data revealed positive correlations between pheromone differences and nucleotide divergence between species, and between the number of pheromone changes along the phylogeny and the branch lengths associated with these changes. These results suggest a gradual rather than saltational mode of evolution. However, remarkable differences in sex pheromones composition exist, even between closely-related species. It appears therefore that the mode of evolution of sex pheromones in *Bactrocera* is best described by rapid saltational changes associated with speciation, followed by gradual divergence thereafter. Furthermore, species that do not overlap ecologically are just as different pheromonally as species that do. Thus, large changes in pheromone composition appear to be achieved, even in cases where other mechanisms to ensure reproductive isolation exist. We suggest that these differences are closely associated with rapid changes in host plant use, which is a characteristic feature of *Bactrocera* speciation.

ADDITIONAL KEYWORDS: chemical communication – reproductive character displacement – saltational – signal – speciation – syntopy.

INTRODUCTION

Chemical signals are arguably the most common form of communication between animals, and are used by myriad species in wide behavioural contexts (Wyatt, 2003). Intraspecific chemical signals (pheromones) comprise blends of chemical components, the exact composition of which can be remarkably diverse even between closely-related species (Schulz, 2004, 2005). Understanding the evolutionary patterns that underlie this diversity is a challenge that has been, surprisingly, rarely tackled (Symonds & Elgar, 2008).

Chemical signals that are used to attract mates (sex pheromones) can be important in species (mate) recognition and isolation. How such signals evolve is a source of continuing interest and debate (Coyne & Orr, 2004). On the one hand, we would expect signals such as these to be under strong stabilizing selection because any mutant individuals that produced modified versions of the signal would be less likely to be recognized by potential mates (Paterson, 1985). However, if change does occur, then small changes in the signal might also be unlikely, especially with sympatric species. This is because, if the signals are too similar, then reproductive interference (e.g. cross-attraction, mismating, and hybridization) can occur (Gröning & Hochkirch, 2008). In such circumstances,

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there should be strong selection driving rapid divergence in the signal (i.e. reproductive character displacement and reinforcement) (Noor, 1999). Of course, if the closely-related species are allopatric, allochronic (i.e. do not mate at the same time), or exploit different niches, then there will be no such selective force driving character divergence. Similarly, if the signal is not important for species recognition, or other mechanisms exist to ensure isolation, perhaps using other communication modalities, then divergent selection would be less likely.

In the context of sex pheromones, theoretical simulations (Butlin & Trickett, 1997; Bengtsson & Löfstedt, 2007) have predicted periods of stabilizing selection punctuated by large, 'saltational' shifts in pheromone composition associated with speciation. These saltational changes result in the chemical components that comprise the pheromone of the new species being substantially different from the antecedent (Baker, 2002). These saltational changes must involve not only the signaller, but also the receiver. There must be individuals in the population who can recognize and respond to novel blends. Under certain conditions, these individuals may spread (e.g. in small populations). Evidence from corn borer moths shows that these rare receivers in the population can, and do, exist (Roelofs *et al.*, 2002). Furthermore, males in a pheromonal signalling system should be strongly selected to track any changes in the signal, or receiver preferences, of the females (Phelan, 1992).

Data on the pheromone compositions of groups of species, coupled with well-resolved phylogenies and ecological information, offer the potential to investigate the mode of pheromone evolution from a comparative perspective. An analysis of aggregation pheromone evolution in *Dendroctonus* and *Ips* bark beetles indicated that there was no phylogenetic pattern to the expression of active pheromone components within genera, and that closely-related species were just as, if not more, different in aggregation pheromone composition than distantly-related species in the same genera (Symonds & Elgar, 2004a). Using simulated models of character evolution for a set of binary characters (akin to hypothetical pheromone components), Symonds & Elgar (2004a) demonstrated that this kind of relationship between phylogenetic distance and character differences fitted a saltational mode of evolution (Fig. 1).

However, a subsequent analysis of *Drosophila* aggregation pheromones (Symonds & Wertheim, 2005) revealed a clear phylogenetic pattern, with closely-related species producing chemically similar pheromones, indicating a gradual mode of evolution. Symonds & Wertheim (2005) explained these results by noting the differences in function of the pheromones in the context of ensuring reproductive isolation. Bark beetles use aggregation pheromones essentially as sex pheromones, with few other mechanisms preventing mismating or hybridization with other species. *Drosophila* flies, however, use aggregation pheromones to promote communal oviposition, which facilitates larval resource exploitation (Wertheim, Dicke & Vet, 2002), and these pheromones frequently attract heterospecifics. Therefore, a gradual mode of evolutionary change is observed for these aggregation pheromones in the absence of a selective force driving saltational changes. Symonds & Wertheim (2005) predicted that *Drosophila* cuticular hydrocarbon profiles, which indeed are used in mate choice as sex pheromones, would show a saltational mode of evolution. A comparative review of these sex pheromones (Ferveur, 2005) confirmed the tendency for closely-related *Drosophila* species to have very dissimilar cuticular hydrocarbon profiles.

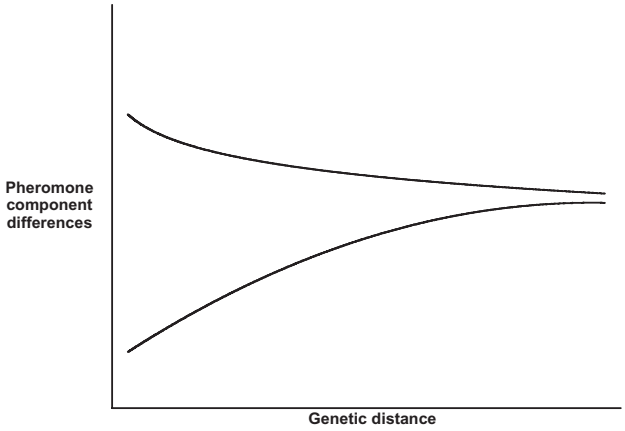

Figure 1. Predicted relationship between phenotypic (pheromonal) difference and genetic distances given by two different modes of evolution: gradual (bottom line) and saltational (top line). Based on the results of simulations performed by Symonds & Elgar (2004a).

In the present study, we examined the evolution of chemical composition of male-produced sex pheromones in *Bactrocera*, a genus of tephritid fly. This highly speciose genus (approximately 500 species) is primarily found in tropical South Asia, Australia, and the South Pacific, and they are major pests of a wide variety of fruit and other crops (Drew, 1989). In most species of *Bactrocera*, males release volatile chemicals from a rectal sac at mating time, which, combined with other courtship behaviours, attracts females (Fletcher, 1969; Drew & Hancock, 1994). The role of these rectal sac secretions as sex pheromones has been established for a number of species (Fletcher, 1969; Hee & Tan, 1998; Wee & Tan, 2005a) and their chemical composition has been ascertained in even more (Fletcher & Kitching, 1995). Although behavioural assays of the secretions have not been con-

ducted in every species, the analogous behavioural scenarios under which they are secreted make it highly unlikely that they are not involved in sexual communication (Krohn *et al.*, 1991, 1992).

We assessed the mode of evolution of these putative sex pheromones by mapping chemical component information onto a phylogeny for *Bactrocera* species constructed from DNA sequence data. Although these pheromones are considered to be important in mate recognition (Drew, Raghu & Halcoop, 2008), it appears there are a number of other mechanisms ensuring reproductive isolation between *Bactrocera* species. For example, two closely-related species, *Bactrocera neohumeralis* and *Bactrocera tryoni* have identical pheromone compositions but sexual activity in these two sympatric species takes place at different times of the day (Bellas & Fletcher, 1979). Although the two species can potentially interbreed (Pike & Meats, 2002), hybridization in their natural environment has not been demonstrated (Gilchrist & Ling, 2006). Furthermore, *Bactrocera* flies exploit a wide range of ecological niches. The evolutionary history of the genus is characterized by rapid and recent speciation, involving large degrees of host-shifting and range changes, particularly in the South-east Asian archipelago (Drew, 2004; Clarke *et al.*, 2005).

We predicted that, given these alternative means of ensuring reproductive isolation, there should be weak selection for character divergence in pheromone composition. Consequently, we would expect to observe a gradual mode of evolution, with considerable overlap in chemical components used between closely-related species (as seen in *B. neohumeralis* and *B. tryoni*).

We analysed the mode of evolution in two ways. First, as a comparison with previous analyses (Symonds & Elgar, 2004a; Symonds & Wertheim, 2005), we related pheromonal differences to phylogenetic distance (in this case, estimates of nucleotide divergence based on mitochondrial and nuclear sequences). Second, we used ancestral state reconstruction to estimate amounts of phenotypic change along each branch of the molecular phylogeny. Under a gradual mode of evolution, there should be a positive correlation between amount of change and the branch length (Pagel, 1999). Finally, we also investigated pheromone composition in the context of other potential reproductive isolating mechanisms. We predicted that geographically or ecologically separated closely-related species should exhibit smaller differences in pheromone composition than species that overlap.

MATERIAL AND METHODS

Pheromonal and phylogenetic information

We collated data on male-produced sex pheromone chemical composition for 19 species of *Bactrocera*. This information was taken from *The Pherobase* (El-Sayed, 2008), an online database of semiochemicals that have been identified for over 7000 mainly arthropod species. The database delineates interspecific chemical attractants from pheromones by providing full details on the structures of the components and their biological function. It also lists all the primary sources from which this information was gathered, enabling us to verify the information. For *Bactrocera*, the Pherobase lists sex pheromone composition for 23 species. However, in four cases, a lack of DNA sequence data prevented us from including the species in the phylogeny (see below) and subsequent analysis. The pheromone data are presented in Table 1. Each chemical component was considered as a 'character' to be mapped onto the phylogeny for the group.

For the phylogenetic reconstruction we accessed GenBank (http://www.ncbi.nlm.nih.gov) and concatenated mitochondrial sequences of Smith, Kambhampati & Armstrong (2003, 2005) and Spanos *et al.* (2000) and the protein coding nuclear gene White Eye (J. Pagadala & S. Kambhampati, unpubl. data), a combined dataset representing 46 *Bactrocera* species and an outgroup species *Ceratitis capitata* (see Supporting information, Appendix S1). For *B. neohumeralis*, the 16S and 12S sequences in GenBank come from M. Muraji and S. Nakahara (unpubl. data). Sequences were first aligned using CLUSTAL W (Thompson, Higgins & Gibson, 1994) followed by manual appraisal. A total of 1962 (16S = 441, 12S = 431, ND1 = 520, COII = 243, White Eye = 327) nucleotide positions were unambiguously aligned, of which 609 were variable and 403 phylogenetically informative. Phylogenetic analysis was conducted using MrBayes, version 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), with model selection determined for each gene partition using MrModelTest (Nylander, 2004). The ribosomal genes 16S and 12S were concatenated into one partition, as were the mitochondrial protein coding genes COI and ND1. The GTRIG model of sequence evolution was applied to both mitochondrial partitions respectively, whereas the HKY-IG model was applied to the White Eye gene. Two simultaneous runs were executed with convergence determined by the average standard deviation of split frequencies declining to < 0.01.

For the final analyses, we employed a consensus phylogram consisting only of the 19 species of *Bactrocera* (plus *Ceratitis capitata*) for which we had pheromone data. The consensus phylogram was derived by removing all nonfocal taxa from the Markov chain Monte Carlo tree samples using the program PAUP, version 4.0 (Swofford, 2001) prior to consensus tree derivation in MrBayes.

Table 1. Chemical components of male sex pheromones of 19 species of *Bactrocera*

Number	Component	Species
1	1,7-Dioxaspiro[5.5]undecane (olean)	*cacuminata*, *oleae*, *umbrosa*
2	(4*S*,6*S*)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane	*cacuminata*, *oleae*
3	(4*R*,6*S*)-4-Hydroxy-1,7-dioxaspiro[5.5]undecane	*cacuminata*, *oleae*
4	(3*S*,6*S*)-3-Hydroxy-1,7-dioxaspiro[5.5]undecane	*cacuminata*
5	(3*R*,6*S*)-3-Hydroxy-1,7-dioxaspiro[5.5]undecane	*cacuminata*, *nigrotibialis*, *oleae*
6	3-Hydroxy-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane	*cucumis*, *dorsalis*
7	1,6-Dioxaspiro[4.5]decane	*cucumis*
8	1,7-Dioxaspiro[5.6]dodecane	*cucumis*
9	(*E*,*E*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	*cucumis*, *kirki*, *latifrons*, *nigrotibialis*
10	(*E*,*Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	*cucumis*, *kirki*
11	(*Z*,*Z*)-2,8-Dimethyl-1,7-dioxaspiro[5.5]undecane	*cucumis*
12	(*E*,*E*)-2-Ethyl-7-methyl-1,6-dioxaspiro[5.5]decane	*cucumis*
13	(*Z*,*E*)-2-Ethyl-7-methyl-1,6-dioxaspiro[5.5]decane	*cucumis*
14	(*E*,*E*)-2-Ethyl-8-methyl-1,7-dioxaspiro[5.5]undecane	*dorsalis*, *kirki*, *latifrons*, *occipitalis*
15	7-Ethyl-2-methyl-1,6-dioxaspiro[5.5]decane	*occipitalis*
16	2,7-Dimethyl-1,6-dioxaspiro[4.4]nonane	*umbrosa*
17	(*E*)-2-Methyl-1,6-dioxaspiro[4.5]decane	*umbrosa*
18	(5R,7S)-7-Methyl-1,6-dioxaspiro[4.5]decane	*xanthodes*
19	6-Butyl-3,4-dihydro-2H-pyran	*cacuminata*
20	1-Hydroxynonan-5-one	*cacuminata*
21	6-oxo-nonan-1-ol	*carambolae*, *occipitalis*
22	Nonane-1,3-diol	*cucumis*, *tau*
23	*N*-2-Methylbutylpropanamide	*dorsalis*, *neohumeralis*, *tryoni*
24	*N*-3-Methylbutylpropanamide	*neohumeralis*, *tau*, *tryoni*
25	*N*-(3-Methylbutyl)-2-methylpropanamide	*neohumeralis*, *tryoni*
26	*N*-(2-Methylbutyl)-2-methylpropanamide	*neohumeralis*, *tryoni*
27	2-Methoxy-N-3-methylbutylacetamide	*tau*
28	*N*-2-Methylbutylacetamide	*neohumeralis*, *tryoni*
29	*N*-3-Methylbutylacetamide	*cacuminata*, *carambolae*, *dorsalis*, *facialis*, *neohumeralis*, *passiflorae*, *tau*, *tryoni*
30	2-Isopropyl-4,5-dimethyloxazole	*latifrons*
31	2-(1-Methylpropyl)-4,5-dimethyloxazole	*latifrons*
32	Ethyl 4-hydroxybenzoate	*cucurbitae*
33	Propyl 4-hydroxybenzoate	*cucurbitae*
34	2-Ethoxybenzoic acid	*cucurbitae*
35	2,3,5,6-Tetramethylpyrazine	*cucurbitae*
36	2,3,5-Trimethylpyrazine	*cucurbitae*, *dorsalis*
37	2-Methylpyrazine	*cucurbitae*
38	Pentacosane	*cucurbitae*
39	Heptacosane	*cucurbitae*
40	Nonacosane	*cucurbitae*
41	(*E*)-5-(3,6-heptadienyl)-dihydro-2(3H)-furanone	*cucurbitae*
42	Dimethyl succinate	*dorsalis*
43	Diethyl 5-oxononanedioate	*oleae*
44	Trimethyl phosphate	*dorsalis*
45	2-Allyl-4,5-dimethoxyphenol	*dorsalis*, *papayae*
46	4-((*E*)-3-Hydroxyprop-1-enyl)-2-methoxyphenol	*dorsalis*
47	(*Z*)-3,4-Dimethoxycinnamylalcohol	*dorsalis*
48	3-Methylbutan-1-ol	*umbrosa*
49	3-Methyl-2-butenylacetate	*visenda*
50	3-Methyl-3-butenylacetate	*visenda*
51	3-Methyl-2-butenylproprionate	*visenda*
52	3-Methyl-2-butenylformate	*visenda*
53	3-Methyl-2-buten-1-ol	*visenda*
54	3-Methyl-2-butenal	*visenda*
55	3-Methylbutylacetate	*visenda*
56	(1*R*,5*S*)-5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	*facialis*, *passiflorae*
57	(1*S*,5*S*)-5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	*facialis*, *passiflorae*
58	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	*facialis*, *passiflorae*

Assessing the mode of evolution

We constructed distance and difference matrices to assess the relationship of pheromonal differences to estimates of pairwise nucleotide divergences. The genetic pairwise matrix was derived from our phylogeny by summing branch lengths (expected substitutions per site) between each species pair using the program PHYLOCOM, version 4 (Webb, Ackerly & Kembel, 2008). The number of pheromonal differences is measured as the binary squared Euclidean distance; the number of components that are absent in one species but present in the other, and vice versa. We then used Mantel tests to examine the correlation between the two measures, the rows and columns of the distance matrix being randomly perturbed and the correlation coefficient recalculated 999 times to generate a null reference distribution. These tests were carried out using GenAlEx (Peakall & Smouse, 2001).

We calculated ancestral states for each component at every node in the phylogeny using a Bayesian approach as implemented in SIMMAP (Bollback, 2006). From these data, we were able to estimate the amount of change (i.e. the number of components that switched state from present to absent or vice versa) along each branch. We then compared these data with the branch lengths, testing for any correlation, aiming to assess the mode of evolution (Pagel, 1999).

Influence of other potential reproductive isolating mechanisms

We collected information, where known, on the geographic distribution, the host plant species, and temporal patterns of mating behaviour for the *Bactrocera* species in our analysis. This information was taken from Drew (1989), White & Elson-Harris (1992), Perkins *et al.* (1990), Allwood *et al.* (1999) and the Pacific Fruit Fly Web (http://www.pacifly.org). The data are presented in the Supporting information (Appendix S2).

We used these data to identify the species pairs that overlap geographically, share host plants and mate synchronically. These species are termed syntopic, *sensu* Rivas (1964), because they are sympatric, share the same habitat, and can potentially interbreed. We repeated the analysis relating pheromonal differences to nucleotide divergences, separating our species pairs into those that are syntopic and those that are allotopic, and comparing the results between the two groups. Allotopic species possess alternative reproductive isolating mechanisms that prevent cross-attraction. Hence, they are predicted to show fewer differences in pheromone composition between closely-related species.

RESULTS

There is a remarkable diversity in sex pheromone composition within *Bactrocera*. For the 19 species in our analysis, 58 different components have been identified, 37 of which are unique to individual species. Only 21 components are shared between two or more species. Species blends comprise 4.89 ± 0.65 (mean ± SE) components. The average pheromonal difference between species is remarkably high (9.01 ± 0.30 components).

In terms of phylogeny, the results of our mixed model Bayesian analysis are entirely consistent with the parsimony based analysis of Smith *et al.* (2005) (results not shown), particularly the monophyly of subgenus *Bactrocera* and the polyphyly of the subgenus *Zeugodacus*. This phylogenetic structure is upheld in the pruned consensus tree of the focal taxa (Fig. 2). A comprehensive revision of the supraspecific classification within *Bactrocera* is near completion (S. Kambhampati & J. Pagadala, pers. commun.) and will discuss further phylogenetic inference.

We found a weak positive correlation between the number of pheromone component differences and the nucleotide divergence between species (Mantel test: $r = 0.263$; $P = 0.075$; Fig. 3). The most closely-related species tend to have the most similar pheromones, which suggests a gradual mode of evolution. However, there is very large variance around this trend. More significantly, the elevation of the best-fit line indicates that even quite closely-related species can be highly dissimilar. For example, on average, the five most closely-related species in our analysis have seven components different between them.

We found a weakly significant positive correlation between the number of pheromone component changes along each branch and branch length ($r = 0.326$; $N = 36$; $P = 0.050$; Fig. 4), indicating that larger amounts of change tend to be associated with longer branches, which again suggests a gradual mode of evolution. Again, however, there is considerable variation. Additionally, the majority of changes occur at the tips of the tree (average number of changes on terminal branches = 3.9, on internal branches = 1.2).

A relatively small proportion (17 out of 171) of our species comparisons are between syntopic species (i.e. species that overlap geographically, share the same host plants, and mate at the same time of day, and hence might interbreed). Allotopic (i.e. non-overlapping) species exhibit a stronger positive correlation between pheromonal differences and nucleotide divergence than do syntopic species (allotopic species, Mantel test: $r = 0.288$; $P < 0.001$; syntopic species, Mantel test: $r = 0.088$; $P = 0.657$; Fig. 5), which

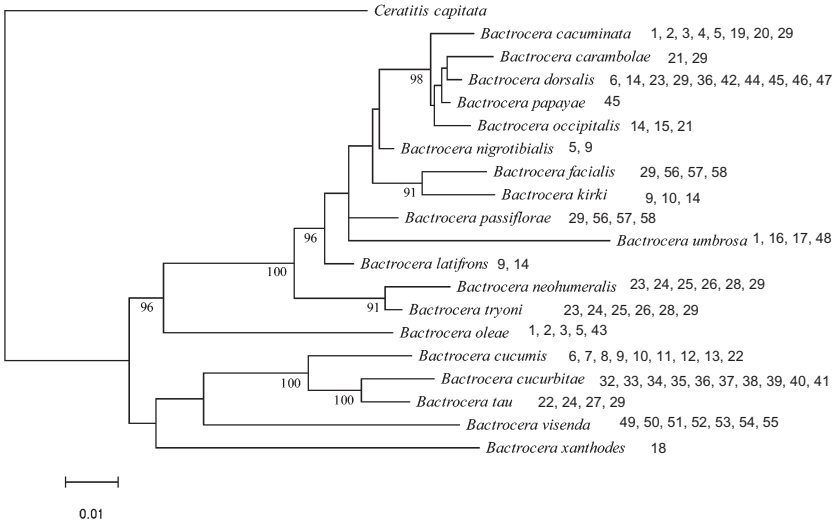

Figure 2. Bayesian posterior Majority Rule (all compatible partitions) consensus tree pruned to the 20 focal taxa. Phylogenetic reconstruction is based on a three partition (1, 16S + 12S; 2, ND1 + COII; 3, White Eye) mixed model analysis with topology and branch lengths linked across partitions. Nodes having > 90% posterior probability are indicated. The branch length scale bar indicates number of substitutions per site. Numbers after species names indicate the chemical components of the pheromone for each species (for component information, see Table 1).

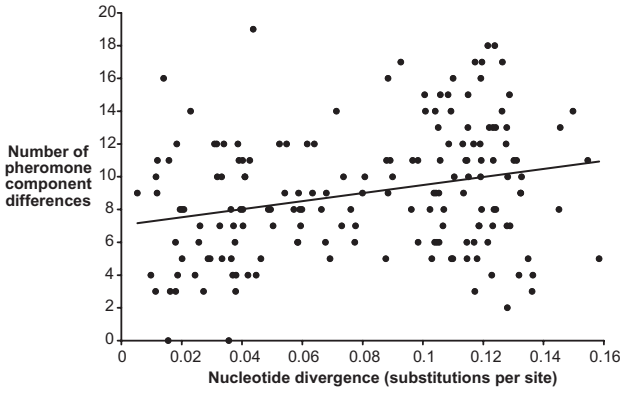

Figure 3. Relationship between number of differences in pheromone components and nucleotide divergence between *Bactrocera* species.

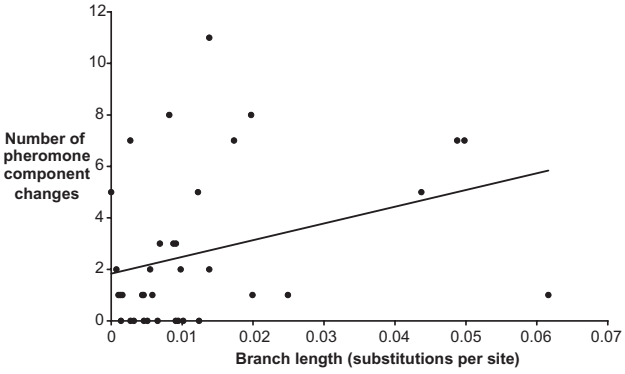

Figure 4. Estimated number of changes in pheromone components along each branch in the phylogeny in relation to the length of the branch. Longer branches tend to show greater amounts of change.

implies that allotopic species show a more gradual mode of evolution in their pheromones. Surprisingly, however, the elevations of the two slopes are similar, indicating that even very closely-related but reproductively isolated species exhibit large differences in pheromone composition.

DISCUSSION

The present study revealed weak correlations between both pheromone difference and nucleotide divergence, and number of chemical component changes and branch length, both of which might indicate a gradual mode of evolution. These findings contrast with the pattern observed in bark beetle aggregation pheromones (Symonds & Elgar, 2004a), but are comparable to the result observed for *Drosophila* aggregation pheromones (Symonds & Wertheim, 2005), although those pheromones are not important in species recognition.

However, there are still remarkably large differences in sex pheromone composition in *Bactrocera*. The average number of chemical component differences across *Bactrocera* species (9) is almost two-fold greater than the amount found in *Drosophila* (4.3; Symonds & Wertheim, 2005), or across two genera of bark beetles for aggregation pheromones (4.6; Symonds & Elgar, 2004a). Indeed, *The Pherobase*

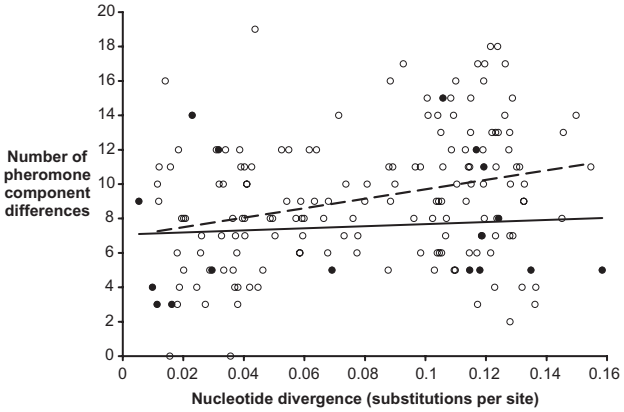

Figure 5. Relationship between number of difference in pheromone components and nucleotide divergence between species for syntopic (filled circles, solid line) and allotopic (open squares, dotted line) *Bactrocera* species.

(El-Sayed, 2008) lists as many as 77 components in the 23 species of *Bactrocera* for which sex pheromone composition has been reported. This represents an enormous 'palette' of chemicals capable of being produced by *Bactrocera* species, and partly explains the large diversity of blends that have been observed.

The relationship between component differences and nucleotide divergence between species (Fig. 3) suggests that even very closely-related species tend, on average, to exhibit considerable differences. We suggest that these results indicate a mode of evolution whereby there are rapid saltational changes in chemical composition at speciation events, with greater divergence gradually being added subsequently. The pheromonally identical sibling species *B. neohumeralis* and *B. tryoni*, as discussed earlier, therefore appear to be an exception to this pattern.

The positive correlation between branch length and amount of change (Fig. 4) indicates a gradual mode of evolution, although, again, even the very shortest branch length (approximately zero) is associated with some change (two components change predicted). Intriguingly, most of the zero change branches in our phylogeny are at deeper points in the tree, whereas a more extensive change appears to be taking place in the terminal branches. This implies that rapid divergence in chemical composition is associated with comparatively recent speciational events.

The recent speciational history of *Bactrocera* has been characterized by sudden and profound changes in host-plant use (Drew, 2004; Clarke *et al.*, 2005). Changes in host-plant use generally are a significant element in speciation, particularly in sympatry (Drès & Mallet, 2002). Host adaptation in turn tends to lead to reproductive isolation (Nosil *et al.*, 2007), with reinforcement occurring through reproductive character displacement, including in sexual signals. For example, host-plant changes have recently been demonstrated to be important to changes in vibrational mating signals in two closely-related species of treehoppers (McNett & Cocroft, 2008). Host-plant related chemical differences would be expected also because these chemicals have, in part, diet-related precursors (e.g. for *Bactrocera*, see Nishida *et al.*, 1988). Thus, *Bactrocera passiflorae* and *Bactrocera facialis* have identical pheromone profiles, despite being phylogenetically separate. This similarity may be attributed to the species' similar ecological habits, feeding primarily on mango, guava, and papaya (Fletcher *et al.*, 1992). We argue, therefore, that the large and rapid changes observed in *Bactrocera* sex pheromone profiles are associated with the similarly extensive host plant changes that have contributed to the diversity of this genus.

Furthermore, there is some question as to the extent to which these signals are important in mate recognition. Although no natural hybrids of the pheromonally identical *B. neohumeralis* and *B. tryoni* have been found (Gilchrist & Ling, 2006), presumably because they mate allochronically, there is clear evidence of hybridization in the field among *Bactrocera papayae*, *Bactrocera dorsalis*, and *Bactrocera carambolae* (Wee & Tan, 2005b). Yet these species have very different pheromone compositions (Fig. 2). Differences in these sex pheromones may neither be necessary, nor sufficient, to ensure mate recognition and reproductive isolation, and may relate more to other factors.

That there have clearly been many changes in host-plant use (see Supporting information, Appendix S2) in *Bactrocera* accounts for why so few of the species in our analysis are syntopic. Furthermore, if allotopy is associated with changes in host-plant use, this may explain why the allotopic species in our analysis are no less different in pheromone profile than syntopic species. An unresolved issue is to what extent species overlap drives divergence in *Bactrocera* chemical signals, and to what extent divergence related to host-plant changes. Even in bark beetle aggregation pheromones, where a saltational mode of evolution occurs, the role of syntopy as an ecological factor driving saltational change is unclear (Symonds & Elgar, 2004b). Furthermore, present-day patterns of syntopy or allotopy may not reflect conditions at the time of speciation. There is equally strong evidence that, in some cases, speciation in *Bactrocera* has been allopatric and, in other cases, sympatric and associated with host-plant changes (Drew, 2004). Similarly, genetic mechanisms have been uncovered that can result in temporal changes in sympatry (Miyatake *et al.*, 2002). These different types of speciation would have a strong influence on changes in pheromone profile. A more detailed speciational

history of *Bactrocera* needs to be established if we are to derive a more accurate picture of the evolution of these signals.

One factor not considered is that many of the pheromone components that we have analysed are structurally related and produced via the same biosynthetic pathways (Fletcher *et al.*, 1992; Fletcher & Kitching, 1995). For example, 12 of the 19 species in our analysis (Table 1) employ spiroacetal compounds such as 1,7-dioxaspiro[5.5]undecane (olean). Differences in pheromone profiles might then be relatively easily obtained via small changes in biosynthetic pathways. Even given the relatively large differences in specific components, we might expect closely-related species to utilize the same groups of chemicals. Hence, a strong pattern of gradual evolution might be expected if the structural and biosynthetic pathways were taken into account. Indeed, a separate analysis by performed us (results not shown) indicated no such trend and suggests that other factors, such as diet, may play a stronger role in determining the pheromone profiles of particular species.

The pattern of evolution of sex pheromones in *Bactrocera* that our analysis has highlighted continues to build the picture regarding the evolution of these chemical signals (Symonds & Elgar, 2008). Our analysis indicates rapid saltational shifts in composition followed by more gradual changes, with an accumulation of differences in pheromone components over time. Although these sex pheromones may be selected to provide mate recognition, we suggest this diversity is more closely associated with host shifts. At the very least, the enormous diversity in pheromone composition observed suggests that, in ecologically diverse genera such as *Bactrocera*, a wide range of ecological factors can play important roles in shaping pheromone profiles.

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REFERENCES

Allwood AJ, Chinajariyawong A, Drew RAI, Hamacek EL, Hancock DL, Hengasawad C, Jipanin JC, Jirasurat M, Kong Krong C, Kritsaneepaiboon S, Leong CTS, Vijaysegaran S. 1999. Host plant records for fruit flies (Diptera: Tephritidae) in southeast Asia. *Raffles Buletin of Zoology Supplement* **7:** 1–92.

Baker TC. 2002. Mechanism for saltational shifts in pheromone communication systems. *Proceedings of the National Academy of Sciences of the United States of America* **99:** 13368–13370.

Bellas TE, Fletcher BS. 1979. Identification of the major components in the secretion from the rectal pheromone glands of the Queensland fruit flies *Dacus tryoni* and *Dacus neohumeralis* (Diptera: Tephritidae). *Journal of Chemical Ecology* **5:** 795–803.

Bengtsson BO, Löfstedt C. 2007. Direct and indirect selection in moth pheromone evolution: population genetical simulations of asymmetric sexual interactions. *Biological Journal of the Linnean Society* **90:** 117–123.

Bollback JP. 2006. SIMMAP: stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7:** 88.

Butlin RK, Trickett AJ. 1997. Can population genetic simulations help to interpret pheromone evolution? In: Cardé RT, Minks AK, eds. *Insect pheromone research*. New York, NY: Chapman and Hall, 548–562.

Clarke AR, Armstrong KF, Carmichael AE, Milne JR, Raghu S, Roderick GK, Yeates DK. 2005. Invasive phytophagous pest arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology* **50:** 293–319.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Drew RAI. 1989. The tropical fruit flies (Diptera: Tephritidae: Dacinae) of the Australasian and Oceanic regions. *Memoirs of the Queensland Museum* **26:** 1–521.

Drew RAI. 2004. Biogeography and speciation in the Dacini (Diptera: Tephritidae: Dacinae). *Bishop Museum Bulletin in Entomology* **12:** 165–178.

Drew RAI, Hancock DL. 1994. The *Bactrocera dorsalis* complex of fruit flies in Asia. *Bulletin of Entomological Research Supplementary Series* **2:** 1–68.

Drew RAI, Raghu S, Halcoop P. 2008. Bridging the morphological and biological species concepts: studies on the *Bactrocera dorsalis* (Hendel) complex (Diptera: Tephritidae: Dacinae) in South-east Asia. *Biological Journal of the Linnean Society* **93:** 217–226.

Drès M, Mallet J. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **357:** 471–492.

El-Sayed AM. 2008. *The pherobase: database of insect pheromones and semiochemicals*. Available at: http://www.pherobase.com

Ferveur JF. 2005. Cuticular hydrocarbons: their evolution and roles in *Drosophila* pheromonal communication. *Behavior Genetics* **35:** 279–295.

Fletcher BS. 1969. The structure and function of sex pheromone glands of the male Queensland fruit fly, *Dacus tryoni*. *Journal of Insect Physiology* **15:** 1309–1322.

Fletcher MT, Kitching W. 1995. Chemistry of fruit flies. *Chemical Reviews* **95:** 789–828.

Fletcher MT, Wells JA, Jacobs MF, Krohn S, Kitching W, Drew RAI, Moore CJ, Francke W. 1992. Chemistry of fruit flies. Spiroacetal-rich secretions in several *Bactrocera* species from the South-West Pacific. *Journal of the Chemical Society Perkin Transactions* **1:** 2827–2831.

Gilchrist AS, Ling AE. 2006. DNA microsatellite analysis of naturally occurring colour intermediates between *Bactrocera tryoni* (Froggatt) and *Bactrocera neohumeralis* (Hardy) (Diptera: Tephritidae). *Australian Journal of Entomology* **45:** 157–162.

Gröning J, Hochkirch A. 2008. Reproductive interference between animal species. *Quarterly Review of Biology* **83:** 257–282.

Hee AKW, Tan KH. 1998. Attraction of female and male *Bactrocera papayae* to conspecific males fed with methyl eugenol and attraction of females to male sex pheromone components. *Journal of Chemical Ecology* **24:** 753–764.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Krohn S, Fletcher MT, Kitching W, Drew RAI, Moore CJ, Francke W. 1991. Chemistry of fruit flies: nature of glandular secretion and volatile emission of *Bactrocera (Bactrocera) cacuminatus* (Héring). *Journal of Chemical Ecology* **17:** 485–495.

Krohn S, Fletcher MT, Kitching W, Moore CJ, Drew RAI, Francke W. 1992. Chemistry of fruit flies: glandular secretion of *Bactrocera (Polistomimetes) visenda* (Hardy). *Journal of Chemical Ecology* **18:** 2169–2176.

McNett GD, Cocroft RB. 2008. Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers. *Behavioral Ecology* **19:** 650–656.

Miyatake T, Matsumoto A, Matsuyama T, Ueda HR, Toyosato T, Tanimura T. 2002. The period gene and allochronic reproductive isolation in Bactrocera cucurbitae. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269:** 2467–2472.

Nishida R, Tan KH, Serit M, Lajis NH, Sukari AM, Takahashi S, Fukami H. 1988. Accumulation of phenylpropanoids in the rectal glands of males of the Oriental fruit fly, *Dacus dorsalis*. *Experientia* **44:** 534–536.

Noor MAF. 1999. Reinforcement and other consequences of sympatry. *Heredity* **83:** 503–508.

Nosil P, Crespi BJ, Gries R, Gries G. 2007. Natural selection and divergence in mate preference during speciation. *Genetica* **129:** 309–327.

Nylander JAA. 2004. *Mrmodeltest V2*. Program distributed by the author. Uppsala: Evolutionary Biology Centre, Uppsala University.

Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401:** 877–884.

Paterson HEH. 1985. The recognition concept of species. In: Vrba ES, ed. *Species and speciation*. Pretoria: Transvaal Museum. Transvaal Museum Monograph no. 4, 21–29.

Peakall R, Smouse PE. 2001. *GenAlEx v5: genetic analysis in excel. Population genetic software for teaching and research*. Canberra: Australian National University. Available at: http://www.anu.edu.au/BoZo/GenAlEx/

Perkins MV, Kitching W, Drew RAI, Moore CJ, Konig WA. 1990. Chemistry of fruit flies – composition of the male rectal gland secretions of some species of south-east Asian Dacinae – reexamination of *Dacus cucurbitae* (melon fly). *Journal of the Chemical Society Perkin Transactions* **1:** 1111–1117.

Phelan PL. 1992. Evolution of sex pheromones and the role of asymmetric tracking. In: Roitberg BD, Isman MB, eds. *Insect chemical ecology: an evolutionary approach*. New York, NY: Chapman & Hall, 265–314.

Pike N, Meats A. 2002. Potential for mating between *Bactrocera tryoni* (Froggatt) and *Bactrocera neohumeralis* (Hardy) (Diptera: Tephritidae). *Australian Journal of Entomology* **41:** 70–74.

Rivas LR. 1964. A reinterpretation of the concepts 'sympatric' and 'allopatric' with proposal of the additional terms 'syntopic' and 'allotropic'. *Systematic Zoology* **13:** 42–43.

Roelofs WL, Liu W, Hao G, Jiao H, Rooney AP, Linn CE Jr. 2002. Evolution of moth sex pheromones via ancestral genes. *Proceedings of the National Academy of Sciences of the United States of America* **99:** 13621–13626.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Schulz S, ed. 2004. *The chemistry of pheromones and other semiochemicals I. Topics in Current Chemistry*, Vol. 239. Berlin: Springer.

Schulz S, ed. 2005. *The chemistry of pheromones and other semiochemicals II. Topics in Current Chemistry*, Vol. 240. Berlin: Springer.

Smith PT, Kambhampati S, Armstrong KA. 2003. Phylogenetic relationships among *Bactrocera* species (Diptera: Tephritidae) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **26:** 8–17.

Smith PT, Kambhampati S, Armstrong KA. 2005. Phylogenetic relationships and character evolution among selected species of *Bactrocera* (Diptera: Tephritidae) based on multiple mitochondrial genes. *Insect Systematics and Evolution* **36:** 343–360.

Spanos L, Koutoumbas G, Kosyfakis M, Louis C. 2000. The mitochondrial genome of the Mediterranean fruitfly, *Ceratitis capitata*. *Insect Molecular Biology* **9:** 139–144.

Swofford DL. 2001. *PAUP*. Phylogenetic analysis using parsimony (*and other methods),* Version 4. Sunderland, MA: Sinauer Associates.

Symonds MRE, Elgar MA. 2004a. The mode of pheromone evolution: evidence from bark beetles. *Proceedings of the Royal Society of London Series B, Biological Sciences* **271:** 839–846.

Symonds MRE, Elgar MA. 2004b. Species overlap, speciation and the evolution of aggregation pheromones in bark beetles. *Ecology Letters* **7:** 202–212.

Symonds MRE, Elgar MA. 2008. The evolution of pheromone diversity. *Trends in Ecology and Evolution* **23:** 220–228.

Symonds MRE, Wertheim B. 2005. The mode of evolution of aggregation pheromones in *Drosophila* species. *Journal of Evolutionary Biology* **18:** 1253–1263.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL

W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

Webb CO, Ackerly DD, Kembel SW. 2008. *Phylocom: software for the analysis of phylogenetic community structure and character evolution*, Version 4.0. Available at: http://phylodiversity.net/phylocom/

Wee SL, Tan KH. 2005a. Female sexual response to male rectal volatile constituents in the fruit fly *Bactrocera carambolae* (Diptera: Tephritidae). *Applied Entomology and Zoology* **40:** 365–372.

Wee SL, Tan KH. 2005b. Evidence of natural hybridization between two sympatric sibling species of *Bactrocera dorsalis* complex based on pheromone analysis. *Journal of Chemical Ecology* **31:** 845–858.

Wertheim B, Dicke M, Vet LEM. 2002. Behavioural plasticity in support of a benefit for aggregation pheromone use in *Drosophila melanogaster*. *Entomologia Experimentalis et Applicata* **103:** 61–71.

White IM, Elson-Harris MM. 1992. *Fruit flies of economic significance: their identification and bionomics*. Wallingford: CAB International.

Wyatt TD. 2003. *Pheromones and animal behaviour: communication by smell and taste*. Cambridge: Cambridge University Press.

SUPPORTING INFORMATION

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

Appendix S1. List of taxa used in the phylogenetic analysis and GenBank accession numbers.
Appendix S2. Ecological information for the 19 species of *Bactrocera* used in the analysis.

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