Intraguild interactions promote assortative mating and affect sexual attractiveness in a phytophagous fly

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Changes in acoustic and substrate-borne sexual signals in phytophagous insects associated with host plant shifts are known to have the potential to promote assortative mating, reproductive isolation and speciation. In this article, we ask whether the switch between pure herbivory and intraguild predation (IGP), which is common amongst phytophagous insects, has similar potential. Male flies in the genus *Lipara* (Diptera: Chloropidae) search for females by vibrating reed stems and waiting for a reply. By kleptoparasitizing other phytophagous species in the genus (a form of IGP), *Lipara rufitarsis* can increase its nonsexual fitness considerably. We looked at the impact of IGP on the timing of hatching, body size and attractiveness of male calls in *L. rufitarsis*. *L. rufitarsis* males that had engaged in IGP hatched significantly earlier than purely phytophagous flies and were significantly larger, but their calls were less likely to elicit responses from females during playback experiments. We conclude that, although behavioural observations of females provided no evidence of 'like preferring like', changes in phenology associated with IGP are likely to promote assortative mating in this system. The general preference of females for the calls of smaller males is a phenomenon worthy of further study: it may have no adaptive significance, or it may indicate that mating with large males is associated with a fitness cost. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **98**, 171–180.

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INTRODUCTION

Mate choice on the basis of sexual traits has the potential to enhance the fitness of individuals of the choosing sex, because such traits can provide information about the condition, genetic quality, compatibility and species identity of potential mates (reviewed in Andersson, 1994). When mate choice evolves, it can play an important role in the processes of speciation and diversification, especially if the different desirable characteristics indicated by sexual traits are positively associated (West-Eberhard, 1983; Andersson, 1994). For example, coevolution of mate preference and sexual traits can reinforce the reproductive isolation of closely related species if phenotypes which make individuals of the correct species more readily recognizable to potential mates also indicate good condition or 'good genes'. However, the desirable characteristics indicated by sexual traits do not necessarily correlate positively, meaning that different selection pressures may have conflicting effects on the evolution of mate choice. For example, traits which indicate the highest genetic quality or best condition in a potential mate may also be those which are most easily confused with the traits of other species (Ryan & Rand, 1993; Pfennig, 1998; Schmeller, O'Hara & Kokko, 2005). Empirical evidence suggests that a conflict between species and mate quality recognition can explain mate choice strategies which might otherwise be considered maladaptive (Barlow & Siri, 1997; Pfennig, 2000).

Many phytophagous insects communicate via acoustic or substrate-borne signals. Such species have provided a number of model systems in which to study the different kinds of selection pressure affecting sexual traits and mate preferences (Cocroft & Rodriguez, 2005; Drosopoulous & Claridge, 2006).

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Females are known to use the characteristics of male calls to identify mates of the correct species (for example, Dewinter & Rollenhagen, 1990; Talyn & Dowse, 2004; Safi, Heinzle & Reinhold, 2006) and those of high quality (for example, Brown *et al.*, 1996; De Luca & Morris, 1998). There is also evidence to suggest that the use of male sexual signals by females to avoid heterospecific matings can influence the value of these signals for distinguishing among conspecific males (Safi *et al.*, 2006).

The study of phytophagous insects in general, and those that rely on substrate-borne communication in particular, has highlighted the potential of host plant shifts to generate patterns of assortative mating and reproductive isolation (Berlocher & Feder, 2002; Funk, Filchak & Feder, 2002; Rodriguez *et al.*, 2008). For example, when different plant species have different effects on insect phenology, a host shift may lead to the temporal separation of sympatric subpopulations (for example, Wood & Keese, 1990). Alternatively, host plant shifts may be accompanied by a divergence in sexual signals used to attract mates (Etges & Ahrens, 2001; McNett & Cocroft, 2008). This raises the question: do other changes in the trophic ecology of species have similar potential to bring about assortative mating and reproductive isolation, and hence perhaps to play a role in speciation? Host shifts in parasitoids can certainly affect phenology in such a way as to generate assortative mating (Henry, 2008), but, of particular interest in the context of this study, is the frequent occurrence of intraguild predation or parasitism (IGP) amongst phytophagous insects (Polis, Myers & Holt, 1989; Arim & Marquet, 2004). In theory, a switch from herbivory to parasitism or predation, with all the possible associated changes in spatial and temporal distribution and nutrition, should be able to generate assortative mating and reproductive isolation in just the same way as a host shift in a herbivore. This possibility has apparently yet to be explored explicitly.

In this article, we explore the impact of a change in trophic ecology on male body size, and on the characteristics and attractiveness of male mating calls, in a phytophagous insect, *Lipara rufitarsis* Meigen (Diptera, Chloropidae). *Lipara rufitarsis* is facultatively kleptoparasitic on a congeneric species, and can be considered to engage in a form of IGP (see below). We focus on three questions. First, how does a shift to a kleptoparasitic mode of life affect body size and the characteristics of male calls? Second, do differences in body size and call characteristics between phytophagous and kleptoparasitic males impact on their attractiveness to females? Third, do the effects of trophic ecology on *L. rufitarsis* mating calls and phenology have the potential to generate assortative mating (flies mating preferentially with individuals with the same trophic history), which might lead to reproductive isolation?

Lipara rufitarsis and its congener *Lipara lucens* are monophagous herbivores which form characteristic apical galls on the stems of the common reed *Phragmites australis* (Chvála *et al.*, 1974). They have an annual life cycle. Eggs are laid and hatch in June, and each individual initiates and excavates a single gall during the summer. *Lipara lucens* galls are large and heavily defended against predators and parasitoids. *Lipara rufitarsis* galls are much smaller, and larvae are vulnerable to parasitism and are easily extracted by avian predators (Chvála *et al.*, 1974; Reader, 2001). Fully grown larvae overwinter within galls and pupate in spring. Adults hatch out and leave their galls in late spring.

The highly mobile adult males locate sedentary females in often dense stands of *P. australis* by moving from stem to stem, producing vibratory signals and waiting for responses from females (Mook & Bruggemann, 1968). Virgin females respond to the relatively complex male signals by producing a simple signal of their own, during or immediately after the male call. On detection of a response, a male will walk along a stem searching for the female. Mating usually takes place rapidly once a male has located a receptive female. Although males will continue to search for females once copulation is complete, mated females will no longer respond to male signals (Kanmiya, 1990).

Because the vibratory signals of *L. lucens* and *L. rufitarsis* appear to be the primary (and perhaps only) mechanism by which a male locates potential mates, opportunities for mating are probably controlled largely by the female. There is marked interspecific variation in the characteristics of male signals, and this variation is used by females to distinguish between conspecifics and heterospecifics (Chvála *et al.*, 1974). At least in *L. lucens*, male (but not female) signals also vary significantly among geographically isolated populations of the same species (Kanmiya, 1990), but the consequences of this variation for the reproductive compatibility of allopatric populations are unknown.

In some habitats, *L. lucens* and *L. rufitarsis* compete strongly for *P. australis* stems (Reader, 2003). A theoretical study of the dynamics of competition between the two species showed that *L. rufitarsis* can only persist in such habitats because it is able to kleptoparasitize *L. lucens* (Reader, Cornell & Rohani, 2006). When larvae of both species are present on the same stem, an *L. lucens* gall forms, but *L. rufitarsis* kills its competitor and takes possession of the gall. *Lipara rufitarsis* individuals developing in *L. lucens* galls (henceforth referred to as 'kleptoparasitic' flies) are more likely to survive to maturity, and

emerge significantly heavier than those developing in *L. rufitarsis* galls ('phytophagous' flies) (Reader, 2003). Kleptoparasitic females are also substantially more fecund than phytophagous females (Reader, 2003). Despite the advantages to *L. rufitarsis*, evidence from competition experiments and observed egg distributions suggests that interspecific interactions between the two species occur more or less at random, and many *L. rufitarsis* populations persist in the absence of *L. lucens* (Reader, 2001). Although it is possible that some *L. rufitarsis* individuals possess traits which make interactions more likely or more profitable, we have no evidence that a tendency to kleptoparasitize is heritable.

In this study, we examine the consequences of interspecific interactions for the phenology and mating behaviour of *L. rufitarsis*. Given the clear fitness benefits of kleptoparasitism to *L. rufitarsis*, we might expect natural selection to favour traits which make interactions with *L. lucens* more common. Such traits might not evolve, however, if mating success is reduced in kleptoparasitic flies. By experimentally assessing female preferences, we tested the hypothesis that, although enjoying enhanced growth and survival, kleptoparasitic males produce vibratory signals which are not typical of their species and are less attractive to potential mates. Measurements of the effect of interspecific interactions on *L. rufitarsis* mating behaviour and phenology also allow us to test for mechanisms by which sympatric populations of kleptoparasitic and phytophagous flies might become reproductively isolated.

MATERIALS AND METHODS

Approximately 500 *L. lucens* and 500 *L. rufitarsis* galls were collected from Chippenham Fen Nature Reserve in Suffolk, UK in April 2007, just prior to the emergence of adult flies. Pupae were extracted from galls and kept in individually labelled Petri dishes in the laboratory at 20–25 °C until adult flies emerged. On eclosion, flies were sexed and the date was recorded. Interspecific interactions were common in the population sampled: 33.9% of the 489 *L. lucens* galls collected contained *L. rufitarsis* pupae, and only 26.2% of 225 surviving *L. rufitarsis* pupae were found in their own galls.

### RECORDINGS OF MALE SIGNALS

The vibratory signals of 41 adult males were recorded 2 or 3 days after eclosion using a modification of the method employed by Kanmiya (1990). A male–female pair of virgin flies was placed in a small (4 cm long × 3 cm wide × 1 cm high) plastic box resting on top of a paper membrane stretched across a metal tripod. The stylus on a ceramic stereo cartridge (Model BSR SC12H, Maplin Electronics) was brought into contact with the paper membrane beneath the box. The output from the cartridge was amplified using an Isleworth A101 AC amplifier, and digitized with a Lab-trax 4/16 A to D converter (World Precision Instruments). In the presence of a female fly, males readily produced vibratory signals which were recorded and analysed using Data-trax recording software (World Precision Instruments). Recordings continued for 30 min, or until at least six calls had been recorded. Measurements of signal amplitude made using this technique are affected by the pressure with which the stylus is pressed against the paper membrane (Cocroft & Rodriguez, 2005). Therefore, to ensure that any variation in amplitude was random with respect to the identity of the fly being recorded, the stylus was repositioned for each recording. The technical limitations of our method mean that variation in the absolute amplitude of the calls did not necessarily indicate biological differences among males. For this reason, we only included information about the relative amplitudes of the components of the calls in our analysis (see below).

Signals were characterized using measurements taken from oscillograms and power spectra generated by fast Fourier transformation. Each signal comprised a 'preliminary vibration' and a 'main vibration' (Fig. 1). The main vibration consisted of a series of 'bursts', each separated from the next by a pause (the 'inter-burst interval') and a 'spike'. In some but not all recordings, a small 'pre-burst spike' could be distinguished immediately before the burst. For each signal, we measured the following: (1) the total length of the signal; (2) the length of the preliminary signal; (3) the length of the main signal; (4) the number of bursts in the main signal; (5) the fundamental frequency of the main signal; (6) the period of the first, second and final bursts (measured as the time between the beginning of one burst and the beginning of the next); (7) the length of the first, second and final inter-burst intervals; and (8) the ratio between the maximum amplitudes of the burst (excluding any pre-burst spike) and the subsequent spike for the first, second and penultimate bursts. This final measurement (the burst to spike amplitude ratio) was used to quantify what was to the human eye the most variable characteristic of the recorded signals; some males produced signals with very low-amplitude bursts, whereas others produced signals in which the burst was nearly as loud as the spike.

PLAYBACK EXPERIMENTS

The responses of females to recorded male signals were assessed using playback experiments. The first
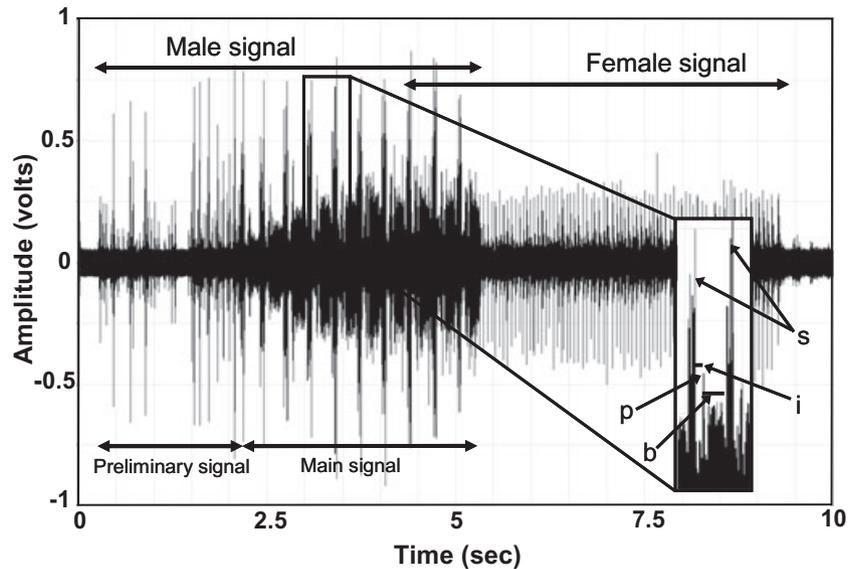
Figure 1. Oscillogram of a typical male *Lipara rufitarsis* vibratory signal, and a typical female response. In this example, the female begins to respond before the male has finished signalling. The magnified inset shows the detail of the iterated components of the male's main signal. A relatively lengthy 'burst' (b) ends in a short, high-amplitude 'spike' (s). The spike is followed by an 'inter-burst interval' (i). In this case, the burst is immediately preceded by a small 'pre-burst spike' (p).

signal recorded from each male was converted into a sound file using Scilab (Copyright © 1989–2007 Institut National de Recherche en Informatique et en École des Ponts ParisTech; see http://www.scilab.org). Signals from randomly chosen males were then played back to individual virgin females, 2 or 3 days after eclosion. Lone females were placed in a small plastic box (as above) resting on a paper membrane stretched over the housing of a small loudspeaker (Yamaha model YST-M15). After a period of 10 min to adjust to their environment, the membrane was vibrated by playing the male signal through the speaker at a standard volume. The playback was repeated ten times (the same call each time), with an interval of 30 s between signals. The responses of females were recorded via the stylus of a ceramic stereo cartridge using the method described above, except that the stylus was placed in contact with the membrane adjacent to rather than below the box housing the fly. Each female was used in only one playback trial.

STATISTICAL ANALYSIS

Measurements taken from male signals, many of which were obviously intercorrelated, were subjected to principal components analysis (PCA) in order to produce a small number of new variables which described the measured variance in the characteristics of the signals and could be used in subsequent analysis. Of the components generated by PCA, those with eigenvalues which plotted to the left of the beginning of the tail on a scree plot, and which were strongly associated (with factor loadings of > 0.6) with at least three of the original variables, were retained (Field, 2005). Varimax orthogonal rotation was performed on the extracted component matrix in order to improve the ease with which factors could be interpreted. This analysis was performed using SPSS Version 12. The extracted components, together with other data describing fly phenology, body size and female responses to playback experiments, were analysed using analysis of variance (ANOVA) and generalized linear mixed effects models in R version 2.6.1 (The R Core Development Team, 2007). Mixed models were used when pseudoreplication within individuals necessitated the fitting of random effects. Model selection broadly followed Venables & Ripley (1997). First, a full model was fitted, containing one or two fixed effects, an interaction where appropriate and one random effect. Fixed effects were then removed sequentially: interaction first, then the main effects in order of likely significance (as indicated by estimated coefficients in the full model). The significance of the effect of each deletion was assessed with likelihood ratio (LR) tests. Finally, the significance of the random effect was assessed by testing the impact of its deletion from the mixed model containing only those main effects that were significant. Error structures were assumed to be Gaussian, except where stated.

Table 1. The mean (±standard error of the mean, SEM) hatch dates, wing lengths and thorax lengths of male and female *Lipara rufitarsis* individuals that developed as larvae in *L. rufitarsis* and *L. lucens* galls. Hatch date is given in days after 1 May 2007

	L. rufitarsis gall		*L. lucens* gall	
Measure	Male	Female	Male	Female
Hatch date (days)	20.84 (±0.65)	21.52 (±0.68)	16.68 (±0.42)	16.68 (±0.40)
Wing length (mm)	2.74 (±0.23)	3.13 (±0.27)	2.96 (±0.25)	3.41 (±0.29)
Thorax length (mm)	1.87 (±0.04)	2.13 (±0.04)	2.08 (±0.03)	2.36 (±0.03)

Table 2. Results of analysis of variance in hatch dates and two measures of body size of male and female kleptoparasitic and phytophagous flies

	Hatch date		Wing length		Thorax length	
Term	$F_{(DF)}$	P	$F_{(DF)}$	P	$F_{(DF)}$	P
Sex	0.43 (1,105)	0.514	155.76 (1,103)	< 0.001	74.47 (1,104)	< 0.001
Gall type	74.62 (1,105)	< 0.001	54.61 (1,103)	< 0.001	46.31 (1,104)	< 0.001
Sex × gall type	0.43 (1,105)	0.515	0.86 (1,103)	0.356	0.09 (1,104)	0.766

RESULTS

EFFECTS OF INTERSPECIFIC INTERACTIONS ON DEVELOPMENT

Male and female *L. rufitarsis* hatched simultaneously (see Tables 1 and 2). Individuals that had developed in their own gall hatched significantly later than individuals that had developed in *L. lucens* galls, regardless of sex. Females had longer wings and thoraces than males, and kleptoparasitic individuals had longer wings and thoraces than phytophagous individuals, regardless of sex.

CHARACTERIZATION OF MALE CALLS

The first three components generated by PCA together explained over 60% of the variance in the original 14 variables that were used to describe calls (see Table 3). Calls which scored highly on the first component (PC1) had long, low-frequency bursts. Calls scoring highly on the second component (PC2) lasted for a long time, and those scoring highly on the third component (PC3) had relatively loud bursts and quiet spikes.

There were highly significant differences among males in all three principal components (mixed effects model; PC1: LR = 189.377, $P < 0.001$; PC2: LR = 113.468, $P < 0.001$; PC3: LR = 94.562, $P < 0.001$; see Fig. 2). There were, however, no consistent differences between the calls of phytophagous and kleptoparasitic males (PC1: LR = 0.870, $P = 0.351$; PC2: LR = 0.969, $P = 0.325$; PC3: LR = 0.158, $P = 0.691$).

Table 3. Results of principal components analysis (PCA) of 14 variables describing a total of 220 calls from 41 different males. Details are shown for the three most important extracted components which, together, explained over 60% of the variance

	Component		
	PC1	PC2	PC3
Eigenvalue	3.277	2.977	2.480
Percentage of variance explained	23.405	21.262	17.714
Loadings:			
Total call length	0.018	0.970	−0.022
Preliminary call length	−0.029	0.647	−0.069
Main call length	0.061	0.905	0.037
Number of bursts	−0.238	0.872	0.031
Fundamental frequency	−0.732	0.033	0.106
First burst period	0.874	−0.044	0.009
Second burst period	0.868	−0.145	0.027
Mean burst period	0.900	0.076	0.024
First inter-burst interval	0.406	−0.070	−0.083
Second inter-burst interval	0.428	−0.027	0.001
Final inter-burst interval	0.031	0.019	0.008
First burst to spike amplitude ratio	0.012	−0.022	0.891
Second burst to spike amplitude ratio	0.000	0.012	0.931
Penultimate burst to spike amplitude ratio	−0.053	−0.033	0.89
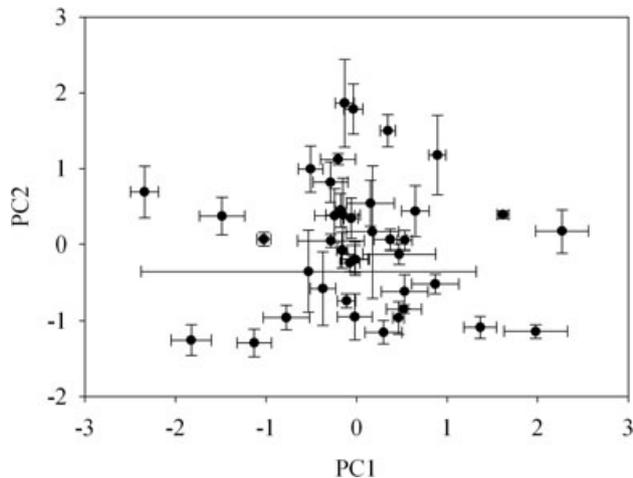
Figure 2. The mean first and second principal component scores (±standard error of the mean, SEM) generated from the calls of 41 male *Lipara rufitarsis* adults. There were similar differences among individuals in PC3 (data not shown). See main text for details of the interpretation of the components shown.

Averaging across calls for each male, there were no significant correlations between wing or thorax size and characteristics of male calls (Pearson's correlations of PC1, PC2 and PC3 versus wing and thorax size: $r < 0.25$, $P > 0.1$, $N = 41$).

FEMALE PREFERENCES

There was a significant effect of male origin on the frequency with which female *L. rufitarsis* responded during the playback experiment (mixed effects model with binomial error structure: LR = 11.975, $P < 0.001$). Females were more likely to respond to calls from phytophagous males (average of 61.8% of calls elicited a response) than to calls from kleptoparasitic males (average of 34.1% of calls elicited a response). There was no significant difference between phytophagous and kleptoparasitic females in the propensity to respond (LR = 1.244, $P = 0.265$), and no interaction between the effects of male and female origin on the propensity to respond (LR = 2.751, $P = 0.097$). Considering only those instances in which females responded to playbacks, there was no effect of male (LR = 1.652, $P = 0.199$) or female (LR = 0.751, $P = 0.386$) origin on the duration of the female response, and no interaction between male and female origin (LR = 0.002, $P = 0.999$).

The propensity of females to respond to male calls, and the duration of responses, were not predicted by the characteristics of the calls (see Table 4). There was, however, a significant negative correlation between the number of female responses to male calls and male wing length and thorax length (see Table 4).

Table 4. Results of correlations between the number and length of responses of females to playbacks, male call characteristics [represented by three components extracted using principal components analysis (PCA)] and two measures of male body size

	Number of responses ($N = 41$)		Average length of response ($N = 24$)	
	Spearman's r_s	P	Pearson's r	P
PC1	0.066	0.684	−0.260	0.220
PC2	−0.051	0.750	−0.031	0.886
PC3	0.188	0.239	0.398	0.054
Wing length	−0.380	0.017	−0.174	0.438
Thorax length	−0.402	0.010	−0.418	0.047

Note that, because playback experiments used only the first recorded call from each male, the PCA described in Materials and methods was repeated using only these calls before conducting the correlation analysis. The first three principal components extracted had very similar eigenvalues and loadings to those shown in Table 3, and can be interpreted in the same way.

The response duration was also negatively correlated with thorax size, but not with wing size.

DISCUSSION

Our results suggest that engaging in IGP has wide-ranging effects on *L. rufitarsis*. Kleptoparasitic flies hatched earlier and were larger than purely phytophagous individuals. Furthermore, we found evidence that engaging in IGP reduced the attractiveness of male calls to both kleptoparasitic and phytophagous females. Analysis of call structure failed to reveal which components of male calls were used by females to distinguish among possible mates. These results have interesting implications for our understanding of the selection pressures acting on mate preferences and trophic niche in this species.

IGP, ASSORTATIVE MATING AND REPRODUCTIVE ISOLATION

The reproductive isolation of phytophagous insect populations as a result of host plant shifts has stimulated considerable interest in the potential for such shifts to facilitate speciation (Berlocher & Feder, 2002; Dres & Mallet, 2002; Bolnick & Fitzpatrick, 2007). Although other kinds of differences in trophic ecology among individuals have not been studied in detail, it seems reasonable to expect that they have similar evolutionary potential. *Lipara rufitarsis* indi-

viduals that developed in their own galls hatched on average about 5 days later than conspecifics that developed in the galls of *L. lucens*, and this strongly suggests that IGP has the potential to lead to assortative mating in this species. Given that females mate only once, begin laying eggs within 1 or 2 days of eclosion and have a reproductively active life of only about 10 days when kept in optimal conditions in the laboratory (Mook, 1967; Chvála *et al.*, 1974), such a difference in phenology must mean that flies are much less likely to mate with individuals with a different trophic history than with individuals that develop in the same kind of gall.

Temporal reproductive isolation of sympatric insect populations on different host plants, as a result of plant-specific changes in phenology, has been identified as a potentially potent facilitator of speciation in a number of systems (Smith, 1988; Wood & Keese, 1990; Pratt, 1994; Feder & Filchak, 1999). Our results serve as a reminder that ecological changes, other than those associated with host plant shifts, can have an impact on phenology, possibly influencing population genetic structure and conceivably promoting speciation. Such potential can only be realized, however, if the isolation of subpopulations extends over many generations. As yet, we have no evidence that *L. rufitarsis* adults emerging from heterospecific galls are any more likely to have offspring which also kleptoparasitize *L. lucens* than would be expected if interactions between the species occurred at random (Reader, 2001, 2003). Thus, the specific evolutionary consequences of the trophic interaction we describe here remain unclear.

We have shown a difference of 10–15% in wing and thorax length between flies that developed in conspecific and heterospecific galls. Kanmiya (1997) observed mean differences of about 10% in body size among allopatric populations of a closely related species (*Lipara japonica*). He found that it was physically impossible for the males and females of most extreme size from different origins to copulate (Kanmiya, 2006). Therefore, it seems possible that, in *L. rufitarsis*, body size differences may promote a degree of mating incompatibility between flies from phytophagous and kleptoparasitic backgrounds. Such a possibility could easily be tested with appropriate mating experiments.

The potential for the reproductive isolation of phytophagous and kleptoparasitic subpopulations of *L. rufitarsis* would be considerably enhanced if patterns of mate preference in females facilitated assortative mating. However, our results show that there is no tendency for females to choose males with the same trophic history. Instead, both phytophagous and kleptoparasitic females showed a preference for phytophagous males that had developed in *L. rufitarsis* galls.

Although our analysis of call structure showed that individual males had strikingly different calls, it did not reveal which component(s) influenced the propensity of females to respond. As such, we cannot yet say whether the calls of kleptoparasitic males are atypical of their species, or are more similar to those of other species. It may be that the female preference we have detected involves a complex nonlinear response to male call traits, or that there are strong interactions among traits, but our current dataset is not sufficiently large, and our measures of female preference are not sufficiently sensitive, to warrant more detailed analysis of the selection response surface. Further work is obviously needed to clarify the mechanism at work, especially as, in this study, we were unable reliably to record the variation in absolute call amplitude (see Materials and methods). Body size might reasonably be expected to impact, in particular, on amplitude, so that the future analysis of calls using apparatus that measures amplitude in a standardized fashion would be of particular interest.

Despite the lack of assortative mating, the impacts of trophic ecology on phenology and morphology in this study system demonstrate that IGP, at least, has the potential to promote the formation of sympatric subpopulations which are more or less reproductively isolated. Especially in the light of work which has shown that host shifts in brood parasites can facilitate speciation (Sorenson, Sefc & Payne, 2003), we believe that the role played by changes in trophic ecology other than host plant shifts in speciation in general, and sympatric speciation in particular, is worth exploring further.

CONFLICTING SELECTION PRESSURES ON MALE AND FEMALE BEHAVIOUR

Kleptoparasitic *L. rufitarsis* larvae are more likely to survive to adulthood (Reader, 2003), and the resultant adults are more fecund (Reader, 2003) and larger (see Results). Given these benefits, we might expect natural selection to favour the evolution of traits which make encounters with heterospecifics and gall theft more likely. We have yet, however, to find evidence for the existence of such traits (Reader, 2001, 2003). The results from our mate preference experiment suggest a possible cost to the kleptoparasitic way of life which may negate the benefits: the calls of males emerging from the 'wrong' galls are less attractive to females. If kleptoparasitic individuals are less successful in the search for a mate, sexual selection may inhibit the action of natural selection on traits which might facilitate the utilization of what is potentially an underexploited niche. The idea that natural and sexual selection exert conflicting selection pressures on traits has long been recognized (Darwin,

1871). Although 'good genes' models have shown that a positive genetic correlation between sexual and nonsexual fitness can lead to a synergism between the two selective forces (Lorch *et al.*, 2003), the evidence that sexual selection promotes adaptation is not strong (Holland, 2002; Rundle, Chenoweth & Blows, 2006). Our system may turn out to be an example of one in which the need to find a mate constrains the abilities of species to adapt to their environment.

There are two important caveats when considering possible explanations for the absence of a specialized, parasitic way of life in *L. rufitarsis*. First, although we have found evidence to suggest that kleptoparasitic males may be disadvantaged when looking for a mate, we have no evidence of any fitness cost for kleptoparasitic females. Valuable insights into the evolutionary significance of the presence or absence of kleptoparasitic traits in *L. rufitarsis* may be gained by considering the fact that the result of any trade-offs that exist are likely to be different for the two sexes. Second, we have no evidence to suggest that a tendency to kleptoparasitize *L. lucens* is heritable and, as such, it is quite possible that, whatever the *potential* selective forces in this system, there may be no traits on which these forces can act; a more definitive leap from herbivore to parasite may simply not be possible in this particular lineage at this particular point in evolutionary time.

The observed female preference for small males that developed in conspecific galls is potentially maladaptive. Why choose less fit males? There are several possible explanations. Firstly, and most obviously, the observed preference may have no adaptive significance – it may have no consequences for fitness, or there may be no genetic variation in preference on which selection can act. Alternatively, female preference may be adaptive, and may reflect a conflict between the need to find a high-quality mate and the need to find one of the right species (Ryan & Rand, 1993; Pfennig, 1998). Females in this genus are known to be able to distinguish among males of four *Lipara* species, all of which feed on reed stems in the same habitats, on the basis of their calls (Chvála *et al.*, 1974). However, mistakes in species recognition have been observed (Chvála *et al.*, 1974). It is conceivable that females receiving unwanted attention from a male of the wrong species will have reduced fitness, and matings with heterospecific males are certainly likely to yield fewer, less fit offspring (for example, Harrison & Hall, 1993; Noor, 1995; Pfennig & Simovich, 2002). There may therefore be selection pressure on female *L. rufitarsis* to respond to those male calls that are most typical of the species, as is the case in, for example, the grasshopper *Chorthippus brunneus* (Butlin, Hewitt & Webb, 1985). Alternatively, it may benefit females to respond to males whose calls are most unlike those of other species, as is the case in *Chorthippus biguttulus* (Safi *et al.*, 2006). Although our analysis of call structure failed to reveal which components allowed females to distinguish between phytophagous and kleptoparasitic males, it seems quite plausible that changes in body size associated with gall theft subtly modify male calls in some way. Unusually large, kleptoparasitic *L. rufitarsis* males may be avoided because they have calls which are in some way atypical of the species, or which are more easily confused with calls of larger species, such as *L. lucens*. Such a phenomenon is known in spadefoot toads (*Spea multiplicata*), where females in populations which are sympatric with those of a larger species choose smaller mates in order to avoid mating with heterospecifics, even though larger conspecific males are better at fertilizing a female's eggs (Pfennig, 2000).

Female *L. rufitarsis* may be keen to avoid large males for other reasons, such as a correlation between male body size and physical harm caused as a result of copulation (see, for example, Pitnick & Garcia-Gonzalez, 2002); however, in the absence of direct evidence of an impact of mate choice on female fitness, any discussion of adaptive explanations for the observed preference remains speculative.

CONCLUSION

The study of acoustic and substrate-borne signals in phytophagous insects continues to yield insights into fundamental questions about the evolution of traits and the diversification of lineages. Our results highlight the potential of plant–insect and herbivore–herbivore interactions to generate subtle, interesting and informative patterns of behaviour. Specifically, they suggest that switches between pure herbivory and IGP may promote reproductive isolation of sympatric populations. They also add to a growing body of evidence suggesting that patterns of resource use and female mate preference are influenced by a suite of sometimes conflicting selection pressures, and that, as a result, the observed behaviour is not always immediately intuitive.

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REFERENCES

Andersson M. 1994. *Sexual selection*. Princeton, NJ: Princeton University Press.

Arim M, Marquet PA. 2004. Intraguild predation: a widespread interaction related to species biology. *Ecology Letters* **7:** 557–564.

Barlow GW, Siri P. 1997. Does sexual selection account for the conspicuous head dimorphism in the Midas cichlid? *Animal Behaviour* **53:** 573–584.

Berlocher SH, Feder JL. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? *Annual Review of Entomology* **47:** 773–815.

Bolnick DI, Fitzpatrick BM. 2007. Sympatric speciation: models and empirical evidence. *Annual Review of Ecology, Evolution and Systematics* **38:** 459–487.

Brown WD, Wideman J, Andrade MCB, Mason AC, Gwynne DT. 1996. Female choice for an indicator of male size in the song of the black-horned tree cricket *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evolution* **50:** 2400–2411.

Butlin RK, Hewitt GM, Webb SF. 1985. Sexual selection for intermediate optimum in *Chorthippus brunneus* (Orthoptera: Acrididae). *Animal Behaviour* **33:** 1281–1292.

Chvála M, Doskocil J, Mook JH, Pokorny V. 1974. The genus *Lipara* Meigen (Diptera: Chloropidae), systematic morphology, behaviour and ecology. *Tijdschrift voor Entomologie* **117:** 1–25.

Cocroft RB, Rodriguez RL. 2005. The behavioral ecology of insect vibrational communication. *Bioscience* **55:** 323–334.

Darwin C. 1871. *The descent of man, and selection in relation to sex*. London: John Murray.

De Luca PA, Morris GK. 1998. Courtship communication in meadow katydids: female preference for large male vibrations. *Behaviour* **135:** 777–794.

Dewinter AJ, Rollenhagen T. 1990. The importance of male and female acoustic behavior for reproductive isolation in *Ribautodelphax* planthoppers (Homoptera, Delphacidae). *Biological Journal of the Linnean Society* **40:** 191–206.

Dres 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.

Drosopoulous S, Claridge MF, eds. 2006. *Insect sounds and communication: physiology, behaviour, ecology and evolution*. Boca Raton, FL: Taylor and Francis.

Etges WJ, Ahrens MA. 2001. Premating isolation is determined by larval-rearing substrates in cactophilic *Drosophila mojavensis*. V. Deep geographic variation in epicuticular hydrocarbons among isolated populations. *American Naturalist* **158:** 585–598.

Feder JL, Filchak KE. 1999. It's about time: the evidence for host plant-mediated selection in the apple maggot fly, *Rhagoletis pomonella*, and its implications for fitness trade-offs in phytophagous insects. *Entomologia Experimentalis et Applicata* **91:** 211–225.

Field A. 2005. *Discovering statistics using SPSS*. London: Sage Publications.

Funk DJ, Filchak KE, Feder JL. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica* **116:** 251–267.

Harrison JF, Hall HG. 1993. African European honeybee hybrids have low nonintermediate metabolic capacities. *Nature* **363:** 258–260.

Henry LM. 2008. Assortative mating and the role of phenotypic plasticity in male competition: implications for gene flow among host-associated parasitoid populations. *Biology Letters* **4:** 508–511.

Holland B. 2002. Sexual selection fails to promote adaptation to a new environment. *Evolution* **56:** 721–730.

Kanmiya K. 1990. Acoustic properties and geographic variation in the vibratory courtship signals of the European chloropid fly, *Lipara lucens* Meigen (Diptera, Chloropidae). *Journal of Ethology* **8:** 105–120.

Kanmiya K. 1997. *Bioacoustic aspect on dipteran biosystematics in Asia, especially in case of* Lipara *Mg. (Chloropidae). Current situation and prospects of dipterology in Asia*. Seoul: Korean Entomological Institute of Korea University.

Kanmiya K. 2006. Communication by vibratory signals in Diptera. In: Drosopoulos S, Claridge MF, eds. *Insects sounds and communication: physiology, behaviour, ecology and evolution*. Boca Raton, FL: CRC Press, 381–396.

Lorch PD, Proulx S, Rowe L, Day T. 2003. Condition-dependent sexual selection can accelerate adaptation. *Evolutionary Ecology Research* **5:** 867–881.

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

Mook JH. 1967. Habitat selection by *Lipara lucens* Mg. (Diptera, Chloropidae) and its survival value. *Archives Neerlandaises de Zoologie* **17:** 469–549.

Mook JH, Bruggemann CG. 1968. Acoustical communication by *Lipara lucens* (Diptera, Chloropidae). *Entomologica Experimentia et Applicalis* **11:** 397–402.

Noor MA. 1995. Speciation driven by natural-selection in *Drosophila*. *Nature* **375:** 674–675.

Pfennig KS. 1998. The evolution of mate choice and the potential for conflict between species and mate-quality recognition. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265:** 1743–1748.

Pfennig KS. 2000. Female spadefoot toads compromise on mate quality to ensure conspecific matings. *Behavioral Ecology* **11:** 220–227.

Pfennig KS, Simovich MA. 2002. Differential selection to avoid hybridization in two toad species. *Evolution* **56:** 1840–1848.

Pitnick S, Garcia-Gonzalez F. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269:** 1821–1828.

Polis GA, Myers CA, Holt RD. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics* **20:** 297–330.

Pratt GF. 1994. Evolution of *Euphilotes* (Lepidoptera, Lycaenidae) by seasonal and host shifts. *Biological Journal of the Linnean Society* **51:** 387–416.

Reader T. 2001. Competition, kleptoparasitism and intraguild predation in a reedbed community. PhD Thesis, University of Cambridge.

Reader T. 2003. Strong interactions between species of phytophagous fly: a case of intraguild kleptoparasitism. *Oikos* **103:** 101–112.

Reader T, Cornell SJ, Rohani P. 2006. Aggregation, intraguild interactions and the coexistence of competitors on small ephemeral patches. *Oikos* **115:** 321–333.

Rodriguez RL, Sullivan LM, Snyder RL, Cocroft RB. 2008. Host shifts and the beginning of signal divergence. *Evolution* **62:** 12–20.

Rundle HD, Chenoweth SF, Blows MW. 2006. The roles of natural and sexual selection during adaptation to a novel environment. *Evolution* **60:** 2218–2225.

Ryan MJ, Rand AS. 1993. Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* **47:** 647–657.

Safi K, Heinzle J, Reinhold K. 2006. Species recognition influences female mate preferences in the common European grasshopper (*Chorthippus biguttulus* Linnaeus, 1758). *Ethology* **112:** 1225–1230.

Schmeller DS, O'Hara R, Kokko H. 2005. Male adaptive stupidity: male mating pattern in hybridogenetic frogs. *Evolutionary Ecology Research* **7:** 1039–1050.

Smith DC. 1988. Heritable divergence of *Rhagoletis pomonella* host races by seasonal asynchrony. *Nature* **336:** 66–67.

Sorenson MD, Sefc KM, Payne RB. 2003. Speciation by host switch in brood parasitic indigobirds. *Nature* **424:** 928–931.

Talyn BC, Dowse HB. 2004. The role of courtship song in sexual selection and species recognition by female *Drosophila melanogaster*. *Animal Behaviour* **68:** 1165–1180.

The R Core Development Team. 2007. *R: a language and environment for statistical computing*. Vienna: Available from http://www.R-project.org. Accessed 3 June 2009.

Venables WN, Ripley BD. 1997. *Modern applied statistics with S-plus*. New York: Springer-Verlag.

West-Eberhard MJ. 1983. Sexual selection, social competition, and speciation. *Quarterly Review of Biology* **58:** 155–183.

Wood TK, Keese MC. 1990. Host-plant-induced assortative mating in *Enchenopa* treehoppers. *Evolution* **44:** 619–628.